Université de Liège

Faculté de Médecine Vétérinaire

Département des Maladies Infectieuses et Parasitaires

Épidémiologie et Analyse de risques appliquées aux sciences vétérinaires

CONTRIBUTION TO THE WILDLIFE INFECTIOUS DISEASE RISK ANALYSIS ASSOCIATED WITH THE REINTRODUCTION OF THE SCIMITAR-HORNED ORYX (*ORYX DAMMAH*) FROM THE UNITED ARAB EMIRATES TO CHAD

Louis LIGNEREUX

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Front Cover: (Figure 1): Bas-relief depicting a scimitar-horned oryx hunting scene, east wall of the Ptahhotep's chapel, Mastabas of Saqqara, Egypt.

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CONTRIBUTION À L'ANALYSE DU RISQUE DE MALADIES INFECTIEUSES ANIMALES ASSOCIÉ À LA RÉINTRODUCTION DE L'ORYX ALGAZELLE (*ORYX DAMMAH*) DES ÉMIRATS ARABES UNIS AU TCHAD

Louis LIGNEREUX

Supervisor
Prof. Claude Saegerman
Research Unit of Epidemiology and Risk Analysis Applied to Veterinary (UREAR - ULiège)
Fundamental and Applied Research for Animals & Health (FARAH)
Faculty of Veterinary Medicine
Liège University
Belgium

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Acronyms

ADAFSA	Abu Dhabi Agriculture Food Safety Authority
ADFCA	Abu Dhabi Food Control Authority
BTV	Bluetongue virus
CCHF	Crimean-Congo haemorrhagic fever
CCPP	Contagious caprine pleuropneumonia
CDC	Centers for Disease Control and Prevention
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
EAD	Environment Agency - Abu Dhabi
EFSA	European Food Safety Authority
FMD	Foot-and-mouth disease
GDP	Gross domestic product
ICCT	Intradermal comparative cervical tuberculin
IPBES	Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services
IUCN	International Union for the Conservation of Nature
km	Kilometer
MIRU-VNTR	Mycobacterial Interspersed Repetitive Unit – Variable Number Tandem Repeat
MTBC	*Mycobacterium tuberculosis* complex
OROAFR	Ouadi Rimé - Ouadi Achim Faunal Reserve
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
SHO	Scimitar-horned oryx
SSC	Species Survival Commission
UAE	United Arab Emirates
UNEP	United Nation Environment Programme
USA	United States of America
USD	United States Dollar
WAHIS	World Animal Health Information System
WDRA	Wildlife disease risk analysis
WHO	World Health Organisation
WOAH	World Organisation for Animal Health

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Abstract

The reintroduction of the scimitar-horned oryx (*Oryx dammah*) (SHO) from the United Arab Emirates to Chad provides a unique opportunity for in-depth exploration of various aspects related to the ecological, environmental, and health dynamics associated with this reintroduction process.

Prompted by the global decline in biodiversity, this endeavour aims to reinstate a native species and mitigate the negative impacts of human activities on ecosystems. The SHO, recognized as a potential keystone species, which might play a pivotal role in shaping ecosystem structure and functioning. The reintroduction initiative not only focuses on the oryx's well-being but also underscores broader implications for ecosystem health, biodiversity, and the harmony between human and environmental interests.

However, the reintroduction process introduces inherent risks, particularly concerning the potential for pathogen pollution and the release of zoonotic pathogens. The increased interaction between the oryx, other wildlife, livestock, and human populations elevates the risk of disease transmission. This necessitates a systematic risk assessment, providing insights to inform strategies for mitigating the potential impacts of disease emergence on both wildlife, livestock, and human health.

Brucellosis poses a significant zoonotic risk, with the first confirmed outbreak in SHO described in article 1. Serological testing revealed a high seroprevalence rate, with females and older individuals showing higher probabilities of being seropositive in article 2. In article 6, a controlled trial administering the Rev.1 vaccine demonstrated safety, with both subcutaneous and conjunctival routes eliciting long-term cellular responses. However, the conjunctival route exhibited a shorter humoral response.

Furthermore, the presence of Bluetongue virus genome in seronegative oryx post-transport highlights risks associated with translocation in appendix 1, necessitating pre-import risk assessment for wild ruminant species susceptible to orbiviruses not only in the country of destination but also where transit happens.

Foot-and-mouth disease outbreaks underscore the susceptibility of SHO to this economically impactful disease in article 3, emphasizing the need for robust surveillance and control measures.

Contagious caprine pleuropneumonia outbreaks further demonstrate the susceptibility of related wildlife species in article 4 and highlight transmission dynamics previously undocumented in article 5.

Our findings provide valuable insights into the epidemiology of pathogens circulating in the United Arab Emirates, informing risk management strategies for the reintroduction of the SHO. Through comprehensive risk assessment and transparent communication, stakeholders play a pivotal role in shaping the success of this conservation endeavour.

Résumé

La réintroduction de l'oryx algazelle (*Oryx dammah*) des Émirats arabes unis au Tchad offre une opportunité unique pour une exploration approfondie des différents aspects liés aux dynamiques écologique, environnementale et sanitaire associées à ce processus de réintroduction.

Motivé par le déclin mondial de la biodiversité, ce projet vise à rétablir une espèce clef de voûte et à atténuer les impacts négatifs des activités humaines sur les écosystèmes. L'oryx algazelle, reconnu comme une espèce clef de voûte, joue un rôle crucial dans la structuration et le fonctionnement des écosystèmes. L'initiative de réintroduction ne se concentre pas uniquement sur le bien-être de l'oryx, mais souligne également des implications plus larges pour la santé des écosystèmes, la biodiversité et l'harmonie entre les intérêts humains et environnementaux.

Cependant, le processus de réintroduction présente des risques inhérents, notamment au potentiel de pollution pathogène et la libération de pathogènes zoonotiques. L'interaction accrue entre l'oryx, d'autres animaux sauvages, le bétail et les populations humaines accroît le risque de transmission des maladies. Cela nécessite une évaluation systématique des risques, fournissant des informations pour éclairer les stratégies d'atténuation des impacts potentiels de l'émergence des maladies sur la faune, le bétail et la santé humaine.

La brucellose induit un risque zoonotique significatif, avec le premier foyer confirmé chez l'oryx algazelle décrit dans l'article 1. Les tests sérologiques ont révélé un taux de séroprévalence élevé, les femelles et les individus plus âgés présentant des probabilités plus élevées d'être séropositifs (article 2). Dans l'article 6, un essai contrôlé administrant le vaccin Rev.1 a démontré sa sécurité, les voies d'administration sous-cutanée et conjonctivale induisant des réponses cellulaires à long terme. Cependant, la voie conjonctivale a induit une réponse humorale plus courte.

De plus, dans l'annexe 1, la présence du génome du virus de la fièvre catarrhale ovine (maladie de la langue bleue) chez des oryx séronégatifs après transport depuis les États Unis d'Amérique souligne les risques associés à la translocation, nécessitant une évaluation préalable des risques d'importation pour les espèces de ruminants sauvages sensibles aux orbivirus non seulement dans le pays de destination mais aussi dans les pays de transit.

Les épizooties de fièvre aphteuse décrites dans l'article 3 soulignent la susceptibilité de l'oryx algazelle à cette maladie qui a un impact économique, soulignant la nécessité de mesures de surveillance et de contrôle robustes.

Les épizooties de pleuropneumonie contagieuse caprine démontrent en outre la susceptibilité d'espèces sauvages apparentées (article 4) et mettent en évidence des dynamiques de transmission précédemment non documentées (article 5).

Nos résultats fournissent des informations précieuses sur l'épidémiologie des agents pathogènes circulant aux Émirats Arabes Unis, éclairant les stratégies de gestion des risques infectieux pour la réintroduction de l'oryx algazelle.

À travers une évaluation complète des risques et une communication transparente, les parties prenantes jouent un rôle crucial dans le façonnement du succès de ce projet de conservation.

General preamble

The decline in global biodiversity prompts a rigorous examination of human impacts on the environment. Over the course of human history, spanning more than a million years, our activities - deforestation, changes in land use and urbanization - have significantly affected ecosystems. The onset of the industrial era intensified these impacts, propelled by hygienic practices and medical advancements, including vaccine discoveries and anti-malarial prevention. Concurrently, progress in mechanical and chemical sciences gave rise to challenges such as resource overexploitation, hunting and poaching, invasive species introduction, waste generation, pollution, water management issues, acidification, and climate change.

In this contemporary context, the reintroduction of the scimitar-horned oryx (*Oryx dammah*), a large Sahelian antelope, from the United Arab Emirates to Chad provides a unique opportunity for in-depth exploration of various aspects related to the ecological, environmental, and health dynamics associated with the reintroduction process. Once native to Chad but declared extinct in the wild in 2000, the oryx's reintroduction serves as a focal point for understanding the complex interplay between human-induced environmental changes and wildlife health. This doctoral thesis aims to make a substantial contribution to the field of wildlife infectious disease risk analysis, specifically delving into the intricacies of the reintroduction process.

The SHO, is recognised as a potential keystone species, for its possible pivotal role as a large herbivore in shaping the structure and functioning of its arid and semi-arid ecosystem. By influencing the abundance and distribution of other species, a keystone species exerts a disproportionate effect on biodiversity, meaning its effect is more significant than would typically be expected based solely on its abundance or population size. The reintroduction of such a species has far-reaching implications, not only for the oryx's well-being but also for the overall health of the ecosystem it inhabits. In this regard, this research initiative contributes to mitigating the negative impacts of human activities on ecosystems by reinstating a native species. This fosters biodiversity, supports natural habitat restoration, and underscores the importance of harmonizing human and environmental interests.

However, the reintroduction process also poses inherent risks, particularly with regard to the potential for pathogen pollution and the release of zoonotic pathogens. The increased interactions between the scimitar-horned oryx, other wildlife, and human populations during the reintroduction process may elevate the risk of disease transmission. This thesis will systematically provide evidences to assess and manage these risks, to inform strategies to mitigate the potential impacts of disease emergence on both wildlife and human health. Insights derived from this study contribute not only to the successful reintegration of a species into its native habitat but also inform broader discussions on the preservation of biodiversity and the imperative for sustainable coexistence between human societies and the natural world. This research seeks to provide

comprehensive insights that inform conservation strategies, wildlife management policies, and broader ecological considerations in the face of ongoing environmental transformations

Chapter 1 - Introduction

1.1. What is a scimitar-horned oryx?

The scimitar-horned oryx (*Oryx dammah*) (SHO) is a large antelope species, with males measuring up to 126cm high at the shoulder and weighing up to 165kg. Females are usually slightly smaller and lighter. It has a sandy to pale whitish coat and long and gently curved horns, measuring up to 150cm (see Figure 2). It belongs to the oryx genus with three other species, two African species: the beisa (*Oryx beisa*), and the gemsbok (*Oryx gazella*) and one from the Middle East: the Arabian oryx (*Oryx leucoryx*) within the Hippotraginae subfamily.

It was once widespread in the Sahelian belt, the arid and semi-arid regions of North Africa, from Mauritania to the Nile River in Egypt (see Front Cover: (Figure 1)) and Sudan (see Figure 3). Its population might have reached one million in the early Holocene (9500-4500 before Christ) (Iyengar et al., 2007).

The suitable habitat for SHO typically includes arid and semi-arid steppes with sparse vegetation, and annual grassland, with sandy or gravelly soils. These ecosystems are characterized by low and sporadic rainfall, high rates of evaporation, and temperatures that can fluctuate dramatically between day and night (Devillers & Devillers-Terschuren, 2005). The SHO is well adapted to open plains and grasslands where it can graze on a variety of herbaceous plants and endure waterless environments for up to ten months yearly. It displays a flexible foraging strategy, exploiting a variety of plant materials such as browse, legume seedpods, bulbs, and tubers. It can forage for succulent vegetation, like the wild gourd *Citrullus colocynthis* during the dry season. The SHO undertook extensive migrations spanning up to 1300 km per year in search of adequate food and water resources. Previous observations from Chad and reintroduction programs in Tunisia contribute valuable insights into their dietary preferences (Gilbert & Woodfine, 2004; Beyouli & Neffati, 2016).

Because it is a large herbivore, the SHO might influence the vegetation structure and distribution, and indirectly impact other species, such as small mammals, insects, and birds. After its reintroduction, the SHO is likely to be considered a keystone species in its native Chadian habitat.

Due to a combination of habitat loss, conflict with livestock, hunting and poaching for its meat and for its trophy, and finally civil unrest in Chad such as the Chadian Libyan conflict between 1978 and 1987, its population declined (Newby, 1980; Newby, 1988; Woodfine & Gilbert, 2016). The SHO has been listed in the appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) since its creation on July 1st, 1975. This appendix reassembles species that are threatened with extinction, and international trade in these species is generally prohibited except in exceptional circumstances. In 2000, the species was declared “Extinct in the Wild” on the International Union for the Conservation of Nature (IUCN) red list (Woodfine & Gilbert, 2016), meaning there was no known population surviving and reproducing in its native habitat. This is the last conservation status category before “Extinct”, when there is no reasonable doubt that the last individual has died, and the species no longer exists.

Thanks to global breeding efforts, the species survived in captivity (East, 1999) and from reportedly no more than 60 wild-caught individuals in the 1960's, the entire global population is estimated between 15,000 and 19,000 heads (Woodfine & Gilbert, 2016). This population is distributed in 444 institutions across 48 countries: about 11,000 individuals are found in extensive private ranches in Texas, United States of America (USA) with minimum level of population management. They exist for ecotourism, hobbies and hunting (Wildt et al., 2019). Wildlife collections in the United Arab Emirates (UAE) account for another 4,000 animals, including the Environment Agency - Abu Dhabi (EAD) collection, DDCR (Dubai Desert Conservation Center) and Al Ain Zoo. On the other side, breeding centres and zoological institutions in Europe, Australia and North America, provide a higher level of population management, including health and breeding, to a lower number of SHO (Humble et al., 2020; Humble et al., 2023).

Genetic studies based on mitochondrial and nuclear DNA markers (Ogden et al., 2020), as well as whole genome sequencing (Humble et al., 2020), have found that the genetic diversity of the global SHO population is higher than previously anticipated, possibly due to a larger number of wild-caught founders (Ogden et al., 2020). With only seven mitochondrial DNA haplotypes found, the EAD population has the lowest genetic diversity when compared to the European and the Australian populations but its nuclear diversity estimates are relatively high, possibly due to the population's large size of several thousands (Ogden et al., 2020).

There have been a handful of projects to reintroduce this species in several countries (Devillers & Devillers-Terschuren, 2005), usually in fenced areas and with limited number of founders individuals: in Morocco (Souss Massa National Park, over 300 SHO in 2015); Tunisia (Bou Hedma National Park, 40 SHO in 2020; Oued Dekouk Nature Reserve, 53 SHO in 2020; Sidi Toui National Park 74 SHO in 2020, Dghoumes National Park, 100 SHO in 2020) and Senegal (Ferlo Nord Wildlife Reserve, 120 SHO in 2012; Guembeul Natural Reserve, 40 SHO in 2009) (ArcGIS StoryMaps, 2022).

Figure 2: Picture of scimitar-horned oryx in the source population in the United Arab Emirates

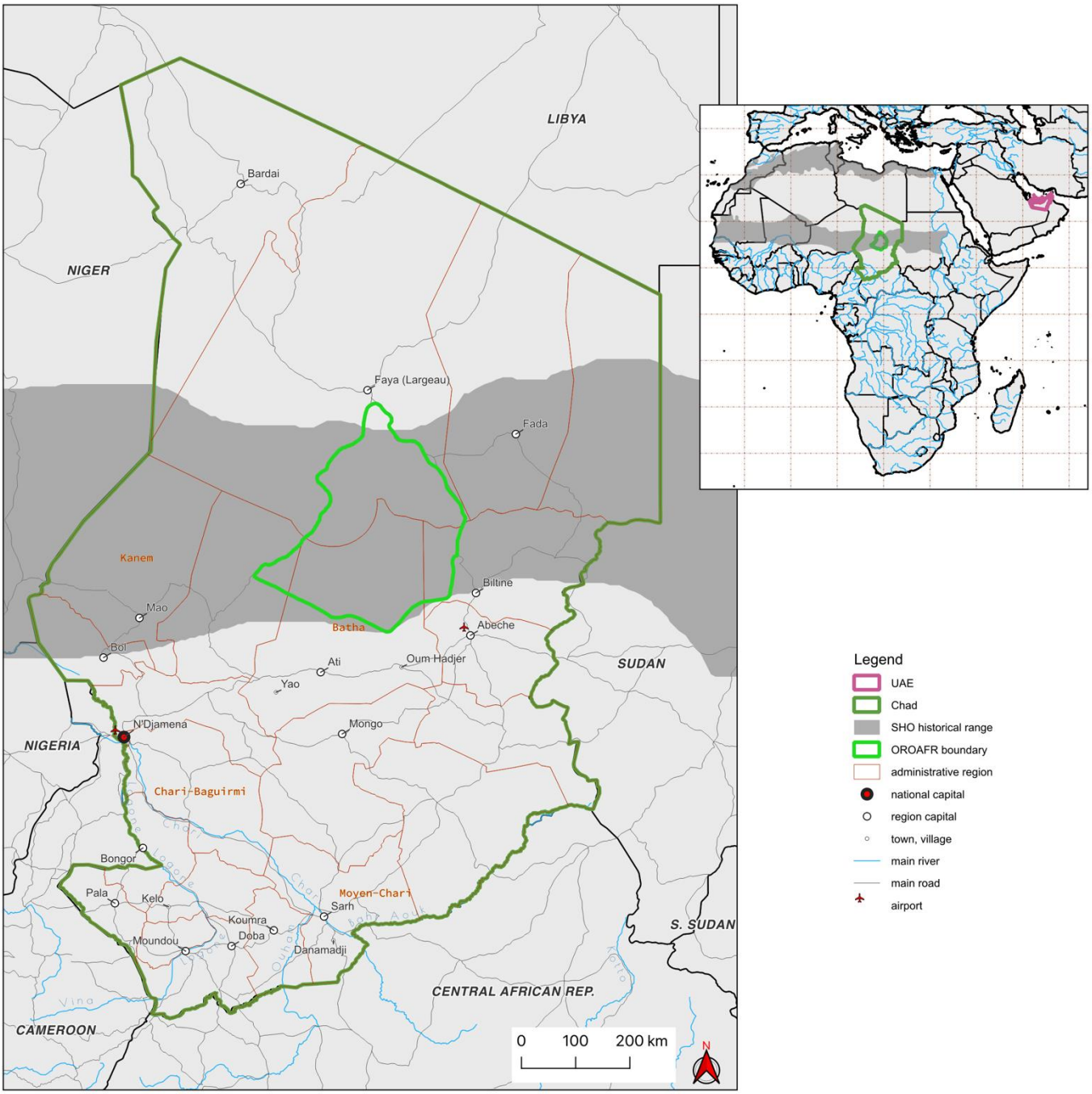

Figure 3: Map of Chad

The relative positions of the United Arab Emirates and Chad are displayed on the overview map. The Ouadi Rimé - Ouadi Achim Faunal Reserve (OROAFR) is shown in green. Its shapefile was obtained from (UNEP-WCMC & IUCN, 2024). The SHO historical range appears in dark grey. Its shapefile was adapted from (IUCN SSC Antelope Specialist, 2023). The Chadian regions discussed in this thesis are displayed in dark red. Note: this map in not an authority on countries' boundaries. Map created with QGIS 3.34.

1.2. Why are the United Arab Emirates and Chad partnering on this project?

1.2.1. United Arab Emirates

Sheikh Zayed bin Sultan Al Nahyan, the founder of the nation ruled the country from its independence from the United Kingdom in 1971 to 2004. Each of the seven emirates is ruled by its own hereditary monarch, known as emir.

The UAE has had an oil-based economy since large reserves of fossil fuel were discovered offshore in the emirate of Abu Dhabi in 1958. The gross domestic product (GDP) of the UAE was USD 507.54 billion in 2022, and USD 51,310 per capita. Its population was 9.89 million in 2022 (IMF, 2023).

Numerous wildlife collections are found in the UAE, either public or privately owned. Cultural and traditional factors, status and prestige, passion for conservation, personal interest and hobbies, tourism and entertainment explain this propensity, along with local wealth.

Sheikh Zayed privately owned Sir Bani Yas Island (location: latitude:24.324, longitude: 52.599), five kilometres (km) off the shore of the Abu Dhabi emirate. This island was home to one of his private wildlife collections. After his passing, a large number of wild ungulates were translocated to an inland location, called al Faya in 2008 (location: latitude: 24.219, longitude: 54.792).

This facility is 6,000 m long and 800 m wide with one single point of entry fitted with guardians and wheel dip. All contained within a perimeter fence, the single mesh fenced enclosures are spread on both sides of a 50 m wide central corridor. The largest enclosures measure 450m by 300 m and they are separated from each other by a 15 m wide access corridor. Some enclosures are subdivided in two, three or six smaller enclosures and the total number of enclosures is 46. A description of this facility can also be found in rrticles 2 and 3 included in this manuscript thesis.

In 2013, there were approximately 4,000 SHO in this new location, explaining why The UAE are involved in this project. Other wildlife species were housed in this facility, including 7,931 Indian blackbucks (*Antelope cervicapra*), 1,300 sand gazelles (*Gazella marica*), 258 mountain (*Gazella gazella*) and Indian (G*azella bennetti*) gazelles and 11 Urial sheep (*Ovis orientalis*).

Due to the nation's prosperity, ample funding is accessible for various facets of this project, including animal transportation, field equipment and facilities, animal feed and water supplementation, staff salaries, permitting, and more. The leaders and decision-makers in the UAE are genuinely motivated, driven by their own education and a commitment to realizing the vision of the country's founder, Sheikh Zayed. His dedication to breeding this species for preservation purposes has instilled a sincere conservation aspiration among those steering this initiative.

1.2.2. Chad

In 2022, the GDP of Chad was USD 11.91 billion, its estimated population was 17.41 million (IMF, 2023). Its GDP per capita was USD 683.89 and the country ranked 11th poorest countries in the world.

Chad is a central African country that gained independence from France in 1960. It is over 1,284,000 km2 with arid Saharan desert climate in the most northern part, semi-arid Sahel in the central region, and a tropical climate in the remaining southern third.

The Sahelian region of Chad was historically within the natural habitat range of the SHO (see Figure 3) and the species was last seen in the wild in the Ouadi Rimé - Ouadi Achim Faunal Reserve (OROAFR) around 1989. The OROAFR is an 83,511 km2 reserve (European Commission, Joint Research Centre, 2023) created in 1969 (IUCN PAPACO, 2009). Located in the Batha administrative region, the limits of this reserve were virtual, with no management of the resources. This reserve is home to a small remaining population of "Critically Endangered" dama gazelle (*Nanger dama*), to the « Vulnerable » dorcas gazelle (*Gazella dorcas*) and to the "Critically Endangered" addax (*Addax nasomaculatus*).

There is an important transhumant and nomadic pastoralism (Luizza, 2017), with pastoral tribes (See pictures C, D and E in Figure 4) moving to the vast grassland of this reserve during the wet season extending from May to October (World Bank, 2023), driving large numbers of small ruminants, cattle, camels, as well as horses and donkeys for transportation, increasing the interface between the local wildlife and the livestock.

The majority of the SHO individuals captured in the wild in the 1960s which eventually became the founding members of the present-day population, originated from Chad (Woodfine & Gilbert, 2016).

Outside the rainy season, access to drinking water is limited in this region to traditional wells and newly created boreholes, surrounded by small human settlements (Newby, 1980) (see Picture D in Figure 4).

There is a strong local connection to this iconic species (see Picture F in Figure 4), as older people continue to share vivid memories and stories.

For a synthetic tabulated comparison between the UAE and Chad, please refer to Annex 1.

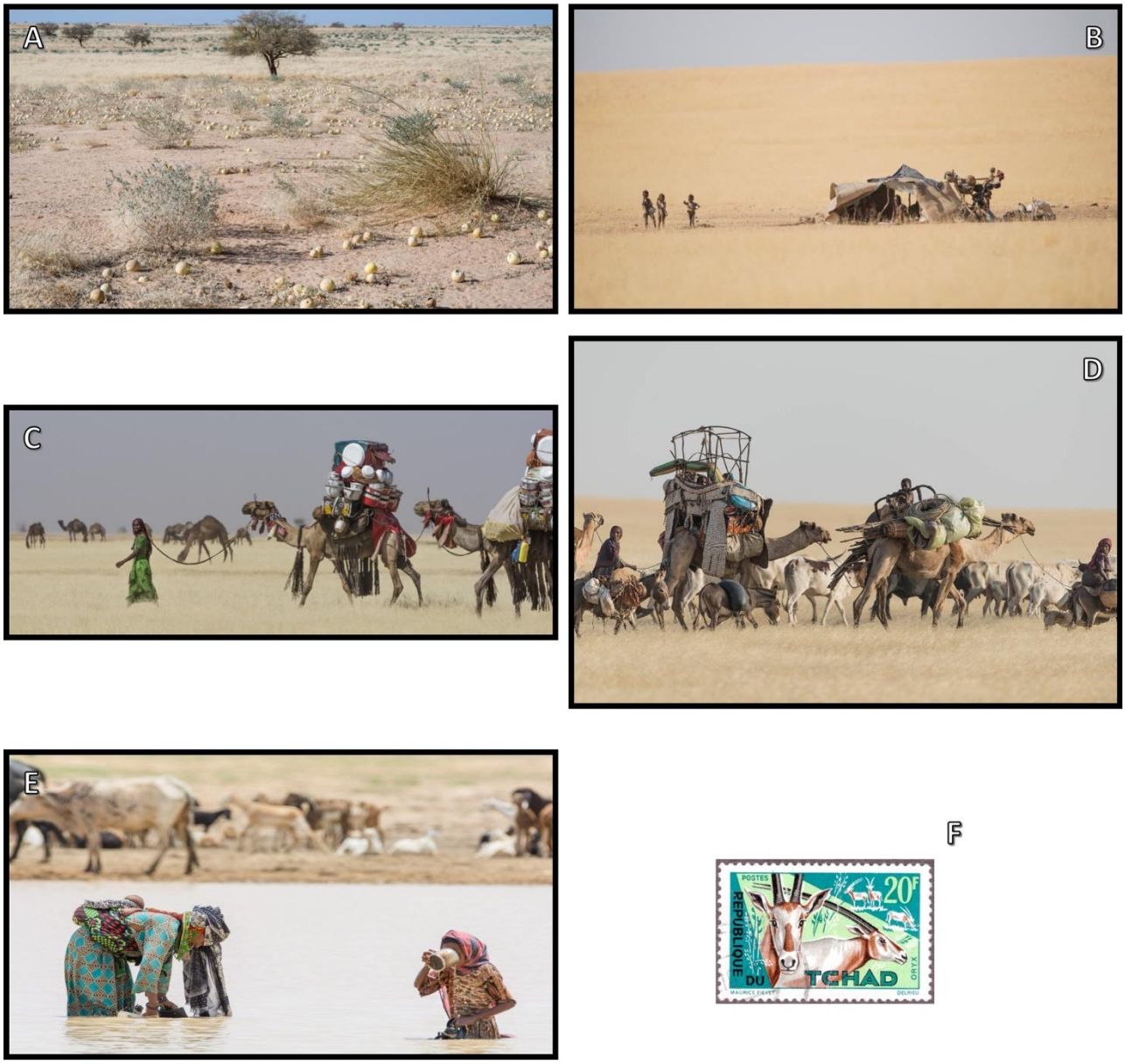

Figure 4: Ouadi Rimé – Ouadi Achim Faunal Reserve (OROAFR) presentation

Picture A: typical Sahelian habitat in the OROAFR constituted of a sub-desert steppe, with wild gourd *Citrullus colocynthis* in the foreground. Picture B: nomad household during the dry season (September to June) in the OROAFR. C and D: Nomads migrating to and from the OROAFR before and after the wet season. Picture E: Human settlement at a borehole. F: Chadian postal stamp depicting scimitar-horned oryx.

1.3. What is a reintroduction and why reintroduce the scimitar-horned oryx?

Animal translocation refers to the deliberate movement of individual animals or populations from one location to another. The primary goal of translocation is to conserve or manage populations by establishing new populations, augmenting existing ones, or relocating individuals to suitable habitats, within their native range or relocating them to areas outside their historical range.

A reintroduction is a particular form of translocation: “it is the intentional movement and release of an organism inside its indigenous range from which it has disappeared.” (*IUCN/SSC*, 2013).

It is a conservation strategy and action that is usually undertaken to reverse the population decline or to restore a species to an area where it was once naturally present and from which it has been extirpated.

The most obvious reason to reintroduce a species is to improve its conservation status. Regarding the SHO, it is to prevent its extinction and contribute to the conservation of biodiversity. Earth is affected by a massive extinction of its living diversity, including plants and animals of all species. This phenomenon is not recent. Over 20 years ago, scientists already alerted about the disappearance of mammal species (Ceballos & Ehrlich, 2002), but it tends to accelerate. The Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES), established by the United Nation Environment Programme (UNEP), accepted in May 2023 during a plenary session, a report (IPBES, 2019) stating that approx. 1,000,000 species are facing extinction – 25% of all assessed species.

Because it is essential to reintroduce a species to its natural habitat, reintroduction projects actively participate in the restoration of ecosystems, with the primary goal of safeguarding and maintaining the inherent natural ecological processes, and ultimately, healthy ecosystems are more resilient to environmental changes (Cardinale et al., 2012). An improved management of the habitat as well as protection from patrolling rangers to reduce poaching might be integral components of the project itself to ensure success. Additionally, the protection provided by these measures extends benefits to other endangered species, like the dama gazelle, the dorcas gazelle and the addax. As the SHO reintroduction progresses and infrastructure is established, opportunities will probably emerge for implementing further conservation initiatives, such as population reinforcement efforts for these species.

Restoring populations of endangered species offers several other benefits and reasons which might have strong implications for motivating and sustaining such projects. Firstly, the presence of rare or charismatic species like the SHO can draw tourists to the area, generating economic benefits for local communities by creating opportunities for sustainable income and employment in rural areas. Additionally, involving local communities fosters trust and cooperation, enhancing social cohesion and reducing conflicts, thereby contributing to stability.

Moreover, reintroduction programs entail scientific research and monitoring of released animals, providing valuable insights into the behaviour, habitat preferences, and conservation needs of the species. This research informs future conservation efforts and helps address conservation challenges effectively.

Furthermore, collaboration with international organisations and agencies, often associated with large reintroduction projects, strengthens diplomatic ties and cooperation between countries, enhancing regional stability and security.

Moreover, reintroduction programs serve as educational tools to raise awareness about endangered species and conservation challenges, engaging the public and garnering support for broader conservation initiatives.

Lastly, the SHO possibly holds cultural significance in some African countries and serves as a traditional symbol, contributing to cultural heritage preservation and the promotion of traditional values within local communities (Gascon et al., 2015).

1.4. Planning a reintroduction and management of the drivers that lead to the extinction

To help plan a reintroduction, guidelines have been published (IUCN & SSC, 2013). As indicated in this document, a comprehensive feasibility study is essential to evaluate the sustainability and viability of a reintroduction project within a specified timeframe. This study, outlined by Miquelle et al. (2018), encompasses various key components, that are also highlighted in the guidelines. Firstly, the assessment of habitat suitability, considering factors such as vegetation, water sources, climate, and topography, along with potential threats like predators or competing species, is crucial. Additionally, evaluating the population to be reintroduced, including its health, genetic diversity, and availability, is paramount. Legal and regulatory aspects must also be addressed to ensure compliance with local, national, and international laws and obtain necessary permits. Engaging stakeholders, such as local communities and conservation organizations, is imperative to garner support and involvement. Addressing predator risk, including natural predators and domestic threats like dogs and poaching, is vital while recognizing the role of natural predators in ecological balance. Establishing a comprehensive monitoring program to assess project success and progress, as well as researching the oryx's behaviour, health, and habitat interaction, is essential. Providing initial feed and water support until the SHO adapts to its environment is crucial. Evaluating financial requirements for ongoing monitoring, research, and conservation efforts is also necessary. Ensuring understanding and acceptance from local communities and the public through awareness and education programs is vital. Developing contingency plans for unexpected challenges or setbacks is essential, as is considering the long-term sustainability of the reintroduced population, including potential growth, genetic diversity, and habitat management..

The recovery of an animal population is a gradual process that demands a long-term commitment, both in terms of financial resources and manpower (Miquelle et al., 2018).

Regarding the reintroduction of the SHO to Chad, most of these points were discussed during a workshop held in N'Djamena in May 2012 (Bemadjim et al., 2012).

In cases where the habitat is suitable, the initial prerequisite for a successful reintroduction is the absence of the factors that initially caused the species to go extinct. It might be difficult to affirm that the causes which resulted in the extinction of a species have disappeared, but hunting and poaching might be managed by education and law enforcement, as well as protection from patrolling rangers.

In recent years, the Sahel region has been plagued by increased terrorist activity and insecurity, prompting many non-governmental organisations to evacuate their staff and suspend their operations in the area. The deteriorating security situation has posed significant challenges to humanitarian and development efforts in the region.

In this context, Chad is relatively stable and safe. There has been French military involvement through the Serval operation in Mali followed by the Barkhane operation in Mali, Burkina Faso, Niger, Chad and Mauritania to stabilise the region and counter Islamist terrorist groups such as Al Qaeda and the Islamic State, until recently (Ministère des Armées, 2022).

The Sahelian region of Chad has garnered significant humanitarian attention. For instance, the European Commission provides important funding for humanitarian aid, protection of biodiversity and ecosystems, and maintaining peace and security through the EURO 280 million Multiannual Indicative Programme (MIP) (European Commission, 2021; European Commission, 2023), or other development programs such as the one from the World Food Programme (WFP, 2019), or a 14 years program financed by the Swiss Development and Cooperation Department and managed by the Swiss Tropical and Public Health Institute (SDC, 2016), or the USD 4,752,328 vaccination programme (Gates Foundation, 2019).

1.5. What is a wildlife disease risk analysis (WDRA) and why should it be performed in a reintroduction project?

Many hazards might impact the animals involved in a reintroduction project. For instance, injuries during capture, transport, or release; water deprivation or heat stress while the animals are waiting for customs clearance at the airport facility. After the release, animals might be subjected to poaching or exposed to diseases.

In the past, animal translocation projects typically overlooked the impact of pathogens and diseases. For instance, after surveying translocations data from Australia, Canada, New Zealand and the USA between 1973 and 1986, (Griffith et al., 1993) wrote; "It seems inconceivable that in nearly 25% of translocations the animals were not given a physical examination by a professional biologist or veterinarian before release".

Nowadays, there is a growing recognition of the significance of assessing potential disease risks and implementing effective management strategies (Kock et al., 2010).

Specific to the potential threats posed by diseases to livestock and wildlife populations during the displacement of wild animals, through trade or conservation activity, a WDRA is a multidisciplinary approach used to assess and manage those threats.

It is a structured and evidence-based process which involves the evaluation of various factors to understand the likelihood and the potential consequences of disease outbreaks in wildlife.

The uncertainty associated with those risks is also explicitly considered (Jakob-Hoff et al., 2014; Hartley & Sainsbury, 2017).

Performing a WDRA in a reintroduction program serves several important purposes.

Firstly, it helps identify potential health threats that released individuals may encounter in their new environment, ensuring their health and well-being and increasing their chances of survival and adaptation.

Additionally, disease outbreaks can significantly impact population viability, either directly by affecting the released animals. For instance the reintroduced endangered mockingbird in the Galapagos suffering from poultry diseases (Deem et al., 2012) or the canine distemper virus decimating black-footed ferret colony (Viggers et al., 1993) or indirectly if the released animals become a pathogen reservoir and control measures include reservoir culling. A reservoir can be described as one or more epidemiologically linked populations or environments where the pathogen can persist indefinitely and through which infection is transmitted to the specified target population (Haydon et al., 2002). For instance, the European badger (*Meles meles*) reservoir for *Mycobacterium bovis* is culled in the United Kingdom (Roper, 2003) or the Alpine ibex reservoir for *Brucella abortus* in the French Alps (Diguimbaye-Djaibé et al., 2006). However, these animals were not reintroduced.

Moreover, the introduction of pathogens not native to the reintroduction area poses risks to local human populations with zoonotic diseases, wildlife, including the same species as the reintroduced one, and livestock. A WDRA assists in identifying potential sources of disease transmission and developing mitigation strategies to prevent the spread of pathogens from reintroduced animals, safeguarding multiple species.

Furthermore, addressing moral and welfare concerns surrounding the introduction of diseased or carrier animals is crucial. A WDRA helps navigate these ethical dilemmas and ensures responsible decision-making in wildlife reintroduction efforts.

Regulatory requirements might mandate WDRA for obtaining necessary permits for reintroduction programs, highlighting its legal significance.

WDRA aids in resource allocation by prioritizing and focusing on the most significant threats and mitigation strategies, optimizing conservation efforts with limited resources.

Lastly, WDRA also provides a framework for ongoing monitoring and adaptive management, allowing for updates to the analysis in response to changing disease risks or emerging pathogens.

People from different communities or fields of expertise might have different opinions on the hazards and the associated risks, as well as on their level of acceptance. For instance, conservation biologists might emphasize the risks the released animals will face after being reintroduced, while public health specialists might consider only zoonotic diseases the animals might carry to the release site. The WDRA framework released by the World Organisation for Animals Health (WOAH) and IUCN focuses on economic and public health impacts (WOAH & IUCN, 2014).

1.6. General framework of a WDRA

The WOAH in collaboration with the IUCN has provided a framework to perform standardised WDRA (WOAH & IUCN, 2014; Jakob-Hoff et al., 2014) articulated around five key points.

It is crucial to highlight the importance of actively seeking knowledge and expertise from relevant experts and stakeholders throughout all stages of the WDRA process. This collaborative approach effectively embodies the concept of "risk communication" also considered as the 6th point of a WDRA.

1.6.1. Problem description

This constitutes all the relevant information providing background and context for this analysis. The goals, scope, and focus of the DRA are identified, and the assumptions and the limitations, as well as the acceptable level of risk, should be stated at this stage.

Habitat suitability studies and wildlife surveys were conducted in early 2010 by the Sahara Conservation Fund (SCF) and the Zoological Society of London (ZSL).

The feasibility of reintroducing SHO into the OROAFR was supported by historical data and recent wildlife surveys, indicating ample space and suitable habitat to meet the SHO's seasonal and annual needs. Strong commitments from the governments of the UAE and Chad, along with technical support from various organisations such as EAD, SCF, ZSL and Smithsonian Conservation Biology Institute (SCBI), laid the groundwork for this conservation initiative. Before the project, international symposia were held to develop a global strategy for species restoration, including criteria for selecting conservation sites. In 2012, a workshop in Chad engaged stakeholders from government agencies and civil society organisations (Soorae, 2018).

A workshop held at EAD head office in 2012 laid the groundwork for the oryx transportation. Population modelling taking into account elevated mortality during severe drought and a 2,000 SHO carrying capacity established that 500 SHO should be reintroduced to obtain a stable population in the OROAFR (see Figure 3).

New enclosures were designed for animal screening and quarantining at Deleikha Holding Facility (location: latitude: 24.065, longitude 55.048).

The reintroduction was officially launched in 2014.

Transportation was done during the cooler winter months in the UAE, coinciding with the dry season in Chad. This timing aimed to reduce thermal stress before departure from the UAE and facilitate the conveyance on solid ground rather than muddy terrain. Groups slightly larger than 25 animals would be selected and quarantined at the Deleikha Holding Facility. On the day of transport, 25 individuals would be led into individual crates, each fitted with a watering system and feeder. The crates would then be transported by truck to Al Ain airport, loaded onto a cargo plane, and flown to Abéché airport. From there, they would be transferred to other trucks and driven to the pre-release site in the OROAFR. The SHO would be released into a 500m by 500m quarantine pen located near the base camp in the morning, typically spending just over 24 hours in crates.

The oryxes would remain in this pen until the end of the dry season, provided with feed and water, with a gradual transition in their diet. In 2016, the first group of 25 SHO was released, and a total of 285 SHO were reintroduced (see Figure 7). By 2023, the SHO population in Chad had grown to over 600.

1.6.2. Hazard identification

The possible health hazards are listed and prioritized. They can be either infectious or non-infectious, and only infectious hazards will be considered in this study.

Other critical factors will significantly contribute to the risk assessment, including the contagious nature of the disease and whether it is vector-borne or not.

Essentially, the underlying question revolves around -identifying the infectious diseases that occur in the wildlife collection from which the SHO originates, and -determining the infectious diseases prevalent in the OROAFR that might affect SHO considering their known or assumed susceptibility.

When this information is not readily accessible, the geographical distribution of pathogens can be inferred from data available in neighbouring countries or the region. Two opposite categories of factors can influence this distribution: the factors that facilitate the disease spread between countries blurring national borders such as animal, animal products or human movements. Trade can be legal, and possibly documented,

or illegal, and data can be deficient, but natural migration must be considered as well. Animated vector diffusion will also play an important role in vector-borne disease spread, seeing their distribution modified by global warming. On the other hand, are the factors limiting disease propagation, for instance, biosecurity measures within national or regional infectious diseases control programs.

There are multiple sources of information, either direct or indirect, to assess the presence or the absence of pathogens in a specific area, but each of these sources has its limitations.

1.6.2.1. Direct sources of information

- **WOAH reports**

The WOAH has listed diseases of importance to the international animal trade. This list is included in the second volume of the Terrestrial Animal Health Code (Terrestrial Code), 2018, and the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Terrestrial Manual), 2018. More recent versions of both books are freely available online, at the following web addresses: https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/, and https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-manual-online-access/, respectively.

The 183 state members of the WOAH network (2022 data) are legally supposed to notify (Vallat et al., 2013), and the semi-annual reports are accessible through the World Animal Health Information System (WAHIS) at https://wahis.woah.org/#/report-smr. Data related to these notifications since 2005 can be visualised and downloaded at this address: https://wahis.woah.org/#/dashboards/country-or-disease-dashboard.

However, the WOAH does not list certain diseases that might constitute risks in a reintroduction project and the list of hazards should be complemented through other sources.

- **Literature review**

Conducting a comprehensive review of existing literature and research on diseases affecting the target wildlife species, including scientific literature, books, and reports, is crucial for understanding the disease history and prevalence in the region. Species-specific information is often lacking, leading to approximations made on data from phylogenetically related species, typically domestic species.

Scientific literature from sources like PubMed, Google Scholar, Web of Science, etc., is a valuable information source. However, it is worth noting that only recent papers may be readily accessible online, especially those published before the digital era (particularly before the advent of the PDF format), which may not be available. Conducting a literature search in a university library can be beneficial in overcoming this limitation and providing access to older articles.

Scientific papers can cover various aspects relevant to the disease risk analysis, including clinical case reports, disease surveillance (presenting results of incidence, prevalence, or distribution), epidemiological models (including disease freedom), risk factor analysis, monitoring program results, and cost/benefit analysis. However, reports may sometimes lack scientific robustness and books, while potentially valuable, might contain outdated information.

Several factors can prevent publication including language barrier, political reasons, funding, time, personal challenges, ethical concerns. Also, not all information is published or publishable. For instance, we have performed a succinct literature search with PubMed (https://pubmed.ncbi.nlm.nih.gov/) and Google Scholar (https://scholar.google.com/) to evaluate the number of results for a selection of 13 queries that included keywords related to animal infectious diseases, to which either « Chad », « UAE » (for comparison) or « Belgium » (for reference) was added. The keywords and detailed results are presented in **Annex 2**. Overall, PubMed returned a total of 309 and 293 results for « Chad » and « UAE », respectively, while 6,692 results where obtained with « Belgium ». Google Scholar returned a total of 86,222 and 80,773 results for « Chad » and « UAE », respectively, while 415,140 results were obtained with « Belgium ». While rough and subject to discussion, these results might indicate that publications related to Chad and to the UAE are nearly equally numerous and substantially less (five to 20 folds lower) than publications related to Belgium.

A literature review may provide a somewhat generalized overview of the health status in both facets of a reintroduction project: the source and the recipient. While it can be beneficial to sketch the situation broadly, a more nuanced understanding can be achieved through the following two sources of information:

- **Expert opinions**

An expert is “someone with comprehensive and authoritative knowledge in a particular area not possessed by most people” (Caley et al., 2014).

Experts in the field, including wildlife veterinarians, ecologists, and epidemiologists, are consulted to obtain qualitative assessments based on their experience and knowledge.

Nevertheless, there exist inherent limitations and biases that may influence the judgment and recommendations offered by experts. Constituting panels composed of multiple experts, whether in various or similar subject domains, represents a method to address this concern (Caley et al., 2014).

- **Field observations**

Conduct field surveys to observe the health of the wildlife population. Look for signs of diseases, abnormal behaviour, or population decline.

Infectious diseases with subtle or nonspecific clinical signs, as well as those with prolonged incubation periods, often evade detection, especially among wild species. Such animals can mask the presence of the

disease, presenting significant challenges for comprehensive investigation and capture, even in captive settings.

Research has shown that alteration of natural habitats can elevate disease transmission rates (Penczykowski et al., 2014). Additionally, persistent patches of environment-resistant pathogens have been identified in high-quality habitats (Leach et al., 2016).

In a captive situation, a habitat examination might provide information related to the potential sources of disease transmission, such as the proximity to livestock or other wildlife, dominant wind, or suboptimal biosecurity measures. For instance, the proximity to traditional farms with poor biosecurity measures increases the risk of disease transmission (Chaber & Saegerman, 2017).

Arthropod populations, including vectors, can be assessed directly through surveys, and indirectly through bioclimatic and environmental condition analysis (Thomson & Connor, 2000; Hartemink et al., 2015).

1.6.2.2. Sources of indirect information

- **Human health data**

Outbreaks in the human population might reveal the presence of zoonotic diseases, therefore human health data might disclose clues on the presence of such diseases.

There are numerous sources of information regarding human health, including scientific medical literature, international health organisations such as the World Health Organisation (WHO) and the Centers for Disease Control and Prevention (CDC), national health authorities, as well as websites like ourworldindata.org and healthmap.org.

This approach finds its limitations in diagnostic challenges, especially in developing countries or disease reporting.

Additionnally, due to people's movement, cases that are detected in one country might result from a transmission in another country. Therefore, the geographical origin of the transmission must be confirmed.

- **Animal vaccines data**

National control programs aiming at local enzootic pathogens and specific serotypes or strains exist. The data regarding the import, procurement, manufacture or use of animal vaccines might give information on the presence of a disease occurring in a country.

This information has to be linked with official control programs to understand if the pathogen is present. Its interpretation depends on each specific disease. For instance, some countries that are free of a particular disease may produce vaccines for other countries where the disease is prevalent, or to maintain

preparedness in the event of a disease incursion. This is exemplified by the production of foot-and-mouth disease (FMD) vaccine in FMD-free countries that do not vaccinate. Also vaccination may be incorporated into a strategy aiming at preventing the emergence of a pathogen, particularly in border region.

The WAHIS website provides official vaccination data accessible at this address: https://wahis.woah.org/#/dashboards/control-measure-dashboard.

1.6.3. Risk assessment

In an ideal scenario, this process follows the One Health approach and should involve collaboration among a diverse array of professionals, including veterinarians, virologists, microbiologists, parasitologists, disease ecologists, epidemiologists, public health practitioners, economists, and other relevant experts (Dufour et al., 2011; Rinchen et al., 2020) who participate in a facilitated workshop spanning several days. Risks are comprehensively evaluated within the framework of the triad central to the One Health perspective: human, animal, and environmental health (Lebov et al., 2017). It has been emphasized that individuals who make decisions based on the results of a risk assessment should not be the ones performing the assessment, and the assessment itself should remain uninfluenced by those decision-makers (Leighton, 2002).

Conducting interviews with key personnel and collaborators is crucial. For instance, 13 experts were consulted to assess the risk of rabies reintroduction to an area in Bhutan (Rinchen et al., 2020), and six assessors for assessing the risk of SARS-CoV-2 transmission from humans to bats in Australia (Cox-Witton et al., 2021). Engaging in discussions with experienced veterinarians from both the source and the recipient areas of the reintroduction, particularly those actively overseeing the health of the source population, holds significant importance. These individuals are likely to offer valuable insights into the diverse diseases impacting the population. Moreover, they may possess crucial information related to clinical signs such as abortions, lameness, skin conditions, past mortality, and historical details encompassing previous outbreaks and vaccinations.

For anonymity and independence, a questionnaire can be sent to each panel member before and after the workshop (Roche et al., 2015). Each participant is asked to assess individually each of the hazards of concern that has been listed during the previous step. This assessment is qualitative through its possible impact or severity and quantitative by its likelihood or probability to happen. For practicality, each attribute is rendered through a numeric scale, for instance ranging from 1 to 5. The lack of standardization in risk assessment scales has been observed, with variations noted even within the same agency, ranging from 4 to 7 levels (EFSA Scientific Committee, 2012; Horigan et al., 2023). It is recommended to adopt a pre-established, harmonized scale with agreed-upon terminology among the participating panel members. For instance, the likelihood of risk occurrence can be categorized as highly unlikely (score=1), unlikely (2), possible (3), likely (4), or highly likely (5). Similarly, the severity of consequences resulting from the risk can be classified as negligible (1), low (2), moderate (3), high impact (4), or catastrophic (5).

Only the questionnaires that are returned after the workshop are used and the expert opinions are combined (Roche et al., 2015).

Once the list of hazards has been created, the hazards with zero or negligible probability of release or exposure should be removed. A scenario tree for the remaining, higher priorities, hazards of concern can then be drawn.

Finally, each risk is then scored with the simple arithmetic (Dufour et al., 2011):

$$\text{RISK} = \text{LIKELIHOOD} \times \text{IMPACT}$$

With the 5-level scale, a 5x5 likelihood-impact risk matrix is generated (see Figure 5), presenting each level of risk.

The limits for each level of risk, therefore the interpretation, are established collegially during the problem description step. For instance, it can be agreed that risk with a score lower than 4 is considered low, between 4 and 7, it is medium, the risk is high when the score is between 8 and 12, above this value it is extreme.

LIKELIHOOD \ SEVERITY	1 (negligible)	2 (low impact)	3 (moderate impact)	4 (high impact)	5 (catastrophic impact)
1 (highly unlikely)	LOW 1	LOW 2	LOW 3	MEDIUM 4	MEDIUM 5
2 (unlikely)	LOW 2	MEDIUM 4	MEDIUM 6	HIGH 8	HIGH 10
3 (possible)	LOW 3	MEDIUM 6	HIGH 9	HIGH 12	EXTREME 15
4 (likely)	MEDIUM 4	HIGH 8	HIGH 12	EXTREME 16	EXTREME 20
5 (highly likely)	MEDIUM 5	HIGH 10	EXTREME 15	EXTREME 20	EXTREME 25

Figure 5: 5x5 Risk matrix

Depending on whether each hazard is present or absent in either the source population or the recipient area, there will be risks of exposure or risks of release (Dufour et al., 2011).

1.6.3.1. The risk of exposure

This risk arises from pathogens that are absent in the reintroduced population but are known or likely to be present in the area where the reintroduction is planned.

This category encompasses diseases that could impact the viability and sustainability of the reintroduced population (Kock et al., 2010). Since this population is immunologically naive, lacking immunity to these pathogens, it faces potential threats that may compromise its well-being. This includes possible increased morbidity and mortality among the reintroduced animals or reduced reproductive success.

In this exercise, it is crucial to consider the genetics of pathogens as they lead to distinct phenotypes. Therefore, the risk analysis should encompass not only the pathogen's species but also its serotypes and potentially its strains, as these factors can have implications on various aspects such as disease severity, transmission dynamics, immune response, clinical signs, andhost range, as well as antibiotic-resistance genes in bacteria (Haulisah et al., 2021).

For instance, different strains of avian influenza, either highly pathogenic or low pathogenic, have varying impacts on poultry (Abdelwhab et al., 2013). Various strains of the African swine fever virus exhibit different transmission dynamics in pigs (Guinat et al., 2016). Additionally, each serotype of the foot-and-mouth disease virus does not provide cross-immunity against the others (Jamal & Belsham, 2018). Similarly, the different serotypes of bluetongue virus can cause varied clinical signs in susceptible species (Caporale et al., 2014).

The uncertainty is included and described at this step.

The **uncertainty** surrounding the risk of exposure can be attributed to several factors (see below).

The absence of the pathogen in the recipient area may be inaccurately assessed due to a lack of disease reports. This could stem from inadequate veterinary services or a deficiency in notification and reporting mechanisms (Jebara et al., 2012).

Also wildlife species, including the SHO, receive less scientific scrutiny compared to domestic species (Lachish & Murray, 2018). Consequently, there may be a dearth of information regarding the pathobiology of various pathogens in the SHO species. The susceptibility of SHO to a given pathogen is often unknown. Questions arise: How will an infected SHO react to the pathogen? Will it develop a disease or succumb to the infection? What clinical signs will manifest, and how will this impact the reintroduced population—whether on a large scale or across the entire population? Additionally, could the infected SHO become a carrier and potentially a reservoir for humans, livestock, or other wildlife? (Rhyan & Spraker, 2010). For instance, a specific disease might affect domestic ruminants without affecting SHO. The absence of fundamental knowledge regarding host/pathogen interactions can lead to decisions being made either with a risk-averse approach or as risk-takers. For instance, in one scenario, it may be assumed that the SHO is affected by a pathogen, necessitating risk mitigation measures. But in another scenario, the assumption might be that the pathogen poses no risk to the SHO, allowing resources to be allocated to mitigate other potential risks.

1.6.3.2. The risk of release

This risk is constituted by hazards that are absent in the recipient area, but they are present or might be present in the region, in the country, in the area, or in the source population itself (Kock et al., 2010). The reintroduced animals, because they come from a potentially infected area and because they might be carriers or acutely infected, constitute a risk for the recipient area.

Reintroduction might involve animals raised in captivity, where conditions of crowding may enhance the transmission of diseases among them (Griffith et al., 1993; Lu et al., 2024).

There are concerns for public health in case of zoonotic diseases, for the livelihood and economy in case of a pathogen affecting food-producing animals, for the wildlife with possible conservation implications for diseases that might affect other species, and for the environment, with possible contamination of land and water sources. Emerging and transboundary infectious diseases fall in this category.

It is of paramount importance to prevent the spread of alien bacterial species and biotypes, antibiotic-resistance genes, and viral serotypes and strains, for the reasons already mentioned (see "Risk of exposure").

The risk of release of a pathogen is associated with **uncertainty** coming from different sources (see below)

The presence of pathogens in the source population may be uncertain due to a lack of reliable information. While one might extrapolate from neighbouring areas or the wider region, the level of uncertainty increases with distance. Notifiable diseases listed by WHO may go undetected without robust animal health screening systems, which are hindered by factors such as inadequate veterinary services and hidden motives preventing notification. Moreover, varying levels of motivation to notify international bodies may exist, potentially linked to perceived negative consequences such as trade barriers.

Wildlife species may not exhibit clinical signs as prominently as livestock, increasing the risk of underdiagnosis (Sainsbury & Vaughan-Higgins, 2012). For instance, the African buffalo (*Syncerus caffer*) can harbor SAT serotypes FMD virus, and red deer (*Cervus e. elaphus*) can be reservoir for the bluetongue virus (BTV), without displaying clinical signs (Hedger, 1972; Casaubon et al., 2013).

Addressing the issue of underdiagnosis may require systematic and randomized pathogen screening, but this is hindered by cost, labour, and laboratory feasibility challenges (Delgado et al., 2023), as well as unknown test parameters specific to wildlife species (Jia et al., 2020; Michel et al., 2021). This is because these tests were developed for domestic species. In the absence of systematic study, their specificity (Sp) and sensitivity (Se) are approximated from the parameters in domestic species, leading to assumptions (Lachish & Murray, 2018).

Additionally, the epidemiological role of wildlife species in most infectious diseases remains largely unknown (Gortazar et al., 2015).

1.6.4. Risk management

In this stage, the options to minimize or manage potential prioritized risks are explored, evaluating their expected outcomes. The goal is to identify effective measures that can reduce the likelihood of a hazard occurring, lessen its potential impact, or achieve a balance of both, making the associated risk acceptable.

Different strategies to reduce each risk are available, however, the chosen options will depend on the type of risk.

For health hazards that might be released along with the animals, efforts should be made to reduce the likelihood of this pathogen release to happen. This could be done with a combination of the following options:

- Through a control program leading to a specific germ-free population: aiming at eradicating the pathogen in the source population before the animal movement. Different strategies are available depending on the pathogen such as testing and isolation to remove the animals that present clinical signs or vectors (for instance attached ticks), and/or vaccination. The effectiveness of those strategies needs to be assessed.

- Biosecurity and quarantine. For instance, crate and transport equipment disinfection, isolation of animals showing clinical signs, associated with paired serum testing, confirmation of infection through polymerase chain reaction (PCR) or any adequate method etc….

- Drug administration, for example, the use of avermectins to control a recent tick infestation.

Options to reduce the impact can be included in a contingency plan if the release of a pathogen happens, and a revision of the plan is required.

Options to reduce the risk of exposure can focus on the likelihood of happening or on its impacts.

Reducing the likelihood of disease transmission can involve various measures, such as minimizing the interactions with potentially infected livestock, particularly in pastures or near water sources. While limiting interactions with wildlife may not be feasible due to the promotion of natural behaviours, the risk associated with vector-borne diseases can be mitigated by selecting the appropriate time of the year when parasitic burdens are lower. Additionally, efforts to decrease disease prevalence in the release area, such as implementing brucellosis or FMD control plans in domestic livestock, can help minimize the probability of exposure.

To mitigate the impact of disease, one strategy is to increase the immunity of the reintroduced herd against targeted pathogens through vaccination programs.

1.6.5. Implementation and review

Develop and implement a practical action plan to manage the risks prioritized during the previous step, along with a contingency plan. This should include a detailed timeline for monitoring and evaluating the effectiveness of the risk management actions.

For example, if the strategy involves enhanced biosecurity measures, it may be necessary to construct a quarantine facility. The timeline for this objective would encompass conducting a site assessment and designing the quarantine facility, procuring necessary materials and equipment, and conducting staff training to implement biosecurity protocols.

If a vaccination program is part of the strategy to address the knowledge gap regarding vaccine use in wildlife species, it is recommended to include the preliminary vaccine assessment in the targeted species within the timeline.

The timeline for a vaccination program will vary depending on the disease. For instance, after hiring staff and procuring the necessary equipment and vaccine for vaccinating cattle against Foot-and-Mouth Disease (FMD), the timeline would involve administering the initial two-dose primo vaccination (maximum 4 weeks apart) and the first semi-annual booster vaccination after six months (Ulziibat et al., 2023). Controlling brucellosis in livestock through vaccination is a lengthy process that may span several years (Scientific Committee on Animal Health and Animal Welfare, 2001).

If the control strategy involves testing and isolation, screening tests for each prioritized disease should be evaluated in the target species, which also requires time and specialized resources. The timeline for testing and isolation should also be established, considering that repeat testing may be necessary to eliminate all infected animals.

The implementation of the management options should be regularly assessed. Indicators should be defined to measure the effectiveness, allowing refinement of the management plan as needed to achieve better outcomes. For instance, indicators for an effective brucellosis control program would include a decrease in seroprevalence among the tested population over time.

Chapter 2 - Objectives

2.1. General objective

The introduction of this thesis highlights that a WDRA should involve a consultation with experts knowledgeable about animal infectious diseases in both countries involved in the reintroduction. The general objective of this work is the communication regarding infectious animal diseases encountered in the source population, in the context of a reintroduction program.

2.2. Specific objectives

List the infectious diseases that might constitute a risk of release or a risk of exposure during this reintroduction project, based on data publicly available on the internet and through a literature review.

We have identified infectious diseases within the SHO population or in related species within the same animal facility. Each disease poses a risk of release during reintroduction, with varying consequences. For instance, brucellosis raises significant public health concerns, FMD has potential trade and livelihood impacts, and contagious caprine pleuropneumonia can affect the conservation of collateral species

For the pathogens encountered in the source population, confirm and characterize each of them with culture and isolation techniques along with molecular typing techniques. Infer phylogenetic trees to place each pathogen in its geographical and evolutionary context. Evaluate prevalence and incidence with tests primarily designed for domestic livestock.

Provide suggestions for disease control programs in the general context of animal reintroduction.

Additionally, the results will provide important epidemiological clues for evidence-based infectious disease control programs in the UAE.

Chapter 3 - Experimental section

3.1. Hazards identification

Table 1 presents the number of new outbreaks notified to the WOAH (and stated as such) by Chad and the UAE from 2005 to 2023 for diseases likely relevant to this study: the diseases known to affect cattle, small ruminants and multi-species were included in the query. The data were retrieved on January 24, 2024 from https://wahis.woah.org/#/dashboards/qd-dashboard with Chrome (Google, USA), and filtered with Excel (Microsoft, USA).

This list of diseases will provide the backbone for our risk analysis (see Table 1).

Table 1: Number of outbreaks notified by the UAE and Chad to the WOAH between 2005 and 2023

Disease	Reporting country	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Anthrax																				
	Chad	4			2	2	12	2	2	2	2	1	1		2	1	3	3		
Bovine anaplasmosis																				
	UAE										1								4	6
Bovine babesiosis																				
	UAE									1										
Bovine tuberculosis (-2018)																				
	Chad						9	3	1					1						
***Brucella melitensis* infection**																				
	UAE												6	12	4	6	8	8	10	8
Contagious caprine pleuropneumonia																				
	Chad					2	11	8	3		5	2	4	3	1	1	5	3		
Foot and mouth disease																				
	Chad	15	19	4	6	9	20	9	2	2			1	2	2		2	2		
	UAE				1	1				1	2		3	3	3	1		10	7	3
Haemorrhagic septicaemia (*Pasteurella multocida* serotypes 6:b and 6:e)																				
	Chad	3			2	7	14	9	2	9	11	8	7	6	20	16	11	7		
Heartwater																				
	Chad									1								1		
***Mycoplasma mycoides* subsp. *mycoides* SC (Inf. with) (Contagious bovine pleuropneumonia)**																				
	Chad	4	1	2	1	6	13	12	2	1	5	5	4	4	5	3		2		
Paratuberculosis																				
	UAE												11	10	10	8	8	7	16	16
Peste des petits ruminants																				
	Chad	3	6	4	2	5	16	5	2	2	2	1	2	3	5	3	1	2		
	UAE	4	4	4	2	1							6	6	3	2	5	9	9	4
Rift Valley fever																				
	Chad														1	1				
Sheep pox and goat pox																				
	Chad														1	1				
	UAE	4	4	3	3															
Surra (*Trypanosoma evansi*)																				
	UAE									2	3								2	2
***Theileria annulata, Theileria orientalis and Theileria parva* (Inf. with) (2023-)**																				
	UAE									2									4	6
West Nile Fever																				
	UAE			1						2										

3.2. Risk of release of a zoonotic disease. The case of brucellosis

Preamble

Brucellosis, also known as Malta fever, is a debilitating zoonotic disease causing undulant fever, hygroma, arthritis, orchitis and abortion in susceptible species, including humans.

The disease is globally distributed (WOAH, 2023) and various species of Gram-negative coccobacilli *Brucella* bacteria cause it. Among these, *B. melitensis* stands out as the most contagious and significant in terms of human infection. This bacterium primarily affects goats and sheep. Other species of *Brucella* known to cause human brucellosis include *B. abortus*, *B. suis*, *B. canis*, *B. ovis*, and *B. neotomae* (Suárez-Esquivel et al., 2017). Each species exhibits distinct host preferences. For instance, *B. abortus* primarily infects cattle, leading to bovine brucellosis, while *B. suis* is commonly associated with swine but can also infect other mammals, including wild boars and rodents. *B. canis* affects dogs and is a significant concern in veterinary medicine.

Brucellosis poses a substantial economic burden due to reproductive losses in livestock, reduced productivity, and trade restrictions. Control measures typically involve vaccination of susceptible animal populations, surveillance programs, and public health interventions to promote food safety practices and raise awareness about the risks associated with brucellosis transmission.

Transmission to humans typically occurs through the ingestion of raw milk or contact with aborted material and vaginal discharge from infected animals (Zhou et al., 2020). Apart from some sporadic cases of laboratory-acquired (Ackelsberg et al., 2020) or sexually transmitted (Li et al., 2020) human brucellosis, all human cases are associated with an animal reservoir.

The disease is prevalent in the human population in the United Arab Emirates with 3.3 yearly human cases/100.000 inhabitants between 2010 and 2015 in the Abu Dhabi Emirate (Al Shehhi et al., 2016).

Among the 4000 SHO in the source population for this reintroduction project, individuals were suffering from clinical signs evocative of this disease: enlarged testicles, abortion and neonatal death. The findings from our bacteriological investigation are detailed in **Article 1**. In this qualitative study, our research delves into the bacteriological aspects of this outbreak. We used molecular typing techniques and compared the results with worldwide *B.melitensis* strains. A cluster reassembling strains from the UAE, Oman and Qatar was discovered indicating a local enzooty of this pathogen. **Article 2**, on the other hand, is a quantitative study. It presents the magnitude of this outbreak: we evaluated the seroprevalence of brucellosis across six enclosures, housing segments of the source population. This initial step guides our targeted brucellosis screening efforts, focusing on certain enclosures and age categories identified through a risk factor study.

ARTICLE 1

Brucella melitensis biovar 1 isolation in a captive wildlife population in the United Arab Emirates. First isolation in the scimitar-horned oryx (*Oryx dammah*)

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Lignereux Louis, Chaber Anne-Lise, Fretin David, Godfroid Jacques and Claude Saegerman

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Short communication

Brucella melitensis biovar 1 isolation in a captive wildlife population in the United Arab Emirates. First isolation in the scimitar-horned Oryx (*Oryx dammah*)

Louis Lignereux [a,b,*], Anne-Lise Chaber [b], David Fretin [c], Jacques Godfroid [d], Claude Saegerman [a]

[a] Faculty of Veterinary Medicine, Research Unit in Epidemiology and Risk Analysis Applied to Veterinary Sciences (UREAR-ULiège), Fundamental and Applied Research for Animals and Health (FARAH) Center, University of Liège, Liège, Belgium
[b] School of Animal and Veterinary Sciences, University of Adelaide, Adelaide, SA, Australia
[c] Scientific Service Veterinary Bacteriology, Sciensano, Brussels, Belgium
[d] Department of Arctic and Marine Biology, Faculty of Biosciences, Fisheries and Economics, Tromsø, Norway

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ABSTRACT

In 2013, *Brucella melitensis* biovar 1 was recovered from the stomach contents of a scimitar-horned Oryx - SHO (*Oryx dammah*) aborted foetus, and from the articular fluid of a sand gazelle (*Gazella marica*) in a captive wildlife collection near Abu Dhabi, United Arab Emirates. Other evidence of exposure to the pathogen was collected through serological testing (Rose Bengal test) and *B. melitensis*-specific PCR of samples from captive wildlife kept in six different enclosures. A Multiple Locus Variable Number of Tandem Repeats (VNTR) Analysis (MLVA) using 15 markers showed that the two strains isolated in animals kept in enclosures, located 1300 m apart from each other, shared an identical genotype. The phylogenetic analysis of MLVA-15 profiles retrieved from the public database suggested that these strains belong to the African clade, clustering regionally in the UAE, Oman and Qatar. This is the first confirmed case of *B. melitensis* in a SHO, an African antelope extinct in the wild and warrants further investigation.

1. Introduction

Human brucellosis or Malta fever poses a serious health hazard always associated to an animal reservoir. It is caused by small non-encapsulated non-motile, facultative intracellular Gram-negative coccobacilli, that belong to the *Brucella* genus. *Brucella melitensis,* the main causative agent for brucellosis in goat and sheep, is the main cause of human brucellosis (Young, 1995). Importantly, *Brucella melitensis* infections are also found in other farmed species, like camels (Gwida et al., 2012) and in wildlife (Dadar et al., 2021).

In the United Arab Emirates, brucellosis surveys in semi free ranging wildlife have been conducted in the past, without detecting anti-*Brucella* antibodies (Ofner et al., 2007). The disease has been documented in livestock (Mohammed et al., 2013) in a Nubian Ibex (*Capra ibex nubiana*) (Wazed Ali Mollah and McKinney, 2002), in a gazelle (Gyuranecz et al., 2016) and in humans; between 2010 and 2015, 3.3 cases of human brucellosis /100,000 inhabitants were diagnosed yearly in the Abu Dhabi emirate (Al Shehhi et al., 2016).

The Scimitar-horned Oryx (*Oryx dammah*) (SHO) is a large Sahelian antelope that is now extinct in the wild. Global conservation efforts rely heavily on captive stocks for possible re-introduction. Such programs involve conducting wildlife disease risk analysis (Jakob-Hoff et al., 2014).

The aim of this rapid communication is to describe a first confirmed case of *B. melitensis* in SHO.

2. Material and methods

The study site was a 5 km long animal holding facility constituted of more than 50 fenced enclosures (Lignereux et al., 2020) located (location: 24.219 ° N, 54.793 ° E) 45 km east to Abu Dhabi, the capital city of the UAE.

The study site was populated with six different species of wild ungulates after they were translocated from a private island (location: 24.322 ° N, 52.598 ° E) in 2008. In November 2012, there were 7931 indian blackbucks (*Antelope cervicapra*), 3894 SHO, 1300 sand gazelles

* Corresponding author at: School of Animal and Veterinary Sciences, University of Adelaide, Adelaide, SA, Australia.
E-mail address: louis.lignereux@adelaide.edu.au (L. Lignereux).

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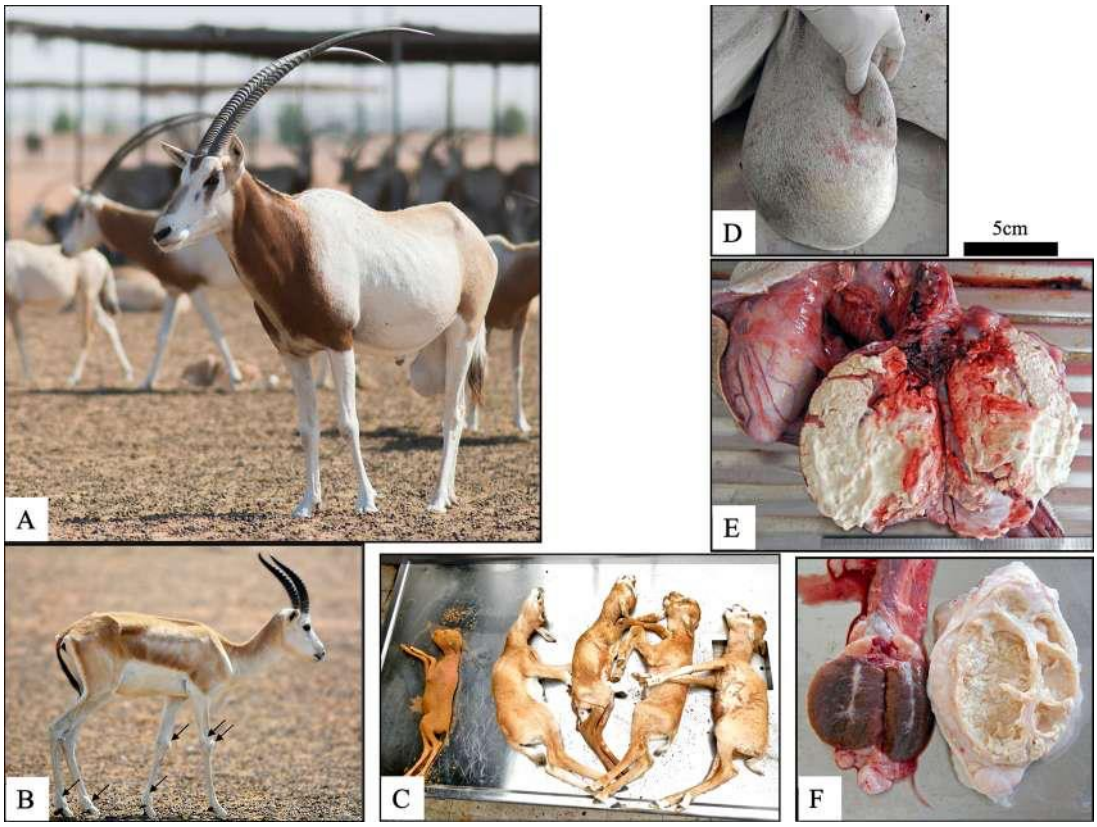

Fig. 1. Brucellosis gross lesions in the wildlife collection.
A: adult male scimitar-horned Oryx (SHO) presenting a bilateral testicular enlargement; B: sand gazelles with distended joints (arrows). Note the poor body condition of the affected gazelles; C: aborted and dead newborn SHO; Picture E: enlarged testicle of a Rose Bengal test positive SHO; F: same testicle after sagittal cut and content left in place. Note the size of the contralateral testicle in the upper left corner; G: the same testicle after removal of the caseous purulent material to visualise the thickened *septa testis*. The contralateral testicle is cut in half for comparison. The same scale is used for pictures D, E and F. The bar represents 5 cm.

(*Gazella marica*), 258 mountain (*Gazella gazella*) and Indian (*Gazella bennetti*) gazelles and 11 Urial sheep (*Ovis orientalis*). Species were separated from each other, and they were all breeding within species groups.

There was no history of testing or vaccination against brucellosis and the health status was unknown. Late 2012, brucellosis was suspected due to observation of enlarged testicles and hygromas, which are external clinical signs compatible with the disease (Garin-Bastuji et al., 1998). To confirm this, animals that presented those signs were restrained (Medetomidine, 0.6 mg and Ketamine, 100 mg per SHO, injected remotely and intramuscularly) and euthanized whenever their condition was detrimental to their welfare (mixture of Embutramide, Mebezonium and Tetracaine injected intravenously). Blood and testes from two males SHO, and blood and articular fluid from a male sand gazelle were collected and sent to a local veterinary laboratory to perform serological screening (RBT) and diagnostic (*Brucella melitensis*-specific PCR) tests for brucellosis. Histopathology was not performed. In addition, the RBT was also performed on blood collected from one aborted foetus and four dead newborn SHO.

Once the presence of *Brucella* was confirmed, another batch of fourteen samples consisting in different organs (SHO foetal spleen, lung, or stomach contents, or sand gazelle articular fluid) was collected randomly and aseptically in different enclosures in November and December 2013 from dead animals. They were sent under controlled temperature to Sciensano (ex. CODA-CERVA), Belgium for culture and biotyping using classical procedures described elsewhere (Alton et al., 1988).

The genotyping analysis was performed by Multiple Locus Variable Number of Tandem Repeats (VNTR) Analysis (MLVA) using 15 markers as described in (Le Flèche et al., 2006). Those markers were divided in three panels: 1, 2A and 2B, with eight, two and five microsatellites, respectively. Band sizes of tandem repeat units longer than 600pb were analysed on a 2% agarose gel. The remaining PCR products were analysed by capillary electrophoresis in a CEQ 8000 automatic DNA Analysis System (Beckman-Coulter) using a commercial kit (GenomeLab™ DTCS-Quick Start Kit, Beckman-Coulter) according to the manufacturer' instructions. Band size and peak numbers were converted to number of units using the *B. melitensis* 16 M reference strain (ATCC 23, 456) (Le Flèche et al., 2006).

Brucella melitensis alleles profiles were retrieved from the public database MLVA Bank (http://mLva.u-psud.fr/mLvav4/genotyping/index.php) as well as from the literature. The genotypes of all the *B. melitensis* strains isolated in Africa, the Middle East and Asia were included in our analysis (Garofolo et al., 2016; Georgi et al., 2017; Gopaul et al., 2014; Gyuranecz et al., 2016; Le Flèche et al., 2006; Menshawy et al., 2014; Mustafa et al., 2017; Osman et al., 2015; Schulze zur Wiesch et al., 2009; Tiller et al., 2009; Vergnaud et al., 2018). A total of 2729 profiles, including our profile, were then imported to Excel (Microsoft, USA) for sorting and cleaning. Loci with incomplete or missing information were not used. The columns presenting values for panel 1, 2A and 2B were copied 10, five and one time to increase their respective weight in the hierachical analysis performed with PHYLOViZ 2.0 software (Francisco et al., 2012). The phylogenetic tree was constructed using the Unweighted Pair-Group Method with Arithmetic mean (UPGMA) algorithm.

3. Results and discussion

Similarly to observations made during a brucellosis outbreak in Dorcas gazelles, (*Gazella dorcas*) (Wieckowski, 2017) and sable antelope

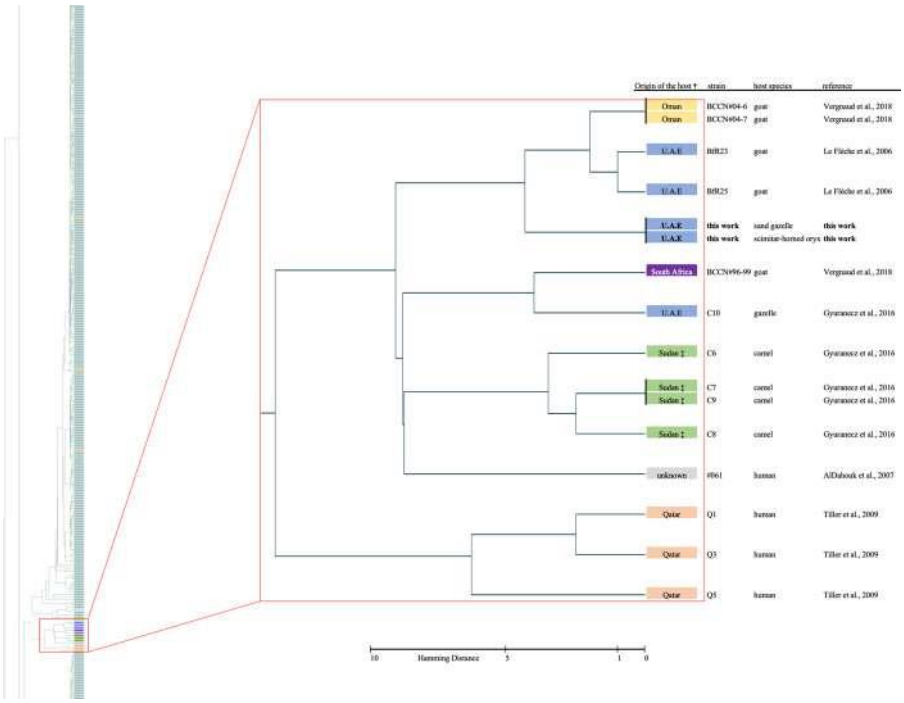

Fig. 2. Cluster analysis based on UPGMA phylogenetic tree inferred from 2729 *Brucella melitensis*. Legend: On the left, a detail of the overall tree showing the cluster (red box) to which our two isolates belong. On the right, this cluster (with country of origin, host, reference). A different colour represents each country of origin. †: Origin of the host as mentioned in the referenced paper. ‡: The host originated from Sudan but brucellosis was diagnosed in the U.A.E.

(*Hippotragus niger*) (Glover et al., 2020), hygromas, i.e. distension of the tibio-tarsal and metacarpo/tarso-phalangeal joints were observed in sand gazelles (Fig. 1**-B**). The presence of hygroma motivated the sampling of one adult male which elicited a positive RBT on serum and a positive *B.melitensis*-specific PCR result on articular fluid collected from the hygroma. Reluctance to move and loss of body condition possibly contributing to death were observed in gazelles.

On November 13, 2013, the carpometacarpal joint synovial fluid collected on another sand gazelle provided a positive culture (with only one colony seen) of *B. melitensis* biovar 1, followed two weeks later by another positive culture performed on the stomach contents of an aborted SHO and in an enclosure located 1300 m away from the gazelle.

In SHO, the clinical picture contrasted from what was observed in gazelles, and was more comparable to lesions described in sheep (Garin-Bastuji et al., 1998), goat (Poester et al., 2013) or alpine ibex (Mick et al., 2014). The uni- or bilateral testicular enlargement triggered the decision to sample the two SHO which appeared otherwise in excellent body condition (Fig. 1**-A**). The affected testicle(s) had undergone important gross lesions with distension due to a caseous purulent material replacing the seminal tissue and separated by thickened septa (Fig. 1**-E and F**). Positive *B.melitensis*-specific PCR results were obtained from the testicular tissues of both animals.

Out of the one aborted SHO foetus and the four dead newborns collected on a single day, the foetus and one newborn were positive to the RBT. None of the four newborns had a stomach content compatible with colostral intake, limiting the transfer of maternal immunity.

The isolation of *B.melitensis* from the stomach content of an aborted foetus (Fig. 1**-C**) reveals the transplacental transmission of the pathogen already reported in sheep (Grilló et al., 1997).

Females SHO did not exhibit obvious external signs and we find no particular external signs were observed in Indian blackbucks from both sexes.

The two isolates showed an identical MLVA-15 profile (Appendix) not described in the MLVA databank. This illustrates the clonal expansion of the bacteria usually observed during brucellosis outbreaks (Dorneles et al., 2014) and suggests a unique origin. They clustered with the genotype of strains previously isolated from the region and belonging to the African cluster (Fig. 2).

The phylogenetically most closely related strains were isolated from goats sampled in the UAE (Le Flèche et al., 2006) and Oman (Vergnaud et al., 2018) and another closely related strain was isolated from a sand gazelle in Abu Dhabi (Gyuranecz et al., 2016). Four strains recovered previously in the UAE from "Sudanese" camels (Gyuranecz et al., 2016) clustered with the strains isolated during this study. Together these strains constitute a cluster coherent with geographical data and limited to Oman, UAE and Qatar.

This study documents the presence of *Brucella melitensis* in at least two species and six enclosures in this wildlife collection. We suggest integrating brucellosis in wildlife disease risk assessment associated with possible reintroduction programs of those species. An effort to limit the risk of transmission to humans and the spread to neighbouring livestock farms should be initiated. Our study highlights the gap in our knowledge in brucellosis and its pathobiology in different wildlife of high conservation value.

Ethics statement

The animal collection health management provided the data presented in this study and this work was not performed primarily for research purposes.

Declaration of Competing Interest

The authors declared that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetmic.2022.109360.

References

Al Shehhi, N., Aziz, F., Al Hosani, F., Aden, B., Blair, I., 2016. Human brucellosis in the emirate of Abu Dhabi, United Arab Emirates, 2010–2015. BMC Infect. Dis. 16 https://doi.org/10.1186/s12879-016-1900-9.

Alton, G.G., Jones, L.M., Angus, R., Verger, J., et al., 1988. Techniques for the Brucellosis Laboratory. Institut National de la recherche Agronomique (INRA).

Dadar, M., Shahali, Y., Fakhri, Y., Godfroid, J., 2021. The global epidemiology of Brucella infections in terrestrial wildlife: a meta-analysis. Transbound. Emerg. Dis. 68 (2), 715–729. https://doi.org/10.1111/tbed.13735.

Dorneles, E.M.S., Santana, J.A., Alves, T.M., Pauletti, R.B., da Silva Mol, J.P., Heinemann, M.B., Lage, A.P., 2014. Genetic stability of Brucella abortus isolates from an outbreak by multiple-locus variable-number tandem repeat analysis (MLVA16). BMC Microbiol. 14 (1), 1–8.

Francisco, A.P., Vaz, C., Monteiro, P.T., Melo-Cristino, J., Ramirez, M., Carriço, J.A., 2012. PHYLOViZ: phylogenetic inference and data visualization for sequence based typing methods. BMC Bioinformatics 13 (1), 87. https://doi.org/10.1186/1471-2105-13-87.

Garin-Bastuji, B., Blasco, J.M., Grayon, M., Verger, J.M., 1998. Brucella melitensis infection in sheep: present and future. Vet. Res. 29 (3–4), 255–274.

Garofolo, G., Fasanella, A., Di Giannatale, E., Platone, I., Sacchini, L., Persiani, T., Boskani, T., Rizzardi, K., Wahab, T., 2016. Cases of human brucellosis in Sweden linked to Middle East and Africa. BMC Res. Notes 9 (1), 277. https://doi.org/10.1186/s13104-016-2074-7.

Georgi, E., Walter, M.C., Pfalzgraf, M.-T., Northoff, B.H., Holdt, L.M., Scholz, H.C., Zoeller, L., Zange, S., Antwerpen, M.H., 2017. Whole genome sequencing of Brucella melitensis isolated from 57 patients in Germany reveals high diversity in strains from Middle East. PLoS One 12 (4), e0175425. https://doi.org/10.1371/journal.pone.0175425.

Glover, B., Macfarlane, M., Bengis, R., O'Dell, J., Steyl, J., van Heerden, H., Abernethy, D., 2020. Investigation of Brucella melitensis in Sable Antelope (Hippotragus niger) in South Africa. Microorganisms 8 (10). https://doi.org/10.3390/microorganisms8101494, 1494.

Gopaul, K.K., Dainty, A.C., Muchowski, J.K., Dawson, C.E., Stack, J.A., Whatmore, A.M., 2014. Direct molecular typing of Brucella strains in field material. Vet. Rec. 175 (11), 2–282. https://doi.org/10.1136/vr.102674, 282.

Grilló, M.J., Barberán, M., Blasco, J.M., 1997. Transmission of Brucella melitensis from sheep to lambs. Vet. Rec. 140 (23), 602–605. https://doi.org/10.1136/vr.140.23.602.

Gwida, M., El-Gohary, A., Melzer, F., Khan, I., Rösler, U., Neubauer, H., 2012. Brucellosis in camels. Res. Vet. Sci. 92 (3), 351–355. https://doi.org/10.1016/j.rvsc.2011.05.002.

Gyuranecz, M., Wernery, U., Kreizinger, Z., Juhász, J., Felde, O., Nagy, P., 2016. Genotyping of Brucella melitensis strains from dromedary camels (Camelus dromedarius) from the United Arab Emirates with multiple-locus variable-number tandem repeat analysis. Vet. Microbiol. 186, 8–12. https://doi.org/10.1016/j.vetmic.2016.02.009.

Jakob-Hoff, R.M., Kock, R., Lees, C., MacDiarmid, S.C., Miller, P.S., Travis, D., 2014. *Manual of Procedures for Wildlife Disease Risk Analysis*. World Organization for Animal Health (OIE) Published in Association With the International Union for Conservation of Nature (IUCN) and the Species Survival Commission (SSC).

Le Flèche, P., Jacques, I., Grayon, M., Al Dahouk, S., Bouchon, P., Denoeud, F., Nöckler, K., Neubauer, H., Guilloteau, L.A., Vergnaud, G., 2006. Evaluation and selection of tandem repeat loci for a Brucella MLVA typing assay. BMC Microbiol. 6 (1) https://doi.org/10.1186/1471-2180-6-9, 9.

Lignereux, L., Chaber, A.-L., Saegerman, C., Heath, L., Knowles, N.J., Wadsworth, J., Mioulet, V., King, D.P., 2020. Foot-and-mouth disease outbreaks in captive scimitar-horned oryx (Oryx dammah). Transbound. Emerg. Dis. https://doi.org/10.1111/tbed.13502.

Menshawy, A.M.S., Perez-Sancho, M., Garcia-Seco, T., Hosein, H.I., García, N., Martinez, I., Sayour, A.E., Goyache, J., Azzam, R.A.A., Dominguez, L., Alvarez, J., 2014. Assessment of Genetic Diversity of Zoonotic Brucella spp. Recovered from Livestock in Egypt Using Multiple Locus VNTR Analysis. Biomed Res. Int. 1–7. https://doi.org/10.1155/2014/353876, 2014.

Mick, V., Carrou, G.L., Corde, Y., Game, Y., Jay, M., Garin-Bastuji, B., 2014. Brucella melitensis in France: persistence in wildlife and probable spillover from alpine ibex to domestic animals. PLoS One 9 (4), e94168. https://doi.org/10.1371/journal.pone.0094168.

Mohammed, M.A., Shigidy, M.T., Al juboori, A.Y., 2013. Sero-prevalence and epidemiology of brucellosis in camels, sheep and goats in Abu Dhabi emirate. Int. J. Anim. Vet. Adv. 5 (2), 82–86. https://doi.org/10.19026/ijava.5.5581.

Mustafa, A.S., Habibi, N., Osman, A., Shaheed, F., Khan, M.W., 2017. Species identification and molecular typing of human Brucella isolates from Kuwait. PLoS One 12 (8), e0182111. https://doi.org/10.1371/journal.pone.0182111.

Ofner, W., Bailey, T., O'Donovan, D., Wernery, U., Zachariah, R., 2007. Absence of antibodies against Brucella sp. In different species of semi free-ranging bovidae in the Emirate of Dubai, United Arab Emirates. Veterinary Medicine Austria / Wiener Tierärztliche Monatsschrift 94, 180–183.

Osman, A.E.F., Hassan, A.N., Ali, A.E., Abdoel, T.H., Smits, H.L., 2015. Brucella melitensis Biovar 1 and Brucella abortus S19 Vaccine Strain Infections in Milkers Working at Cattle Farms in the Khartoum Area, Sudan. PLoS One 10 (5), e0123374. https://doi.org/10.1371/journal.pone.0123374.

Poester, F.P., Samartino, L.E., Santos, R.L., 2013. Pathogenesis and Pathobiology of Brucellosis in Livestock. Revue Scientifique Et Technique (International Office of Epizootics), 32(1), pp. 105–115. https://doi.org/10.20506/rst.32.1.2193.

Schulze zur Wiesch, J., Wichmann, D., Sobottka, I., Rohde, H., Schmoock, G., Wernery, R., Schmiedel, S., Dieter Burchard, G., Melzer, F., 2009. Genomic tandem repeat analysis proves laboratory-acquired brucellosis in veterinary (Camel) diagnostic laboratory in the United Arab Emirates. Zoonoses Public Health 57 (5), 315–317. https://doi.org/10.1111/j.1863-2378.2009.01258.x.

Tiller, R.V., De, B.K., Boshra, M., Huynh, L.Y., Ert, M.N.V., Wagner, D.M., Klena, J., Mohsen, T.S., El-Shafie, S.S., Keim, P., Hoffmaster, A.R., Wilkins, P.P., Pimentel, G., 2009. Comparison of two multiple-locus variable-number tandem-repeat analysis methods for molecular strain typing of human Brucella melitensis isolates from the Middle East. J. Clin. Microbiol. 47 (7), 2226–2231. https://doi.org/10.1128/JCM.02362-08.

Vergnaud, G., Hauck, Y., Christiany, D., Daoud, B., Pourcel, C., Jacques, I., Cloeckaert, A., Zygmunt, M.S., 2018. Genotypic expansion within the population structure of classical Brucella species revealed by MLVA16 typing of 1404 Brucella isolates from different animal and geographic origins, 1974–2006. Front. Microbiol. 9 https://doi.org/10.3389/fmicb.2018.01545.

Wazed Ali Mollah, M., McKinney, P., 2002. Brucellosis and suspected paratuberculosis in a nubian ibex (capra ibex nubiana)-a case report. Proceedings of the World Association of Wildlife Veterinarians Wildlife Sessions at the 27th World Veterinary Congress in Tunisia. http://wmenews.com/Information/Other/Section1Wazedpp19-22pdf1.pdf.

Wieckowski, T., 2017. Brucella Melitensis Infection in Dorcas Gazelles (Gazella Dorcas) in Isolated Semi Free Range Zoological Collection in the UAE, pp. 170–174.

Young, E.J., 1995. An overview of human brucellosis. Clin. Infect. Dis. 21 (2), 283–290. https://doi.org/10.1093/clinids/21.2.283.

ARTICLE 2

Brucellosis seroprevalence in captive scimitar-horned oryx (*Oryx dammah*) in the United Arab Emirates and associated risk factors

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Lignereux Louis, Chaber Anne-Lise, Nemery Quentin, Godfroid Jacques and Claude Saegerman

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Brucellosis seroprevalence in captive scimitar-horned oryx (*Oryx dammah*) in the United Arab Emirates and associated risk factors

Louis Lignereux [a,b,*], Anne-Lise Chaber [b], Quentin Nemery [a], Jacques Godfroid [c], Claude Saegerman [a]

[a] Faculty of Veterinary Medicine, Research Unit in Epidemiology and Risk Analysis Applied to Veterinary Sciences (UREAR-ULiège), Fundamental and Applied Research for Animals and Health (FARAH) Center, University of Liège, Liège, Belgium
[b] School of Animal and Veterinary Sciences, University of Adelaide, Adelaide, SA, Australia
[c] Department of Arctic and Marine Biology, Faculty of Biosciences, Fisheries and Economics, UiT – The Arctic University of Norway, Tromsø, Norway

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ABSTRACT

Background: The scimitar-horned oryx (*Oryx dammah*) (SHO) is a large African antelope that became extinct in the wild just over two decades ago. Conservation of the species is of prime importance, but it might face pathogen stressors.

Methods and principal findings: *Brucella melitensis* biovar 1 was previously confirmed in a high-density captive population of SHO held in Abu-Dhabi emirate. The infection reached 67.0 % (95 % CI: 64.0–70.0) individual seroprevalence (n = 959) during testing performed between January 2013 and January 2015. A model based on a multivariable logistic regression analysis showed that the seroprevalence ranged from 51.2 (95 % CI: 39.6–62.7) to 86.9 % (95 % CI: 82.4–91.4) between six different enclosures, and probability of being seropositive was 1.83 (95 % CI: 1.32–2.55) higher in females than in males, 3.09 (95 % CI: 1.66–5.91) and 9.35 (95 % CI: 4.66–19.44) higher in subadults and adults than in juveniles, respectively. The three serological tests used in this study (Rose Bengal Test, lateral flow assay and in-house i-ELISA) had a perfect or near-perfect agreement (Cohen's Kappa coefficient > = 0.97). Recurrent high seroprevalence in time and congruence of results from three different serological tests point toward a persistent *B. melitensis* infection in a high-density captive SHO population.

Conclusion and significance

Testing strategy (Bengal Test, lateral flow assay or in-house i-ELISA) has no effect on the estimation of the brucellosis seroprevalence in SHO permitting the selection of a practical test. We call for an evidence-based control program, and *Brucella* vaccine efficacy and innocuity studies in this endangered species.

1. Introduction

With an estimated 5,000,000 to 12,000,000 true annual cases (Hull and Schumaker, 2018), human brucellosis or Malta fever is a zoonotic debilitating chronic bacterial disease caused by small non-encapsulated non-motile, facultative intracellular Gram-negative coccobacilli, that belong to the *Brucella* genus. It poses a serious public health hazard always associated with an animal reservoir.

The main cause of human brucellosis is *Brucella melitensis* (Young, 1995), which is also the main causative agent for brucellosis in goats and sheep.

There were on average 3.3 cases of human brucellosis/100,000 inhabitants diagnosed yearly between 2010 and 2015 in the Abu Dhabi Emirate (Al Shehhi et al., 2016).

The scimitar-horned oryx (SHO) (*Oryx dammah*) is a large desert antelope that once inhabited extensive areas of the Sahel from Mauritania to Egypt. It is now extinct in the wild because of intensive hunting, habitat loss, and competition with domestic livestock (IUCN, 2015). Global conservation efforts rely on captive stocks for possible reintroduction.

An outbreak of brucellosis due to *Brucella melitensis* biovar 1 has been confirmed in possibly the world's largest population of SHO, in the Emirate of Abu Dhabi (Lignereux et al., 2022).

* Corresponding author at: Faculty of Veterinary Medicine, Research Unit in Epidemiology and Risk Analysis Applied to Veterinary Sciences (UREAR-ULiège), Fundamental and Applied Research for Animals and Health (FARAH) Center, University of Liège, Liège, Belgium.
E-mail addresses: l_louis77@hotmail.com (L. Lignereux), anne-lise.chaber@adelaide.edu.au (A.-L. Chaber), jacques.godfroid@uit.no (J. Godfroid), claude.saegerman@uliege.be (C. Saegerman).

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Reintroduction programs involve conducting a wildlife disease risk analysis (Jakob-Hoff et al., 2014) and preventing the introduction of exotic disease/pathogen into the host area is probably the most important responsibility of decision-makers (Kock et al., 2007).

As a prelude to control this outbreak, this study aimed at determining the associated risk factors, to concentrate the testing effort on certain enclosures or age groups. A test and isolation strategy aiming at removing infected SHO could be put in place afterwards.

2. Material and methods

2.1. Animals

Two animal holding facilities were sampled for brucellosis during this study.

The first animal holding facility (location: 24.219° N, 54.793° E) was described elsewhere (Lignereux et al., 2020, 2022). It was 6,000 m long and 800 m wide (Fig. 1) and was constituted of single fenced enclosures initially designed for livestock. It is unknown if this facility ever served its intended purpose, but it was devoid of livestock at the time of the study. A local farm compound – "izbas" (Al Shehhi et al., 2016) or "ezbas" (Chaber and Saegerman, 2017), was located 2500 m away from the nearest occupied enclosure. The entire facility was surrounded by a 50 m buffer zone. It received in late 2008 and without prior disease testing over 11,000 wild ungulates from Sir Bani Yas Island (SBYI) (location: 24.322° N, 52.598° E). A further 3000 gazelles were moved later from at least three other locations. The animals were kept on sandy ground and the manure was left to dry. Artificial shade structures were installed. Water and imported feed were provided daily.

The different species were kept separated and direct contact between enclosures was prevented by access corridors of at least 15 m wide. Some fences were in poor condition and animals could sometimes escape their enclosures. Most enclosures contained both sexes. There were 7931 Indian blackbucks (*Antelope cervicapra*), 3894 SHO, 1300 sand gazelles (*Gazella marica*), 258 mountain (*Gazella gazella*) and Indian (*Gazella bennetti*) gazelles and 11 Urial sheep (*Ovis orientalis*) in November 2012.

The SHO population was spread over 11 enclosures and had an important conservation value due to its unique, but low, genetic diversity with only seven haplotypes (Ogden et al., 2020). The enclosures with many animals in poor condition were deemed to be of lower interest for breeding and conservation purposes and were not tested.

This study focused on six pens (pens I to VI in Fig. 1) holding a total of 2537 SHO and spread across three testing campaigns, between January 2013 and January 2015.

A catching pen, alleyway and mobile chute system (Tamer®, Fauna Research, USA) were installed in each of the tested pens. The SHO were driven into the alleyway where they could be sorted: under the assumption that older animals would be more affected than younger animals, it was arbitrarily decided to put the testing effort on younger and better-looking individuals. Females were selected over males to enhance the breeding capacity. This process possibly led to a selection bias in this study.

The SHO were physically restrained in the chute for clinical examination, individual identification, sexing, ageing, and bleeding. Animals exhibiting only deciduous teeth were considered juveniles, those exhibiting one or two pairs of adult incisors, subadults and those exhibiting three or four pairs, adults. Subadults were estimated between 19 and 27 month-old (Lignereux et al., 2020).

The second animal facility was situated on SBYI. It was the source of the translocated population. There were about 1500 SHO among thousands of ruminants from different wildlife species on this island when this survey was done in April 2015.

2.2. Screening tests

Either the Rose Bengal test (RBT) or a lateral flow assay (LFA) was used to evaluate the exposure of each tested SHO to *Brucella* spp.

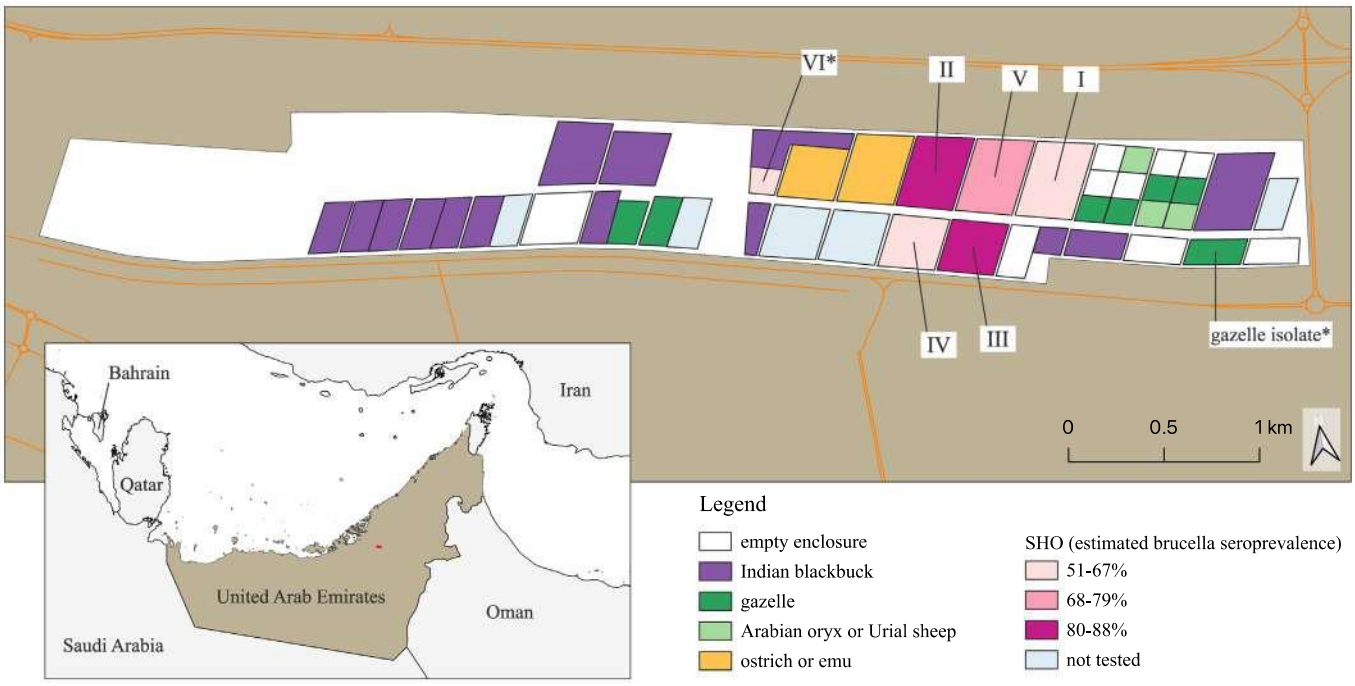

Fig. 1. Schematic representation of the field compound. The animal facility, represented in red, is located in the United Arab Emirates on the general map. On the simplified layout of the animal facility, the space devoid of animals is shown in white and the species are represented in different colours. The tested pens (I to VI) are shown and the estimated brucellosis seroprevalence in scimitar-horned oryx (SHO) is indicated with a pink gradient. *: enclosure where *Brucella melitensis* biovar 1 was isolated in 2013 (Lignereux et al., 2022). The map was made with QGIS 3.23 using a colourblind colour palette. The countries' shapefiles were uploaded from the GADM database (www.gadm.org). Note: the animal facility layout has been rotated as indicated by the north arrow. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The RBT (Bengatest®, Synbiotics, France then Zoetis, USA) cost 0.25US$/test. RBT is a rapid buffered agglutination test, that requires fridge storage and little laboratory work. An inactivated, concentrated solution of *B. abortus* stained with rose Bengal is mixed on a clean single-use microscope slide with an equal volume of serum as described elsewhere (OIE - World Organisation for Animal Health, 2018) and read after four minutes of gentle shaking (see examples in Appendix 1, picture A).

The LFA (Anigen Rapid Bovine *Brucella* Ab Test Kit, RB2301DD, Bionote, South Korea) costs 3.8US$/test. LFA is a chromatographic immunoassay. It is a room temperature storable pen-side test, that can be performed on unclotted heparinized blood or serum.

An invisible band of *B. abortus* 1119-3 lipopolysaccharide (LPS) is deposited on a nitrocellulose membrane held in a plastic casing fitted with perforations to add the sample and visualize the result. A chromogenic reaction occurs within 20 min whenever anti-Brucella immunoglobulins are present, and their concentration dictates the strength of this reaction (see examples in Appendix 1, picture B). Slight reactions are considered positive. The test result was considered positive when the test band was seen by all persons from a panel of two or three to decrease interpretation subjectivity.

A third serological test, an in-house indirect Enzyme-Linked Immuno-Sorbent Assay (i-ELISA) was performed in parallel with RBT and LFA on a subset of serum samples to evaluate the agreement between tests.

This i-ELISA is accredited at the Belgian National Reference Centre (Sciensano, Belgium). It was described elsewhere (Rahman et al., 2012). Briefly, the 1/50 diluted serum samples were deposited in plates previously coated with smooth LPS from *B. abortus* strain Weybridge 99. The binding antibodies were detected with a protein G-horseradish peroxidase conjugate (Biorad, Belgium) and following the addition of O-phenylenediamine, the optical densities were measured at 490 nm and 620 nm with an iMark Microplate Absorbance Reader (Biorad, Belgium). The results were calculated based on the difference between the two measurements. Six dilutions (from 1/270 to 1/8640) of the OIE reference serum provided the standard curve and the cut-off was determined as the mean of 1/8640 dilutions of the standard curve.

2.3. Statistical analysis

2.3.1. Serological tests comparison

In the absence of a "gold standard", i.e. actual bacteriological status of every single animal, the agreement between pairs of serological tests (RBT, LFA, and i-ELISA) was evaluated with the Kappa coefficient κ (Petrie and Watson, 2013).

2.3.2. Risk factors for exposure to brucellosis

Multiple logistic regression analysis (Prism 9, Graphpad, USA) was used to evaluate the effects of sex, age category, tested pen, testing protocol, and testing campaign on the initial individual serological outcome. The odds ratios (OR) were calculated from the estimated model parameters, and a Wald test assessed their significance through Z- and subsequent *P*-values.

The explanatory categories and the interactions that did not affect the outcome (odds ratios not significantly different from 1) were removed from the model and the regression analysis was re-run. The "margins" function in Stata (StataCorp, USA) was used to calculate the predicted seroprevalence associated with each risk factor. The pairwise comparison of the seroprevalences was performed after Bonferroni correction (Petrie and Watson, 2013).

For all statistical tests, a 95 % confidence interval was calculated using a binomial (Clopper-Pearson) exact method and all *P*-values inferior to 0.05 were considered significant.

3. Results

3.1. Initial individual seroprevalence

The first testing campaign spread from January to March 2013, 364 SHO were tested with RBT out of the 1399 SHO present in enclosures I, II, III, and IV (Fig. 1). A total of 424 SHO were tested with LFA during the second campaign from February to April 2014 out of the 726 SHO present in enclosure V. Finally, out of 412 SHO in enclosure VI, 62 subadults and 65 adults were tested with LFA, and 44 adults were tested with RBT during the third and last campaign from November 2014 to January 2015. Details can be seen in Table 1.

In total, 959 SHO were tested including 645 females and 314 males, 231 juveniles, 126 subadults and 602 adults. Also, 408 and 551 SHO were tested with RBT and LFA, respectively.

In addition, *Brucella melitensis* biovar 1 was isolated in 2013 (Lignereux et al., 2022) in enclosure VI – see Fig. 1.

Amongst the 959 SHO tested, 643 elicited a positive result, the overall observed seroprevalence was 67.0 % (95 % CI: 64.0–70.0).

The "testing protocol" (RBT *versus* LFA) did not significantly influence the serological outcome. With RBT as reference level, the odds ratio was 1.130 (95 % CI: 0.489–2.548; p = 0.77). The sampling protocol was therefore not included as an explanatory variable from the multiple logistic regression.

The two variables "testing campaign" and "tested pen" were not independent, and they could not be included together in the analysis, leading to two different scenarios: in the first one, the risk of being seropositive was higher during the second campaign (OR = 1.56 (95 % CI: 0.93–2.61)), and lower (OR = 0.64 (95 % CI: 0.34–1.05)) during the third campaign than it was during the first campaign.

The second scenario was conducted with the independent explanatory variables "sex", "age category" and "tested pen". The results (presented in Table 2) indicate that the likelihood of being seropositive was 1.83 times higher in females than it was in males, and the estimated seroprevalences were 60.0 % in males (95 % CI: 55.0–65.0) and 70.1 % in females (95 % CI: 67.5–73.9). It was also 3.09 and 9.35 times greater in sub-adults and adults than it was in juveniles, respectively, with an estimated seroprevalence of 34 % in juveniles (95 % CI: 23.3–45.0), 58.4 % in sub-adults (95 % CI: 48.8–68.2), and 79.4 % in adults (95 % CI: 75.3–83.4). All those differences were significant. The pen also influenced the seroprevalence: the estimated values ranged between 51.2 % (95 % CI: 39.6–62.7) in pen I and 86.9 % (95 % CI: 82.4–91.4) in pen III. The pairwise comparison with Bonferroni correction (Table 2) indicated that no significant difference existed between the higher seroprevalence in pens II and III, the intermediate seroprevalence in pens II and V, and the somewhat lower seroprevalence in pens I, IV, V and VI (Fig. 1).

3.2. Serological tests agreement

A subset of 67 SHO sera samples, consisting of the 62 samples collected in November 2014 from subadult SHO in pen VI, and five other samples also randomly chosen, underwent RBT, LFA and i-ELISA tests. Twenty-two and 44 samples were classified as positive and negative by all three tests, respectively. (Table 3).

The i-ELISA and LFA were in perfect agreement (κ coefficient = 1; 95 % CI: 0.76–1.24). RBT failed to detect a sample that was found seropositive by both the LFA and the i-ELISA leading to a near-perfect agreement (κ coefficient = 0.97; 95 % CI: 0.73–1.21).

3.3. Seroprevalence on SBYI

Out of the 50 adult females tested on SBYI, one elicited a positive reaction with LFA.

Table 1
Number of tested scimitar-horned oryx and their distribution according to sex, age category and enclosures.

Date	Tested pen	Testing protocol	Male	Female	Juvenile	Subadult	Adult	Negative	Positive	n tested	Observed sero-prevalence (in %)	95 % CI*
2/01/2013 to 22/01/2013	I	RBT	66	68	105	18	11	103	31	134	23.1	16.3–31.2
28/01/2013 to 29/01/2013	II	RBT	22	20	37	5	0	19	23	42	54.8	38.7–70.2
12/02/2013 to 25/02/2013	III	RBT	54	62	78	35	3	32	84	116	72.4	63.3–80.3
27/02/2013 to 19/03/2013	IV	RBT	39	33	10	4	58	30	42	72	58.3	46.1–69.9
10/02/2014 to 24/04/2014	V	LFA	89	335	1	2	421	68	356	424	84.0	80.1–87.3
18/11/2014 to 06/01/2015	VI	LFA	33	94	0	62	65	51	76	127	59.8	50.8–68.4
18/11/2014 to 06/01/2015	VI	RBT	11	33	0	0	44	13	31	44	70.5	54.8–83.2
	TOTAL		314	645	231	126	602	316	643	959	67.0	64.0–70.0

Legend: RBT, Rose Bengal Test; LFA, lateral flow assay.
* 95 % confidence interval (binomial exact).

Table 2
Calculation of the odds ratio and the estimated seroprevalence for each risk factor (n = 959).

		Odds ratio				Estimated seroprevalence		
Risk factor	Variable	Odds ratio	95 % CI*	|Z|	*P*-value	Estimated sero-prevalence (in %)	95 % CI*	Bonferroni groups
	intercept	0.14	0.08–0.23	7,516	<0.0001			
age category	juvenile	1 (reference)				34.2	23.4–45.0	
	subadult	3.09	1.66–5.91	3,493	0.0005	58.5	48.8–68.2	
	adult	9.35	4.66–19.44	6,152	<0.0001	79.4	75.3–83.4	
sex	male	1 (reference)				60.0	55.0–65.0	
	female	1.83	1.32–2.55	3,588	0.0003	70.7	67.5–73.9	
tested pen	pen I	1 (reference)				51.2	39.6–62.7	A
	pen II	5.75	2.69–12.6	4,454	<0.0001	80.6	71.8–89.3	B C
	pen III	10.16	5.60–19.00	7,456	<0.0001	86.9	82.4–91.4	C
	pen IV	1.17	0.52–2.61	0.391	0.6959	54.4	44.2–64.5	A
	pen V	2.57	1.21–5.38	2,478	0.0132	68.7	62.2–75.1	A B
	pen VI	1.27	0.63–2.54	0.661	0.5088	55.8	49.0–62.6	A

* 95% confidence interval (binomial exact).

Table 3
Serological tests agreement (n = 67).

RBT	LFA	i-ELISA	Number of animals
+	+	+	**22**
+	+	–	0
+	–	+	0
–	+	+	**1**
–	+	–	0
–	–	+	0
–	–	–	**44**
Total			67

Legend: RBT, Rose Bengal Test; LFA, lateral flow assay; and i-ELISA, indirect enzyme-linked immunosorbent assay.

4. Discussion

4.1. Screening tests and tests agreement

The disease has never been documented before in the SHO. The screening tests based on *B. abortus* antigens such as the ones used in this study cross-react with anti-*B. melitensis* immunoglobulins (Díaz-Aparicio et al., 1994; Blasco et al., 1994; OIE - World Organisation for Animal Health, 2018). However, brucellosis serological tests might have limited sensitivity (Se), with falsely negative reactions common in vertically or pseudo-vertically infected sexually immature females (Saegerman et al., 2010). For instance, RBT sensitivity (Se) in goats and sheep was 80.2 % and 82.8 % respectively (Rahman et al., 2013). On the other hand, false-positive reactions due to other Gram-negative bacteria have occurred, limiting the specificity (Sp) (Weynants et al., 1996; Saegerman et al., 2004). In *Brucella*-free goats, RBT Sp was 100 % (Blasco et al., 1994).

The results of both the multivariable logistic regression and Kappa coefficient tend to indicate that RBT and LFA are somewhat interchangeable and provide results that are not significantly different.

However, as indicated by the pairwise comparison on 67 serum samples, LFA detected one more positive sample than RBT which might translate into a slightly higher sensitivity of the LFA. The LFA was more expensive than RBT but carried the advantages of a pen-side test: in our experience, it provided a quicker result with positive results usually obtained within three to five minutes and while the animal was still being handled, allowing for immediate segregation of seropositive SHO.

Those practical and important observations might be of prime interest in further steps of a brucellosis control program in wild or wild-captive species. Importantly, LFA is not currently recommended test by the OIE (OIE - World Organisation for Animal Health, 2018).

The three serological tests results matched very well, even perfectly for the LFA and i-ELISA, but in the absence of the actual brucellosis sta-

tus for each individual, it is difficult to further evaluate the tests' parameters, notwithstanding that *B. melitensis* biovar 1 has been isolated from SHO in this setting (Lignereux et al., 2022).

The RBT detects both IgG and IgM, which can be detected first in seroconverting animals, while the protein G used in the i-ELISA will specifically bound IgG. A different testing panel with animals of different age or sex distribution might nevertheless provide a different agreement between tests and could be interesting to investigate further.

4.2. Seroprevalence and risk factors

Brucellosis is a debilitating disease. Favouring animals in good apparent condition over debilitated individuals for testing purposes might have introduced a possible selection bias likely to underestimate the brucellosis seroprevalence.

From our results, both sexes, all age categories and all tested enclosures were affected by brucellosis.

The 67.0 % individual seroprevalence observed is higher than the highest prevalence reported in wild animals in the literature. For instance, 36 % of Alpine ibex (*Capra ibex*) (ANSES, 2015) and 56 % of male bison (*Bison bison*) (Meyer and Meagher, 1995) were seropositive. A substantial difference is that, in this study, the SHO were not living in their natural environment. This high level of prevalence might stem from a recent introduction of the pathogen in a population previously naïve and could evolve towards *equilibrium* at a lower seroprevalence once the disease becomes enzootic and herd immunity is acquired. Nevertheless, such elevated prevalence suggests a high transmissibility of the pathogen, possibly due to a favourable combination of individual factors such as low genetic diversity (Biebach and Keller, 2010), species-specific characteristics (such as host susceptibility and behaviour) and husbandry practices (high animal density, absence of cleansing and animal waste removal including foetal membranes).

The effect of sex on *Brucella* seroprevalence has been observed on multiple occasions: in cattle (Awah-Ndukum et al., 2018; Assenga et al., 2015; Muma et al., 2006; Mai et al., 2012), goats (Solorio-Rivera et al., 2007) or bison (Meyer and Meagher, 1995). In agreement with other studies (ANSES, 2015; Tadesse, 2016), our results suggest that females were more affected than males, but the cause remains unknown. Perhaps it could be related to the longer lifespan of females or/and the dominance of certain males or/and due to the design of the survey with a prior selection of mostly females, younger and better-looking individuals.

As reported in other domestic species like cattle and small ruminants (Boukary et al., 2013), all studied age categories were exposed to brucellosis, and the level of exposure increased with age, possibly due to a repeated risk of becoming infected over time.

Because the two predictors ("testing campaign" and "tested pen") were dependent, the analysis was performed using one predictor or the other at a time. The risk of being seropositive fluctuated with the testing campaign: it was lower during the third campaign when it was expected to increase due to brucellosis biology. On the other hand, the analysis based on the tested enclosures provided a more likely scenario: it appeared that the highest level was found in two geographically close enclosures (Fig. 1, pen II and pen III) and that there may have been a centrifugal gradient of seroprevalence, with intermediate (pen V and pen VI) and lower (pen I and pen IV) levels observed further from the first two enclosures. All enclosures shared the same husbandry conditions, had similar animal composition and density, and the animals were of the same origin. No selection or testing was done before this study. It is important to note that not all enclosures containing *Brucella*-susceptible species were tested and the parameters of brucellosis transmission amongst other species – mainly the Indian blackbucks and the gazelles – remain unknown.

Nevertheless, the serological results suggest that animals from all tested pens were exposed. In this view and unless proven otherwise, it would be appropriate to consider all enclosures of this animal facility as infected. Whole-genome sequencing has provided fundamental insights for examining transmission dynamics of *B. abortus* in bison and elk (*Cervus canadensis*) in the Greater Yellowstone Ecosystem. Such genomic approaches, relying on the analysis of *B. abortus* strains isolated from different wildlife species, obtained during previous and contemporary outbreaks allowed specific epidemiological reconstructions of "who-infected-whom" (Kamath et al., 2016). In the studied population, *Brucella* was isolated only twice: from a SHO in an enclosure with the lowest seroprevalence (Fig. 1 pen VI) and a gazelle located 1300 m far away (Lignereux et al., 2022). In this context, a balanced sampling (i.e. proportional to disease prevalence) is important to make sound transmission inferences for infectious diseases in wildlife (Kamath et al., 2016).

A limited serological investigation in SBYI wildlife showed only one seropositive individual out of 50 in 2015. Yet, in the absence of an epidemiological inquiry and additional testing, it is not possible to conclude whether the single seropositive SHO found on the island was truly or falsely seropositive. We suggest further investigation of brucellosis on SBYI, including culture, molecular typing and phylogenetic analysis for comparison with known genotyping profiles (Kamath et al., 2016; Lignereux et al., 2022).

4.3. Limitations of the study

Our study was limited by the absence of a gold standard and the lack of systematic use of diagnostic tests (*Brucella*-specific PCR and culture/isolation/typing).

5. Conclusion

Constructing *B. melitensis* phylogenies and transmission events based on the analysis of *B. melitensis* isolated from wildlife and livestock (sheep, goat cattle, and camels) in the region will substantiate the claim that brucellosis is likely to have been introduced in the SHO after they have been translocated to the fenced facility. The SHO raised in high-density captive conditions might constitute a maintenance host for *B. melitensis*. However, what would happen under natural circumstances remains unknown.

Our results prompt the implementation of measures to control the infection in this threatened species. In our study, LFA showed a nearly 100 % agreement with the RBT and 100 % agreement with the i-ELISA. This suggests that LFA could be considered for recommendation by the OIE, after further validation.

Ethics statement

The animal collection health management provided the data presented in this study and this work was not performed primarily for research purposes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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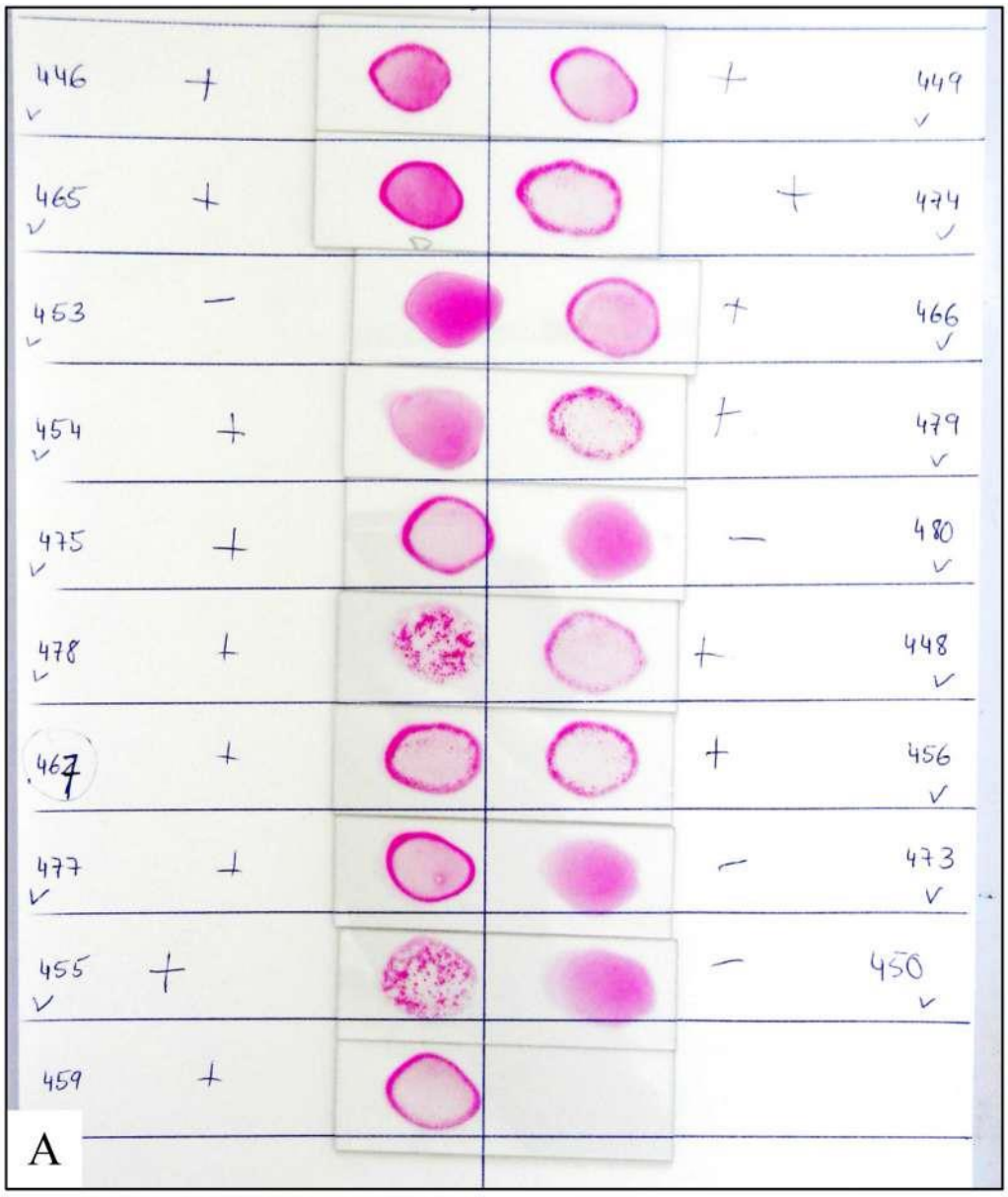
Appendix 1. Examples of brucellosis serological tests performed in scimitar-horned oryx

Picture A: examples of Rose Bengal Test. Picture B: examples of Bionote Anigen Rapid Bovine *Brucella* Ab Lateral Flow Assay (LFA).



References

Al Shehhi, N., Aziz, F., Al Hosani, F., Aden, B., Blair, I., 2016. Human brucellosis in the Emirate of Abu Dhabi, United Arab Emirates, 2010–2015. BMC Infect. Dis. 16. https://doi.org/10.1186/s12879-016-1900-9.

ANSES. 2015. *Mesures de maîtrise de la brucellose chez les bouquetins du Bargy* (No. 2014-SA-0218; Rapport d'expertise collective, p. 164). ANSES. https://www.anses.fr/en/system/files/SANT2014sa0218Ra.pdf.

Assenga, J.A., Matemba, L.E., Muller, S.K., Malakalinga, J.J., Kazwala, R.R., 2015. Epidemiology of Brucella infection in the human, livestock and wildlife interface in the Katavi-Rukwa ecosystem, Tanzania. BMC Vet. Res. 11. https://doi.org/10.1186/s12917-015-0504-8.

Awah-Ndukum, J., Mouiche, M.M.M., Bayang, H.N., Ngwa, V.N., Assana, E., Feussom, K. J.M., Manchang, T.K., Zoli, P.A., 2018. Seroprevalence and associated risk factors of Brucellosis among indigenous cattle in the Adamawa and North Regions of Cameroon. Vet. Med. Int.. https://doi.org/10.1155/2018/3468596.

Biebach, I., Keller, L.F., 2010. Inbreeding in reintroduced populations: The effects of early reintroduction history and contemporary processes. Conserv. Genet. 11 (2), 527–538. https://doi.org/10.1007/s10592-009-0019-6.

Blasco, J.M., Garin-Bastuji, B., Marin, C.M., Gerbier, G., Fanlo, J., Jiménez de Bagués, M. P., Cau, C., 1994. Efficacy of different Rose Bengal and complement fixation antigens for the diagnosis of *Brucella melitensis* infection in sheep and goats. Vet. Rec. 134 (16), 415–420. https://doi.org/10.1136/vr.134.16.415.

Boukary, A.R., Saegerman, C., Abatih, E., Fretin, D., Alambédji Bada, R., De Deken, R., Harouna, H.A., Yenikoye, A., Thys, E., 2013. Seroprevalence and potential risk factors for *Brucella* spp. infection in traditional cattle, sheep and goats reared in urban, periurban and rural areas of Niger. PLoS ONE 8 (12), e83175. https://doi.org/10.1371/journal.pone.0083175.

Chaber, A., Saegerman, C., 2017. Biosecurity measures applied in the United Arab Emirates–a comparative study between livestock and wildlife sectors. Transbound. Emerg. Dis. 64 (4), 1184–1190. https://doi.org/10.1111/tbed.12488.

Díaz-Aparicio, E., Marín, C., Alonso-Urmeneta, B., Aragón, V., Pérez-Ortiz, S., Pardo, M., Blasco, J.M., Díaz, R., Moriyón, I., 1994. Evaluation of serological tests for diagnosis of Brucella melitensis infection of goats. J. Clin. Microbiol. 32 (5), 1159–1165. https://doi.org/10.1128/jcm.32.5.1159-1165.1994.

Hull, N.C., Schumaker, B.A., 2018. Comparisons of brucellosis between human and veterinary medicine. Infect. Ecol. Epidemiol. 8 (1). https://doi.org/10.1080/20008686.2018.1500846.

Iucn, 2015. IUCN Red List of Threatened species. IUCN http://www.iucnredlist.org/details/15568/0.

Jakob-Hoff, R.M., Kock, R., Lees, C., MacDiarmid, S.C., Miller, P.S., Travis, D., 2014. Manual of procedures for wildlife disease risk analysis. World Organization for Animal Health (OIE) Published in Association with the International Union for Conservation of Nature (IUCN) and the Species Survival Commission (SSC).

Kamath, P.L., Foster, J.T., Drees, K.P., Luikart, G., Quance, C., Anderson, N.J., Clarke, P. R., Cole, E.K., Drew, M.L., Edwards, W.H., Rhyan, J.C., Treanor, J.J., Wallen, R.L., White, P.J., Robbe-Austerman, S., Cross, P.C., 2016. Genomics reveals historic and contemporary transmission dynamics of a bacterial disease among wildlife and livestock. Nat. Commun. 7 (1), 11448. https://doi.org/10.1038/ncomms11448.

Kock, R., Soorae, P., Mohammed, O., 2007. Role of veterinarians in re-introductions. Int. Zoo Yearbook 41 (1), 24–37. https://doi.org/10.1111/j.1748-1090.2007.00013.x.

Lignereux, L., Chaber, A.-L., Saegerman, C., Heath, L., Knowles, N.J., Wadsworth, J., Mioulet, V., King, D.P., 2020. Foot-and-mouth disease outbreaks in captive scimitar-horned oryx (*Oryx dammah*). Transbound. Emerg. Dis.. https://doi.org/10.1111/tbed.13502.

Lignereux, L., Chaber, A.-L., Fretin, D., Godfroid, J., & Saegerman, C. (2022). *Brucella melitensis* biovar 1 isolation in a captive wildlife population in the United Arab Emirates. First isolation in the Scimitar-horned Oryx (*Oryx dammah*). *Vet. Microbiol.* https://doi.org/10.1016/j.vetmic.2022.109360.

Mai, H.M., Irons, P.C., Kabir, J., Thompson, P.N., 2012. A large seroprevalence survey of brucellosis in cattle herds under diverse production systems in northern Nigeria. BMC Vet. Res. 8 (1), 144. https://doi.org/10.1186/1746-6148-8-144.

Meyer, M.E., Meagher, M., 1995. Brucellosis in free-ranging bison (*Bison bison*) in Yellowstone, Grand Teton, and Wood Buffalo National Parks: A review. J. Wildl. Dis. 31 (4), 579–598. https://doi.org/10.7589/0090-3558-31.4.579.

Muma, J.B., Samui, K.L., Siamudaala, V.M., Oloya, J., Matope, G., Omer, M.K., Munyeme, M., Mubita, C., Skjerve, E., 2006. Prevalence of antibodies to *Brucella* spp. and individual risk factors of infection in traditional cattle, goats and sheep reared in livestock–wildlife interface areas of Zambia. Trop. Anim. Health Prod. 38 (3), 195–206. https://doi.org/10.1007/s11250-006-4320-9.

Ogden, R., Chuven, J., Gilbert, T., Hosking, C., Gharbi, K., Craig, M., Al Dhaheri, S.S., Senn, H., 2020. Benefits and pitfalls of captive conservation genetic management: Evaluating diversity in scimitar-horned oryx to support reintroduction planning. Biol. Conserv. 241, 108244. https://doi.org/10.1016/j.biocon.2019.108244.

OIE - World Organisation for Animal Health (Ed.). (2018). Chapter 3.1.4. Brucellosis (infection with *Brucella abortus*, *B. melitensis* and *B. suis*). In: *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (8th edition, Vol. 1–3, pp. 355–398). OIE.

Petrie, A., Watson, P., 2013. Statistics for Veterinary and Animal Science. Wiley-Blackwell Publishing.

Rahman, A.K.M.A., Dirk, B., Fretin, D., Saegerman, C., Ahmed, M.U., Muhammad, N., Hossain, A., Abatih, E., 2012. Seroprevalence and risk factors for brucellosis in a high-risk group of individuals in Bangladesh. Foodborne Pathog. Dis. 9 (3), 190–197. https://doi.org/10.1089/fpd.2011.1029.

Rahman, A.K.M.A., Saegerman, C., Berkvens, D., Fretin, D., Gani, M.O., Ershaduzzaman, M.d., Ahmed, M.U., Emmanuel, A., 2013. Bayesian estimation of true prevalence, sensitivity and specificity of indirect ELISA, Rose Bengal Test and Slow Agglutination Test for the diagnosis of brucellosis in sheep and goats in Bangladesh. Prev. Vet. Med. 110 (2), 242–252. https://doi.org/10.1016/j.prevetmed.2012.11.029.

Saegerman, C., De Waele, L., Gilson, D., Godfroid, J., Thiange, P., Michel, P., Limbourg, B., Vo, T.-K.-O., Limet, J., Letesson, J.-J., Berkvens, D., 2004. Evaluation of three serum i-ELISAs using monoclonal antibodies and protein G as peroxidase conjugate for the diagnosis of bovine brucellosis. Vet. Microbiol. 100 (1–2), 91–105. https://doi.org/10.1016/j.vetmic.2004.02.003.

Saegerman, C., Berkvens, D., Godfroid, J., Walravens, K., 2010. Chapter 77: Bovine brucellosis. In: Lefevre Charles, P., Blancou, J., Chermette, R., Uilenberg, G. (Eds.), Infectious and Parasitic Diseases of Livestock. 1st ed. Lavoisier, pp. 991–1011.

Solorio-Rivera, J.L., Segura-Correa, J.C., Sánchez-Gil, L.G., 2007. Seroprevalence of and risk factors for brucellosis of goats in herds of Michoacan, Mexico. Prev. Vet. Med. 82 (3–4), 282–290. https://doi.org/10.1016/j.prevetmed.2007.05.024.

Tadesse, G., 2016. Brucellosis seropositivity in animals and humans in Ethiopia: A meta-analysis. PLoS Negl.Trop. Dis. 10 (10), e0005006. https://doi.org/10.1371/journal.pntd.0005006.

Weynants, V., Gilson, D., Cloeckaert, A., Denoel, P.A., Tibor, A., Thiange, P., Limet, J.N., Letesson, J.J., 1996. Characterization of a monoclonal antibody specific for Brucella smooth lipopolysaccharide and development of a competitive enzyme-linked immunosorbent assay to improve the serological diagnosis of brucellosis. Clin. Diagn. Lab. Immunol. 3 (3), 309–314. https://doi.org/10.1128/cdli.3.3.309-314.1996.

Young, E.J., 1995. An overview of human brucellosis. Clin. Infect. Dis. 21 (2), 283–290. https://doi.org/10.1093/clinids/21.2.283.

3.3. Risk of release of a disease impacting the local economy. The case of foot-and-mouth disease

Preamble

FMD is caused by a single-strand RNA virus belonging to *Aphthovirus* genus within the family *Picornaviridae*. Seven antigenically distinct serotypes occur worldwide, namely O, A, C, Asia-1, Southern Africa Territories (SAT) 1, 2 and 3 (Jamal & Belsham, 2018).

The disease is highly contagious among cloven-hoofed animals and spreads easily through direct contact, contaminated feed or equipment, and long-distance airborne transmission (Alexandersen et al., 2003).

The different serotypes, but also topotypes and strains may have varying levels of virulence and pose different risks to susceptible species, and while the disease is generally not lethal, fatal cases have been observed in gazelle species (Shimshony et al., 1986). However, clinical signs are generally limited to fever, lameness, reduced milk production in females, and painful blisters or vesicular lesions on the hooves, mouth, and teats (Alexandersen et al., 2003).

Its economic impact on the livestock industry is significant, due to reduced productivity, the cost of disease control measures and more importantly, trade restrictions (Belsham et al., 2020).

Apart from countries that have heavily invested in costly control and eradication programs, the disease is present globally. The geographical distribution of the seven serotypes is nevertheless not uniform and seven virus pools (1-7) of locally circulating serotypes have been described (Brito et al., 2017). Serotypes O, A and C were historically the most distributed. While SAT and Asia-1 serotypes are restricted to sub-Saharan Africa and Asia. Serotype C might have disappeared (Jamal & Belsham, 2018).

FMD control programs involve mass vaccination targeting locally circulating serotypes. Because cross-immunity between serotypes does not exist, the vaccination against or the infection by one serotype does not confer protection against the other serotypes (Jamal & Belsham, 2018). It is therefore crucial to prevent the emergence of alien serotypes.

The source population of SHO has been affected by three outbreaks of FMD between 2013 and 2015. In **Article 3**, we depict the clinical signs that SHO showed during the infection, and, in an attempt to understand whether the virus maintained itself in the population or emerged several times, we present the results of the molecular epidemiology investigations through phylogenetic analysis.

ARTICLE 3

Foot-and-mouth disease outbreaks in captive scimitar-horned oryx (*Oryx dammah*)

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Lignereux Louis, Chaber Anne-Lise, Saegerman Claude, Heath Livio, Knowles Nick J., Wadsworth Jemma, Mioulet Valérie and Donald P. King

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SHORT COMMUNICATION

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Foot-and-mouth disease outbreaks in captive scimitar-horned oryx (*Oryx dammah*)

Louis Lignereux[1,2] | Anne-Lise Chaber[1,2] | Claude Saegerman[1] | Livio Heath[3] | Nick J. Knowles[4] | Jemma Wadsworth[4] | Valérie Mioulet[4] | Donald P. King[4]

[1]Faculty of Veterinary Medicine, Research Unit for Epidemiology and Risk Analysis Applied to Veterinary Sciences (UREAR-ULiège), Centre of Fundamental and Applied Research for Animals and Health (FARAH), University of Liège, Liège, Belgium

[2]School of Animal and Veterinary Sciences, University of Adelaide, Adelaide, SA, Australia

[3]Transboundary Animal Disease Program, Onderstepoort Veterinary Institute, Pretoria, South Africa

[4]FAO World Reference Laboratory for Foot-and-Mouth Disease, The Pirbright Institute, Pirbright, UK

Correspondence
Louis Lignereux, Leske building, School of Animal and Veterinary Sciences, University of Adelaide, Roseworthy 5371, South Australia.
Email: l_louis77@hotmail.com

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Abstract

This paper describes three episodes of foot-and-mouth disease (FMD) that were detected during 2013–2015 in scimitar-horned oryx (*Oryx dammah*) (SHO), a large Sahelo-Saharan antelope extinct in the wild housed in a wild ungulate breeding facility located 50 km east of Abu Dhabi, United Arab Emirates. While no mortality attributable to FMD was noted in the population of nearly 4,000 SHO during two of the three outbreaks, the morbidity varied according to the circulating strains and seroconversion reached a plateau of 78.0% within two weeks and remained at this level for at least nine months. Partial or complete sequencing of the VP1 encoding region demonstrated that the three outbreaks were caused by three different FMDV lineages (O/ME-SA/PanAsia-2, A/ASIA/Iran-05 and O/ME-SA/Ind-2001), consistent with FMD viruses that are circulating elsewhere in the region. These findings demonstrate that SHO are susceptible to FMD and highlight the risks of virus incursion into zoos and captive facilities in the Arabian Peninsula.

KEYWORDS

epidemiology, foot-and-mouth disease, Scimitar-horned Oryx (*Oryx dammah*), United Arab Emirates

1 | INTRODUCTION

The scimitar-horned oryx (SHO) (*Oryx dammah*) is a large antelope that along with three other species belongs to the *Oryx* genus within the Hippotraginae subfamily. The SHO had a distribution range across the Sahelian countries, from Mauritania to the Nile river in Egypt and Sudan, and it has been suggested that its population reached one million in the early Holocene period (9500–4500 BC) (Iyengar et al., 2007). Despite being previously widely distributed in large numbers, the twentieth century brought the species to extinction in the wild due to a combination of rangeland degradation, competition with livestock, uncontrolled hunting and civil unrest. Today, survival of this species relies on captive breeding (East, 1999) and the entire global population is estimated between 15,000 and 19,000 head (Woodfine & Gilbert, 2016), distributed in 444 institutions across 48 countries. Trophy hunting ranches in Texas, United States of America (USA) account for about 11,000 individuals and the United Arab Emirates (UAE) for another 4,000. To counter the disappearance of this species from the wild, conservation organizations have initiated several reintroduction projects and the most important of these in terms of number of reintroduced animals is the one currently underway between the UAE and Chad (Newby, 2016).

Foot-and-mouth disease (FMD) is a highly contagious viral disease affecting all cloven-hoofed animals that is caused by a member of the *Aphthovirus* genus belonging to the *Picornaviridae* family. The virus has a positive-sense single-stranded RNA genome (Flather &

	Dates	Pens	FMDV lineage	Percentage of SHO presenting FMD lesions
Outbreak 1	Jan 13	B1 and A	O/ME-SA/PanAsia-2^{ANT-10}	0.6% (0.1%*)
Outbreak 2	Dec 13	B1	A/ASIA/Iran-05^{FAR-11}	0.2%*
Outbreak 3	Mar 15	C and D	O/ME-SA/Ind-2001d	28.5%

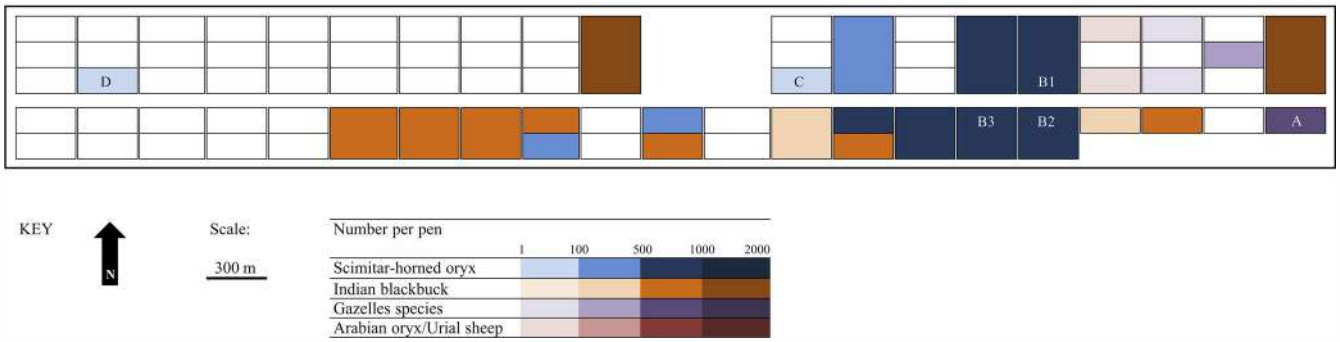

FIGURE 1 Outbreak chronology and schematic representation of the compound. A simplified layout of the animal facility: black lines represent single mesh fence. Empty pens as well as access corridors and perimeter buffer zone appear in white while Pens with scimitar-horned oryx (SHO) appear in blue, Indian blackbucks in orange, gazelles species in purple, Arabian oryx and Urial sheep in pink. Darker colour tones represent pens with larger numbers of animals. For clarity, pen subdivisions are not shown. Pens discussed in this paper as well as locations of FMD outbreaks, dates and isolated FMDV are shown. For each outbreak, the percentage of SHO presenting FMD characteristic lesions was calculated amongst the SHO that underwent clinical examination and/or amongst the SHO that were only visually assessed (*) [Colour figure can be viewed at wileyonlinelibrary.com]

Semler, 2015) that is encapsidated within an icosahedral shell comprising four structural proteins (SP) called VP1, 2, 3 and 4. VP1 is responsible for virus attachment and entry, and also contains many of the determinants that confer protective immunity and serotype specificity (Carrillo et al., 2005). The coding sequence of VP1 (1D) is frequently targeted for sequencing for virus typing and tracing. Seven virus pools (1–7) have been proposed to define the geographical circulation of the seven immunologically distinct FMD virus (FMDV) serotypes: O, A, C, Asia 1, Southern African Territories (SAT) 1, SAT 2 and SAT 3 along with their topotypes, genetic lineages and strains. The Arabian Peninsula (including the UAE) located in Pool 3 is home to regional serotype O, A and Asia 1 lineages (O/ME-SA/PanAsia-2, A/ASIA/Iran-05 and Asia-1/Sindh-08 (Brito, Rodriguez, Hammond, Pinto, & Perez, 2017; Knowles et al., 2009) and has also recently experienced incursions of viral lineages (O/ME-SA/Ind-2001 and A/ASIA/G-VII) from Pool 2 (South Asia) (Bachanek-Bankowska, Di Nardo, Wadsworth, Henry, et al., 2018; Bachanek-Bankowska, Di Nardo, Wadsworth, Mioulet, et al., 2018).

This study describes FMD cases that occurred in high-density captive-bred SHO in the UAE, a country where small ruminants predominate: 1,850,462 sheep and 2,082,926 goats were registered in 2014 compared with 50,103 head of cattle (Ministry of Environment & Water, 2015). This animal collection was located inland in close proximity to an intercity highway and a truck road, 45 km east of Abu Dhabi (United Arab Emirates) and more than 100 km from the borders with neighbouring countries. A separate complex with hundreds of 'ezbas', the local traditional farms (Chaber & Saegerman, 2017) was situated 3,900 m to the east. The facility measured over 6,000 m long by 750 m wide. It could be represented as an elongated chessboard (Figure 1) and comprised more than 50 pens ranging in size from 150 × 150 m to 300 × 450 m. Animals were not present in all pens and an aerial animal-registration survey undertaken at the end of November 2012 indicated there were 7,931 Indian blackbucks (*Antelope cervicapra*) in 13 pens, 3,894 SHO in 11 pens, 1,300 reem gazelles (*Gazella marica*) in four pens, 258 mountain (*Gazella gazella*) and Indian (*Gazella bennetti*) gazelles in five pens, 11 urial sheep (*Ovis orientalis*) in one pen and four Arabian oryx (*Oryx leucoryx*) in one pen. Species were kept separate, but pens contained animals of both sexes and all age categories (apart from one pen with four male Arabian oryx and one pen with 246 male SHO).

Animals displaying only deciduous teeth were considered as juveniles, while those with one or two pairs of adult incisors were categorized as subadults and three or four pairs were defined as adults. Based on those criteria, the subadult category would be estimated between approximately 19 and 27 months old in the closely related Arabian oryx (Ancrenaz & Delhomme, 1997).

There was no history of FMDV vaccination or disease screening for FMD. Typically, no animals went out of the collection but large numbers (hundreds) of sand gazelles were moved in during 2012 and 2013. FMD cases occurred in three distinct episodes (outbreaks) in pens A, B1, 2 and 3, C and D. Pen A was located at one end of the facility, pens B1, 2 and 3, and C were in the middle while pen D was well separated from the high-density pens and located at the other end. In early 2013, a selection process based on morphological criteria and on infectious disease status was initiated to create a breeding herd of SHO that were more intensively managed. This work led to the visual assessment of all 1,952 SHO present in pens B1, B2 and B3. Subsequently between 1 January and 19 March 2013, 351 of these animals were selected and captured; each received an individually numbered ear tag and

a succinct clinical examination including the eyes and oral cavity was performed. Abnormal findings or clinical signs were recorded, and blood was collected from the jugular vein for serological analyses. Sixty-seven passed this first screening test and were moved into pen C. On 5 November 2013, these SHO were blood sampled again and were all re-identified with a subcutaneous microchip because some animals had lost their ear tag, after which they were all moved to pen D, leaving pen C empty. In 2014, the process was repeated in other pens containing SHO resulting in the movement of 41 new SHO into pen C.

Outbreak 1: During the examination that was ongoing between January and March 2013, two juvenile SHO were identified that exhibited lesions compatible with FMD: a 6-month-old male had a 6 mm ulcer on the gum of the upper lip on 28 January 2013 and 8-month-old male displayed ulcers on gum and coronary band the following day. Swab samples from both animals were collected for virology testing. Five days later, on 2 February 2013, four sand gazelles died in pen A, 1,100 m away from pen B1. These animals presented with ulcers in the oral cavity, on the gum or on the tongue (Figure 3). The mortality in that pen during the first quarter of 2013 was very high, with deaths accounting for 663/1095 gazelles (60.7% mortality rate), approximately 10 times the mortality rate observed in a sand gazelle population housed in similar conditions but located in a separate facility that was not affected by FMD (approximately 6% over three months) (Lignereux, Chaber, et al., 2018). It is not possible to determine if FMD accounted for all the recorded mortality, although we speculate it might have played an important role.

After their recapture in pen C in November, the sera from the 67 SHO that met the selection criteria were analysed together with the sera collected from those same animals during Outbreak 1 in pens B1, 2 and 3. Three serum samples collected early 2013 were not available for these analyses; thus 131 sera were analysed using a commercial indirect (blocking) enzyme-linked immunosorbent assay (ELISA) kit 'FMD 3ABC bo-ov' (Idexx, USA; formerly known as 'Chekit-FMD-3ABC' (Bommeli AG, Switzerland) which specifically measures antibodies directed at FMDV non-structural proteins (NSP). The test was performed and interpreted according to the manufacturer's recommendations; however, since this test has not been validated for the SHO species, results should be interpreted with caution. The results shown in Figure 2 demonstrate that all 17 SHO tested until 21 January 2013 were FMDV NSP seronegative, and the first seropositive case appeared on 28 January 2013. Seroprevalence plateaued at 78.0% amongst the 41 SHO tested (32 positive) between 12 February and 19 March 2013. These results are compatible with an introduction of FMDV during the last week of January 2013 and rapid transmission of the virus within the collection due to its high contagiousness which match the observed clinical cases. On 5 November 2013, the seroprevalence was 77.6%, 95% CI [65.8–86.9] (exact binomial distribution) amongst the 67 SHO tested (52 positive, six doubtful, nine negative).

Outbreak 2: On 16 December 2013 a dead juvenile SHO was found in pen B1 amongst approximately 490 SHO. This animal had gum and coronary band ulcerations that were swabbed for testing (Figure 3).

Outbreak 3: On 2 March 2015, 26 out of the 41 SHO in pen C (63.4%) presented with lesions characteristic of FMD infection (Figure 4). The affected animals were predominantly subadults (17 out of 28) but cases also included adults (nine out of 13). Out of 58 lesions seen, the gum was the anatomical region affected the most (39.7% of 58), followed by the dental pad (25.9%), the coronary band (13.8%), the nostril (12.1%), the tongue (6.9%) and the base of the horn (1.7%). In the following 2 weeks, on 9 March and 16 March

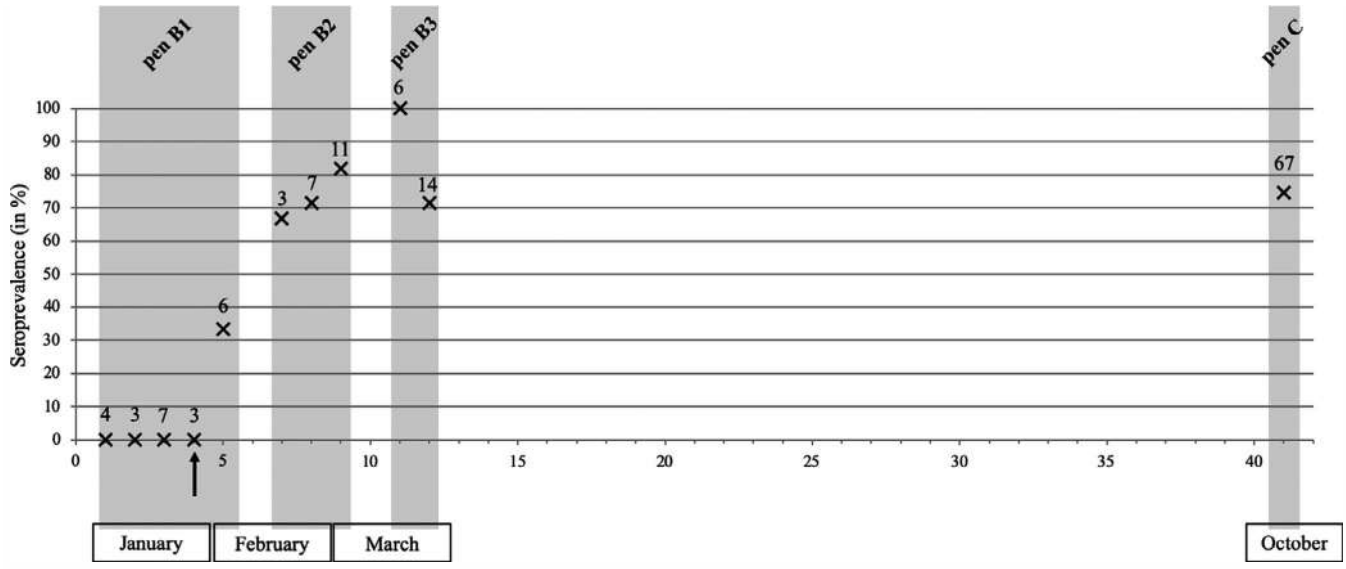

FIGURE 2 FMD seroprevalence in scimitar-horned oryx (SHO) in 2013. Each marker represents the percentage of seropositive results to FMD non-structural protein 3ABC ELISA in SHO tested the same week. The x-axis represents the time, in week, from January to November 2013. The pens where the SHO were blood sampled are indicated by the grey rectangles. The number of SHO tested is given above each marker

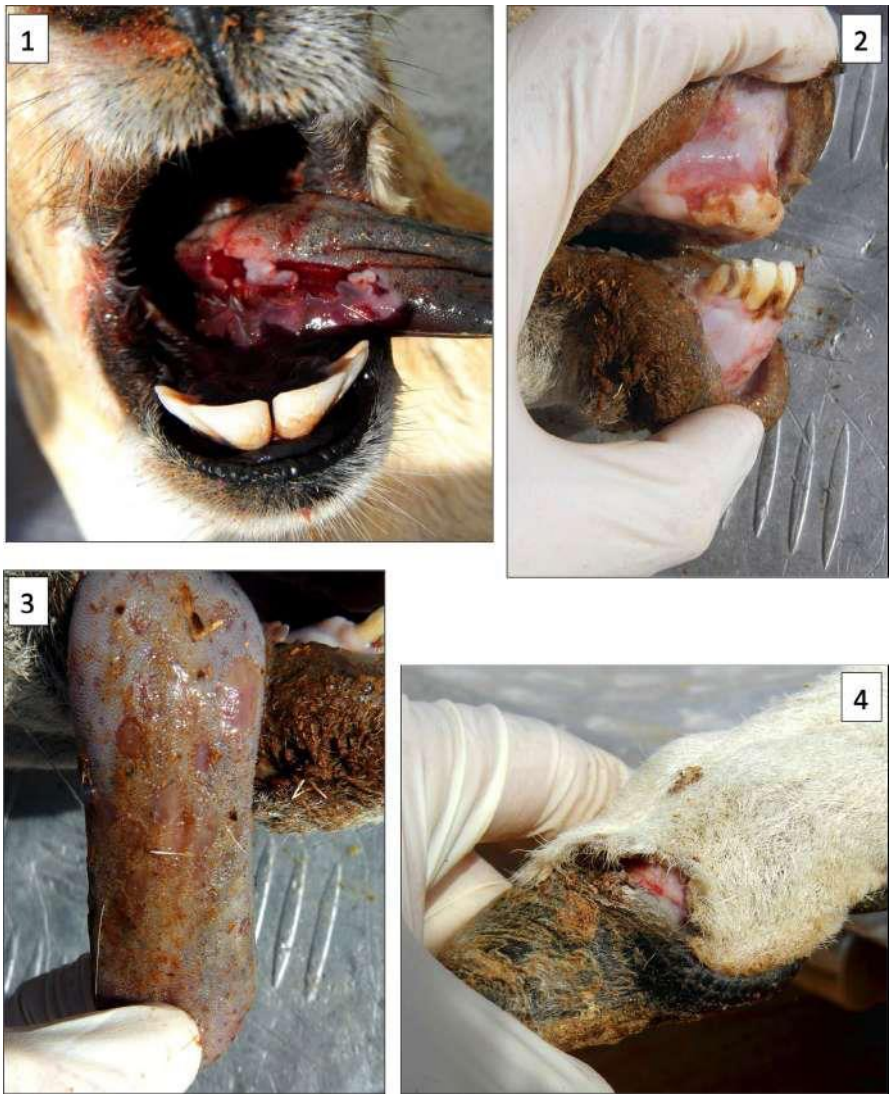

FIGURE 3 FMD gross lesions observed during Outbreaks 1 and 2. Picture 1: mucosal ulceration of the tongue observed on a sand gazelle where O/UAE/2/2013 was collected; Picture 2: gingival mucosal ulceration observed on the gum and dental pad of a scimitar-horned oryx (SHO) where A/UAE/1/2013 was collected; Picture 3: perioplic ulceration and Picture 4: circular mucosal erosions of the tongue of the same SHO [Colour figure can be viewed at wileyonlinelibrary.com]

2015, 12 out of the 89 SHO (13.4%) in pen D (formerly in pen C and affected by Outbreak 1) displayed characteristic FMD lesions: cases included two adults out of 54, one subadult out of 17, nine juveniles out of 18. All animals were in good body condition and their feed intake, while not measured, was considered normal at the time. Other parameters, such as fertility, were not assessed.

The tissue collected after swabbing the oral lesions was kept frozen at −20°C in Universal Viral Transport (Beckton and Dickinson, USA) until laboratory analysis could be carried out. Two oral swabs (one from a SHO, one from a sand gazelle) were submitted for Outbreak 1 and following confirmation of the pathogen at WRLFMD (Pirbright, UK) by virus isolation, antigen-detection ELISA and real-time RT-PCR, the VP1 coding region was amplified by RT-PCR and sequenced as previously described (Knowles, Wadsworth, Bachanek-Bankowska, & King, 2016). FMDV was also confirmed with virus isolation and real-time RT-PCR for Outbreaks 2 and 3 at the Onderstepoort Veterinary Research (Pretoria, South Africa) upon receiving four oral swabs, and viral RNA was reverse-transcribed using AMV-Reverse Transcriptase (Promega, USA) and the partial VP1 gene region was amplified to obtain DNA for sequencing using Go-taq (Promega, USA) combined with the WDA (Beck & Strohmaier, 1987) and VP1O (Rodriguez et al., 1994) Type O-specific or the NK61/A-1C$_{562}$ (Knowles and Samuel, 1998) Type A-specific oligonucleotide sets, respectively.

Sequences were aligned using BioEdit v7.2.5 (Hall, 1999). Maximum-likelihood phylogenetic trees were generated using MEGA7 (Kumar, Stecher, & Tamura, 2016) and were based on the best nucleotide substitution model as implemented in the programme. The Kimura 2-parameter (type O) and Hasegawa-Kishino-Yano (type A) models were chosen. In order to establish the parameters for phylogenetic analyses, a discrete gamma distribution was used to model evolutionary rate differences amongst sites [five categories (+G)] and in the case of the type A tree there was allowance for some sites to be evolutionarily invariable (+I). About 1,000 bootstrap replicates (Felsenstein, 1985) were used to assess branching reliability (only values to 70% and above are shown).

FMD viruses recovered from two samples collected from Outbreak 1 (UAE/1/2013 and UAE/2/2013, GenBank accession

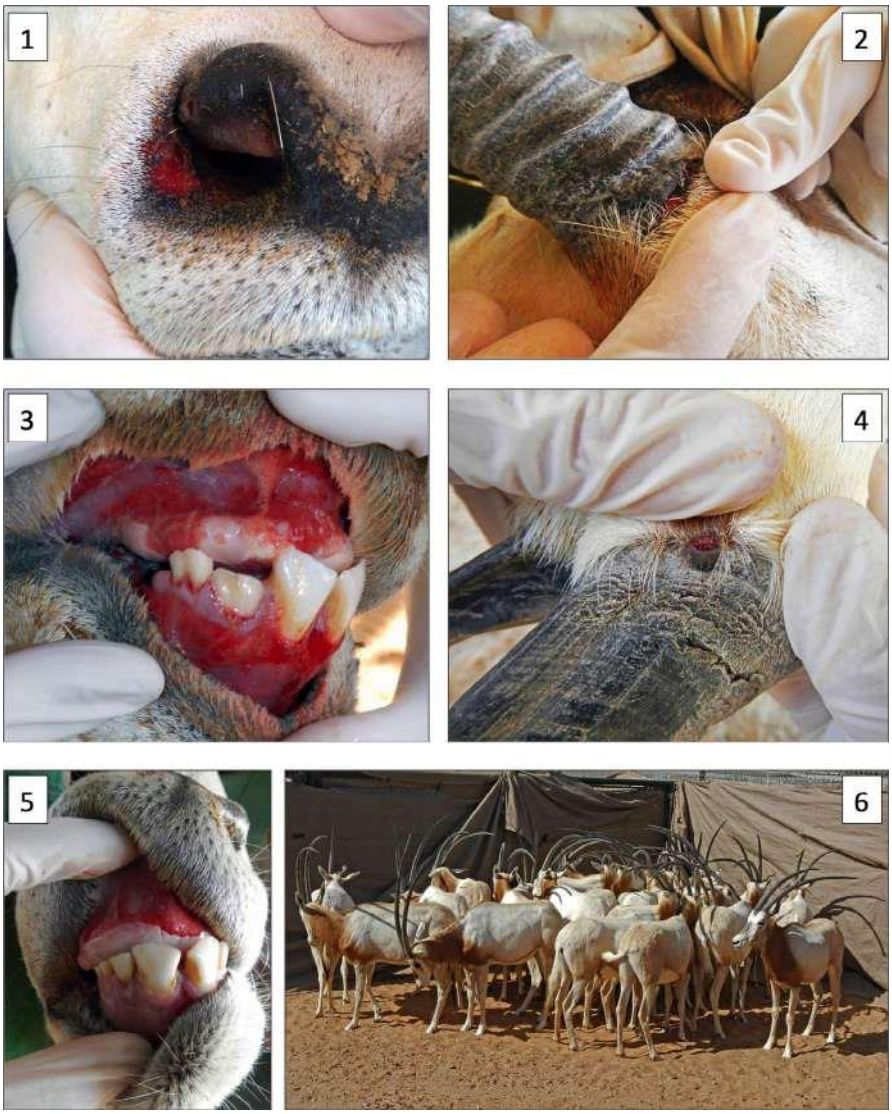

FIGURE 4 External gross lesions observed in a group of scimitar-horned oryx (SHO) during Outbreak 3. Picture 1: mucosal ulceration in a nostril; Picture 2: cutaneous ulceration at the base of a horn; Pictures 3 and 5: gingival mucosal ulceration of the dental pad and the gum; Picture 4: perioplic ulceration; Picture 6: the group of young SHO where the outbreak was described, showing their apparent external good condition [Colour figure can be viewed at wileyonlinelibrary.com]

number MN276040 and MN276041) were characterized as belonging to the O/ME-SA/PanAsia-2^{ANT-10} sub-lineage (Figure 5), sharing closest nucleotide identity (98.8%) with an FMDV isolate collected from Iran (O/IRN/13/2012). The FMDV responsible for Outbreak 2 in December 2013 was a serotype A belonging to the A/ASIA/Iran-05^{FAR-11} sub-lineage (GenBank accession number MN276043) (Figure 6), while partial VP1 sequences (304 nucleotides) recovered from Outbreak 3 were characterized as belonging to the O/ME-SA/Ind-2001d lineage (Figure 5). The sequence for the Outbreak 3 virus (GenBank accession number MN276042) was identical to O/UAE/1/2014 and O/UAE/2/2014, GenBank accession number KM921877 and KM921878, respectively, collected on 8 January 2014 on gazelles in captivity, 40 km north from the collection studied here and is representative of a FMDV lineage that has been recently introduced into the Arabian Peninsula on multiple occasions (Bachanek-Bankowska, Di Nardo, Wadsworth, Mioulet, et al., 2018).

During the 3-year span of this study, no case fatality or clinical signs that could have been attributed to FMD were recorded in the approximately 8,000 Indian blackbucks even in pens contiguous to SHO affected by FMD. This observation contrasts somewhat with the high mortality following an FMD type O outbreak described by Kar, Hota, and Acharjyo (1983) and relayed by Thomson, Vosloo, and Bastos (2003) and Weaver, Domenech, Thiermann, and Karesh (2013). The high mortality observed in the gazelle population, while speculated and not well recorded in our case is more in agreement with other published data (Bailey, O'Donovan, Kinne, & Wernery, 2009; Shimshony et al., 1986). The number of individuals in the collection belonging to other ruminant species is too low to draw conclusions about their epidemiological role. The Arabian oryx was previously documented as being a spill-over following O serotype outbreak (Ostrowski & Anajariyah, 2003) in (Frölich et al., 2005), and high mortality and morbidity due to FMD were recorded in this species (Lignereux, Alzahlawi, Al Kharusi, & Pesci, 2018).

The three FMD outbreaks that are recorded in this study demonstrate that SHO are susceptible to infection with FMDV and may exhibit variable clinical expression depending on the lineage of FMDV. The sequences represent three viral lineages (from two serotypes) currently circulating in Pool 3 where FMD is endemic. Long-distance

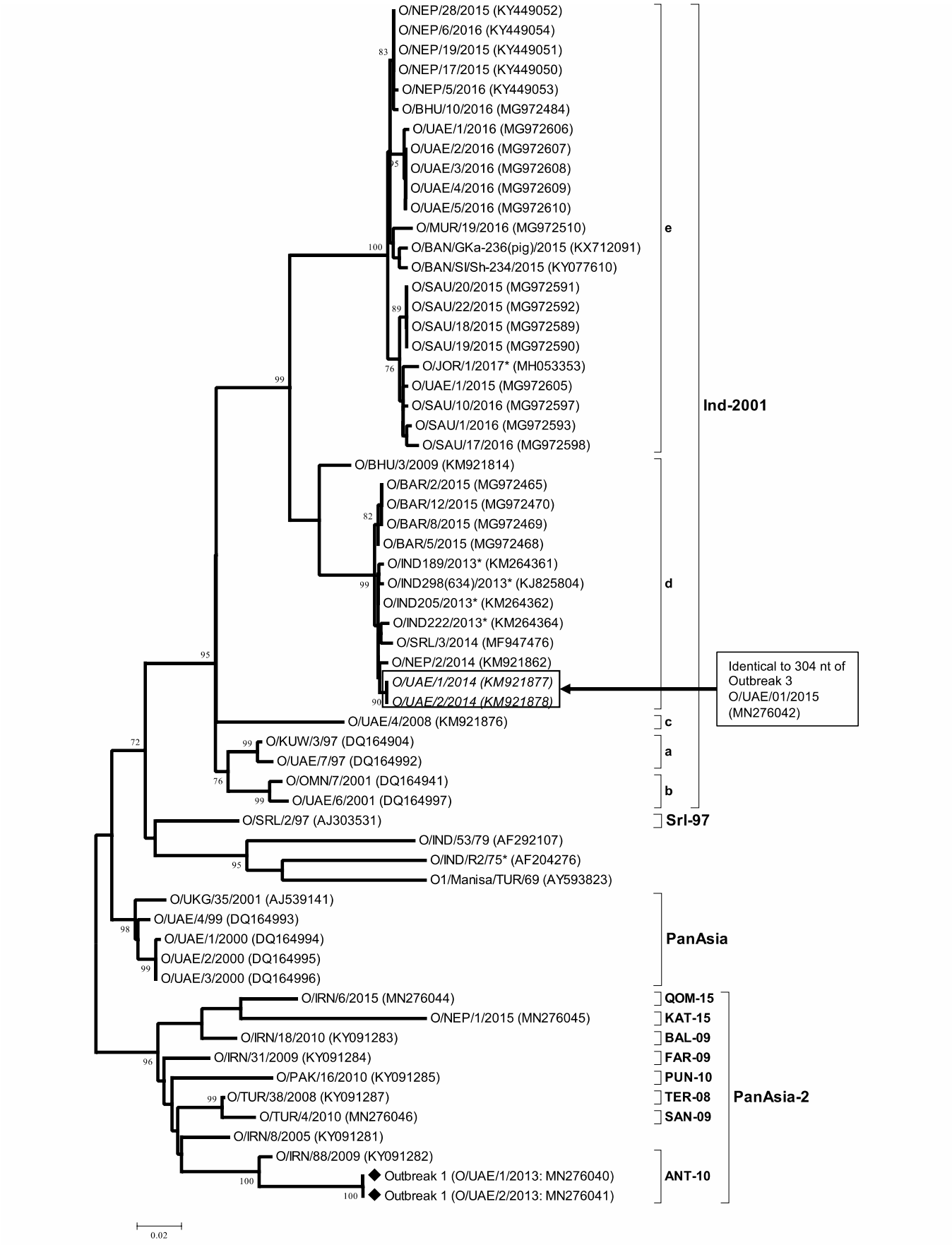

FIGURE 5 Phylogenetic tree based on viral VP1 sequences showing the relationship between the serotype O sequences recovered from Outbreak 1 and Outbreak 3 (shown with a diamond symbol [♦]) * denotes FMD virus sequences that are not WRLFMD codes

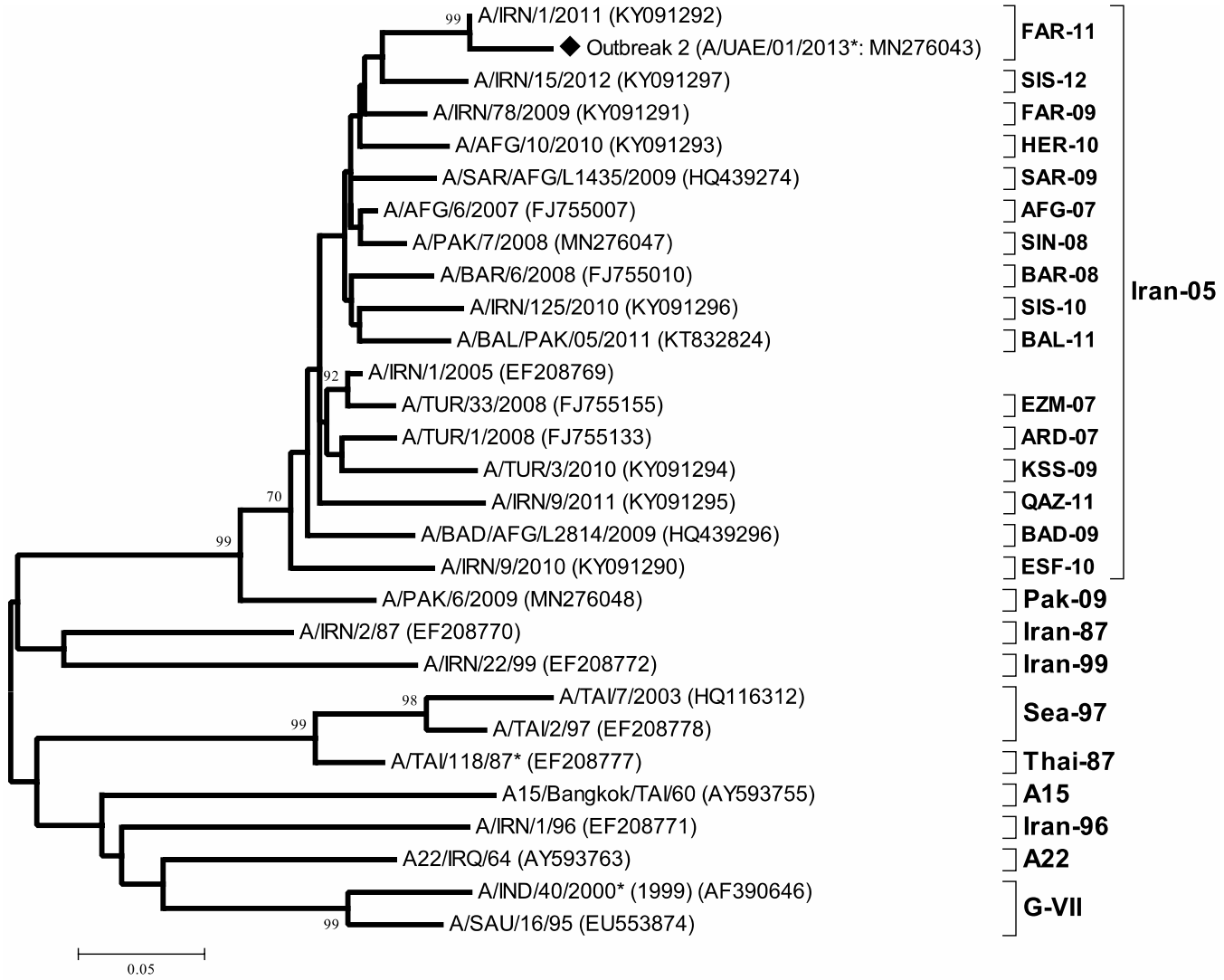

FIGURE 6 Phylogenetic tree based on viral VP1 sequences showing the relationship between the serotype A sequences recovered from Outbreak 2 (shown with a diamond symbol [◆]). * denotes FMD virus sequences that are not WRLFMD codes

FMD transmission has been documented (Gloster, Sellers, & Donaldson, 1982) but vicinity to roadways, where livestock transit and to traditional farms compounds could be seen as potential risk factors for FMD infection for wildlife collection. However, the precise transmission routes by which these viruses have entered the facility are not known. Further studies should focus on evaluating the cost-benefit ratio of vaccination programmes in those wildlife collections as well as systematic investigation of the unlikely maintenance host status of the affected species. Within the UAE, FMD outbreaks in wildlife collections are frequently reported, and results from this study indicate that it would be beneficial to integrate the large wildlife collections present in the UAE as sentinels to provide valuable epidemiological data in the framework of an FMD control programme.

How these different FMD viruses entered and spread unnotified on at least three occasions remains unanswered. The importance of contributing factors that might help explain these patterns is not well understood, such as the presence of large numbers of small ruminants, known to harbour and spread FMDV with mild to inapparent clinical signs (Geering, 1967; Hughes et al., 2002; Stenfeldt et al., 2015) as well as connections to numerous traditional farms in the region, possibly with poor biosecurity (Chaber & Saegerman, 2017) and inadequate FMD vaccination coverage. In light of these gaps, we recommend that efforts are made to identify and quantify the risk factors for FMD importation to, and transmission within the UAE.

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CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

ETHICAL APPROVAL

Ethical statement is not applicable to this study as the data were gathered through the everyday animal handling for population management purposes, as part of an ex situ conservation project.

ORCID

Louis Lignereux https://orcid.org/0000-0001-6493-9226
Anne-Lise Chaber https://orcid.org/0000-0001-9042-8753
Claude Saegerman https://orcid.org/0000-0001-9087-7436
Nick J. Knowles https://orcid.org/0000-0002-6696-1344
Jemma Wadsworth https://orcid.org/0000-0002-1110-3203
Donald P. King https://orcid.org/0000-0002-6959-2708

REFERENCES

Ancrenaz, M., & Delhomme, A. (1997). Teeth eruption as a means of age determination in captive Arabian oryx, Oryx leucoryx (Bovidae, Hippotraginae). *Mammalia*, *61*(1), 135–138.

Bachanek-Bankowska, K., Di Nardo, A., Wadsworth, J., Henry, E. K. M., Parlak, Ü., Timina, A., … Knowles, N. J. (2018). Foot-and-mouth disease in the Middle East caused by an A/ASIA/G-VII virus lineage, 2015–2016. *Emerging Infectious Diseases*, *24*(6), 1073–1078. https://doi.org/10.3201/eid2406.170715

Bachanek-Bankowska, K., Di Nardo, A., Wadsworth, J., Mioulet, V., Pezzoni, G., Grazioli, S., … Knowles, N. J. (2018). Reconstructing the evolutionary history of pandemic foot-and-mouth disease viruses: The impact of recombination within the emerging O/ME-SA/Ind-2001 lineage. *Scientific Reports*, *8*, 14693. https://doi.org/10.1038/s41598-018-32693-8

Bailey, T., O'Donovan, D., Kinne, J., & Wernery, U. (2009). Observations on foot-and-mouth disease in vaccinated and unvaccinated wildlife in the United Arab Emirates. *Wildlife Middle East News*, *4*(1), 4, Retrieved from https://www.wmenews.com/newsletters/WME_V4_I1_en_5.pdf.

Beck, E., & Strohmaier, K. (1987). Subtyping of European foot-and-mouth disease virus strains by nucleotide sequence determination. *Journal of Virology*, *61*(5), 1621–1629. https://doi.org/10.1128/JVI.61.5.1621-1629.1987

Brito, B. P., Rodriguez, L. L., Hammond, J. M., Pinto, J., & Perez, A. M. (2017). Review of the global distribution of foot-and-mouth disease virus from 2007 to 2014. *Transboundary and Emerging Diseases*, *64*(2), 316–332. https://doi.org/10.1111/tbed.12373

Carrillo, C., Tulman, E. R., Delhon, G., Lu, Z., Carreno, A., Vagnozzi, A., … Rock, D. L. (2005). Comparative genomics of foot-and-mouth disease virus. *Journal of Virology*, *79*(10), 6487. https://doi.org/10.1128/JVI.79.10.6487-6504.2005

Chaber, A., & Saegerman, C. (2017). Biosecurity measures applied in the United Arab Emirates–a comparative study between livestock and wildlife sectors. *Transboundary and Emerging Diseases*, *64*(4), 1184–1190. https://doi.org/10.1111/tbed.12488

East, R. (1999). *African antelope database 1998* (p. x+434) [21]. Retrieved from IUCN/SSC Antelope Specialist Group. IUCN, Gland, Switzerland and Cambridge, UK website: https://portals.iucn.org/library/efiles/documents/ssc-op-021.pdf.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, *39*(4), 783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Flather, D., & Semler, B. L. (2015). Picornaviruses and nuclear functions: Targeting a cellular compartment distinct from the replication site of a positive-strand RNA virus. *Frontiers in Microbiology*, *6*, 594. https://doi.org/10.3389/fmicb.2015.00594

Frölich, K., Hamblin, C., Jung, S., Ostrowski, S., Mwanzia, J., Streich, W. J., … Anajariyah, S. (2005). Serologic surveillance for selected viral agents in captive and free-ranging populations of Arabian oryx (Oryx leucoryx) from Saudi Arabia and the United Arab Emirates. *Journal of Wildlife Diseases*, *41*(1), 67–79. https://doi.org/10.7589/0090-3558-41.1.67

Geering, W. A. (1967). Foot-and-mouth disease in sheep. *Australian Veterinary Journal*, *43*(11), 485–489. https://doi.org/10.1111/j.1751-0813.1967.tb04774.x

Gloster, J., Sellers, R., & Donaldson, A. (1982). Long distance transport of foot-and-mouth disease virus over the sea. *Veterinary Record*, *110*(3), 47–52. https://doi.org/10.1136/vr.110.3.47

Hall, T. A. (1999). *BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT* (pp. 95–98). Nucleic Acids Symp. Ser. Oxford University Press.

Hughes, G. J., Mioulet, V., Kitching, R. P., Woolhouse, M. E. J., Alexandersen, S., & Donaldson, A. I. (2002). Foot-and-mouth disease virus infection of sheep: Implications for diagnosis and control. *The Veterinary Record*, *150*(23), 724–727. https://doi.org/10.1136/vr.150.23.724

Iyengar, A., Gilbert, T., Woodfine, T., Knowles, J. M., Diniz, F. M., Brenneman, R. A., … Maclean, N. (2007). Remnants of ancient genetic diversity preserved within captive groups of scimitar-horned oryx (Oryx dammah). *Molecular Ecology*, *16*(12), 2436–2449. https://doi.org/10.1111/j.1365-294X.2007.03291.x

Kar, B., Hota, N., & Acharjyo, L. N. (1983). Occurrence of foot-and-mouth disease among some wild ungulates in captivity. *Indian Veterinary Journal*, *60*, 237–239.

Knowles, N. J., Nazem Shirazi, M. H., Wadsworth, J., Swabey, K. G., Stirling, J. M., Statham, R. J., … Paton, D. J. (2009). Recent spread of a new strain (A-Iran-05) of foot-and-mouth disease virus type A in the Middle East. *Transboundary and Emerging Diseases*, *56*(5), 157–169. https://doi.org/10.1111/j.1865-1682.2009.01074.x

Knowles, N. J., & Samuel, A. R. (1998). *RT-PCR and sequencing protocols for the molecular epidemiology of exotic virus diseases of animals* (pp. 1–37). Pirbright, UK: Institute for Animal Health, Pirbright Laboratory.

Knowles, N. J., Wadsworth, J., Bachanek-Bankowska, K., & King, D. P. (2016). VP1 sequencing protocol for foot and mouth disease virus molecular epidemiology. *Revue Scientifique Et Technique (International Office of Epizootics)*, *35*(3), 741–755. https://doi.org/10.20506/rst.35.3.2565.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, *33*(7), 1870–1874. https://doi.org/10.1093/molbev/msw054

Lignereux, L., Alzahlawi, N., Al Kharusi, Y., & Pesci, M. E. (2018). *Middle East Arabian Oryx Disease Survey*, 3rd ed. https://www.arabianoryx.org/En/Downloads/EAD%20-%20Arabian%20ORYX%20DSR%203rdEd%202018.pdf.

Lignereux, L., Chaber, A.-L., Saegerman, C., Manso-Silván, L., Peyraud, A., Apolloni, A., & Thiaucourt, F. (2018). Unexpected field observations and transmission dynamics of contagious caprine pleuropneumonia in a sand gazelle herd. *Preventive Veterinary Medicine*, *157*, 70–77. https://doi.org/10.1016/j.prevetmed.2018.06.002

Ministry of Environment and Water. (2015, December). *The United Arab Emirates, country presentation*. Presented at the Global Framework for the progressive control of Transboundary Animal Diseases (GF-TADs) consultative meeting on control progress of FMD and PPR, Doha, Qatar. Retrieved from http://www.rr-middleeast.oie.int/download/presentation/11%20Doha_1st%20ME%20PPR%20Roadmap%20meeting_Situation%20in%20UAE.ppt.

Newby, J. (2016, Spring). *Sandscript number 19*. Retrieved from https://www.saharaconservation.org/sites/default/files/2017-08/Sandscript_19_Spring_2016.pdf.

Ostrowski, S., & Anajariyah, S. (2003). *2002 Middle East Arabian oryx disease survey*. Taif, Saudi Arabia: National Wildlife Research Center.

Rodríguez, A., Núñez, J. I., Nolasco, G., Ponz, F., Sobrino, F., & de Blas, C. (1994). Direct PCR detection of foot-and-mouth disease virus. *Journal of Virological Methods*, *47*(3), 345–349. https://doi.org/10.1016/0166-0934(94)90030-2

Shimshony, A., Orgad, U., Baharav, D., Prudovsky, S., Yakobson, B., Moshe, B. B., & Dagan, D. (1986). Malignant foot-and-mouth disease in mountain gazelles. *Veterinary Record*, *119*(8), 175–176. https://doi.org/10.1136/vr.119.8.175

Stenfeldt, C., Pacheco, J. M., Singanallur, N. B., de Ferreira, H. C., Vosloo, W., Rodriguez, L. L., & Arzt, J. (2015). Clinical and virological dynamics of a serotype O 2010 South East Asia lineage foot-and-mouth disease virus in sheep using natural and simulated natural inoculation and exposure systems. *Veterinary Microbiology*, *178*(1), 50–60. https://doi.org/10.1016/j.vetmic.2015.04.004

Thomson, G., Vosloo, W., & Bastos, A. D. (2003). Foot and mouth disease in wildlife. *Virus Research*, *91*(1), 145–161. https://doi.org/10.1016/S0168-1702(02)00263-0

Weaver, G. V., Domenech, J., Thiermann, A. R., & Karesh, W. B. (2013). Foot and mouth disease: A look from the wild side. *Journal of Wildlife Diseases*, *49*(4), 759–785. https://doi.org/10.7589/2012-11-276

Woodfine, T., & Gilbert, T. (2016). The fall and rise of the scimitar-horned oryx: A case study of ex-situ conservation and reintroduction in practice. In *Antelope Conservation: From Diagnosis to Action* (p. 280–296). New York, NY: John Wiley & Sons. https://doi.org/10.1002/9781118409572.ch14

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3.4. Risk of release of a disease that can impact the conservation of collateral species. The case of the contagious caprine pleuropneumonia and its management

Preamble

Contagious caprine pleuropneumonia (CCPP), caused by *Mycoplasma capricolum capripneumoniae* (Mccp), has long been a goat-only disease transmitted by close contact and leading to respiratory distress and death. Since its first documentation in 1873 in Algeria (Thomas, 1873), both its range of hosts and its geographical distribution has expended. The disease has been found in northern and central Africa, the Middle East and Asia, to China (Loire et al., 2020) and in wildlife species more or less related to goats: wild goats (*Capra aegagrus*), Nubian Ibex (*Capra ibex nubiana*), Laristan mouflon (*Ovis orientalis laristanica*) and gerenuk (*Litocranius walleri*), (Arif et al., 2007), Tibetan antelope (*Pantholops hodgsonii*) (Yu et al., 2013).

In this study, we described for the first time that the Arabian oryx (*Oryx leucoryx*), closely related to the SHO, is susceptible to the infection (see **Article 4**), and that CCPP might be lethal in this species.

Nonetheless, a combination of pre-existing debilitation and an elevated infectious load resulting from the outbreak among the gazelles in the neighbouring enclosure may have driven the infected oryx toward an inevitable demise. The precise epidemiological role of the Arabian oryx remains uncertain.

One individual SHO has died of CCPP in the population source for the reintroduction project. This discovery was not published but the bacteriological culture and isolation performed on this animal provided a genotype included in a paper (Loire et al., 2020). This genotype was different than the one obtained on the Arabian oryx. The phylogenetic study revealed that the strains originating from wildlife species in the Middle East formed a cluster, possibly suggesting an adaptation to wildlife species – or under notification of domestic species cases.

The morbidity rate was nonetheless very limited, with a singular case of this highly contagious disease in a high density SHO population. Many questions remained unanswered: What was the source of infection? What is the epidemiological role of the SHO? What was the level of exposure to CCPP in this population?

We also described an outbreak of CCPP in gazelles (see **Article 5**). Several findings in this study are relevant to the reintroduction of the SHO: With CCPP found in two different locations in the UAE, including the source population, the release of CCPP in the OROAFR with the reintroduction of SHO might be considered highly likely, potentially detrimental to local gazelles species, especially the critically endangered dama gazelle or vulnerable dorcas gazelles. We observed a transmission of Mccp over longer distances than previously thought but our observation was made in a high-density captive breeding situation that might be difficult to extrapolate to natural situations. Also, while interspecies behaviour is not well documented between SHO and gazelles, SHO might come in close contact with local gazelles, thus increasing the disease transmission risk. Furthermore, the level of CCPP susceptibility in local gazelles is unknown, but a high

mortality rate in a gazelle species is worrisome for other gazelles species. Lastly, we discovered that efficacy is questionable in commercial CCPP vaccine, while it is as expected with a reference vaccine, This raised the question regarding poor quality control during production, which has possible implications on the effect of CCPP management strategy.

ARTICLE 4

Fatal transmission of contagious caprine pleuropneumonia to an Arabian oryx (*Oryx leucoryx*)

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Chaber Anne-Lise*, Lignereux Louis *, Al Qassimi Mohammed, Saegerman Claude, Manso-Silvan Lucia, Dupuy Virginie and François Thiaucourt

*Both authors contributed equally to this manuscript.

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Short Communication

Fatal transmission of contagious caprine pleuropneumonia to an Arabian oryx (*Oryx leucoryx*)

AL Chaber [a,e,1,*], L Lignereux [b,1], M Al Qassimi [b], C Saegerman [a], L Manso-Silván [c,d], V Dupuy [c,d], F Thiaucourt [c,d]

[a] *Research Unit of Epidemiology and Risk Analysis applied to veterinary sciences (UREAR-ULg), Fundamental and Applied Research for Animals & Health (FARAH), Faculty of Veterinary Medicine, University of Liege, Boulevard de Colonster, 42, B-4000 Liège, Belgium*
[b] *Environment Agency Abu Dhabi, Terrestrial and Marine Biodiversity, P.O. Box 45553, Al Mamoura Building, Abu Dhabi, United Arab Emirates*
[c] *CIRAD, UMR CMAEE, F-34398 Montpellier, France*
[d] *INRA, UMR1309 CMAEE, F-34398 Montpellier, France*
[e] *Wildlife Consultant L.L.C, P.O. Box 13587, Al Ain, United Arab Emirates*

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ABSTRACT

Contagious caprine pleuropneumonia (CCPP) is an infectious respiratory disease mainly affecting domestic goats. As CCPP has never been documented in grazing antelopes (subfamily hippotraginae), they were not considered susceptible. *Mycoplasma capricolum* subspecies *capripneumoniae* (Mccp) was isolated from pleural liquid collected during the necropsy of a severely emaciated Arabian oryx with mild nasal discharge. The Mccp isolate was then genotyped using a multilocus sequence scheme; the sequence type was identical to the Mccp strain previously identified in a sand gazelle from a nearby enclosure. This case shows for the first time that members of the hippotraginae subfamily, here the Arabian oryx, can be affected by CCPP. In addition, genotyping shows that the oryx was most probably infected, at a distance, by sand gazelles.

1. Introduction

Arabian oryx (*Oryx leucoryx*) was considered extinct in the wild in 1972 (Henderson, 1974). This emblematic species recovered part of its territories thanks to re-introduction programs relying heavily on captive stock where veterinary management is crucial. Contagious caprine pleuropneumonia (CCPP) is an infectious respiratory disease mainly affecting domestic goats. CCPP is endemic in the Middle East (World Organization for Animal Health, 2009). In naïve flocks of goats, morbidity and mortality may reach 100% and 80%, respectively (MacOwan and Minette, 1976). The evidence that some wild ungulates are highly susceptible to CCPP is a recent finding (Arif et al., 2007). CCPP has never been documented in the hippotraginae subfamily hence was not considered susceptible. We describe here a fatal case of CCPP in an Arabian oryx (*O. leucoryx*) infected at a distance by neighbouring sand gazelles (*Gazella subgutturosa marica*).

2. Materials and methods

2.1. Study site

A mixed group of 14 Arabian oryx was kept among a large collection of local gazelles in the United Arab Emirates. The studied population was housed within 4 side-by-side enclosures (Fig. 1) that were separated from

* Corresponding author. Wildlife Consultant L.L.C, P.O. Box 13587, Al Ain, United Arab Emirates. Tel.: +971 5 61 25 05 96.
E-mail address: alchaber@hotmail.com (A. Chaber).
[1] Both authors contributed equally to this manuscript.

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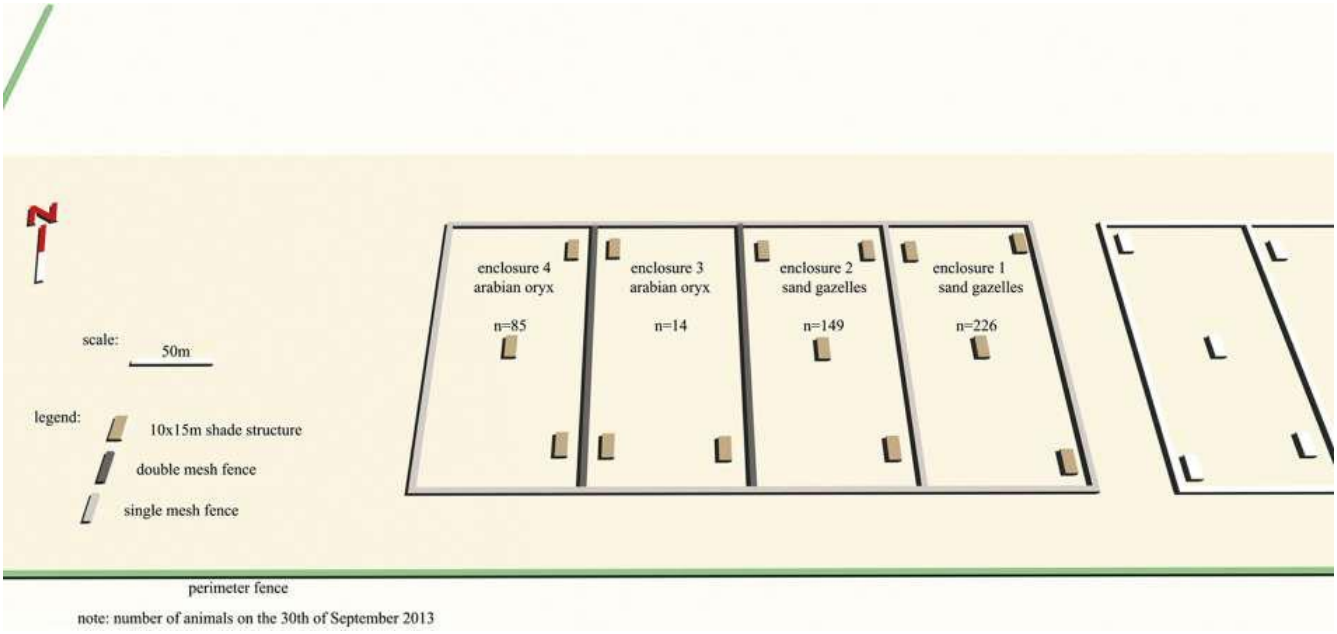

Fig. 1. Details of the animal housing facility.

each other by a double chain link fence with a mesh size of 5 cm. Oryx and gazelles' fences were spaced from one another by 8 cm. No animals were brought in the collection within a year prior to this case.

An outbreak of contagious caprine pleuropneumonia (CCPP) was identified the 15th of June in the adjacent sand gazelle population (enclosure 1), followed by enclosure 2 the 28th of June. The outbreak in the enclosure contiguous to the Oryx (enclosure 2) claimed a total mortality of 34.3%, with a peak the third week of August and a weekly mortality approaching 14.7%. It was controlled only in October after a therapeutical approach initiated months before and involving drastic reduction of gazelle density, mass vaccination and use of oxytetracycline.

2.2. Clinical history

On September the 30th a female adult Arabian oryx showed signs of general depression in enclosure 3. It was emaciated, recumbent and was reluctant to move. A mild sero-haemorrhagic bilateral nasal discharge was observed. Thoracic auscultation revealed unilateral crackling respiratory sounds. No treatments were administered and the animal was euthanized on welfare grounds. No other oryx was affected.

2.3. Post-mortem examination and sample collection

The post-mortem examination revealed unilateral pleuropneumonia characterised by localised pleurisy on both pleurae (Fig. 2A) with profuse pleural fluid, yellowish fibrin deposits and severe consolidation of apical and cardiac lobes of the right lung, associated with pericarditis (Fig. 2A and B). No other gross lesion that may point to concomitant disease was observed. Pleural fluid samples were collected aseptically for pathogen identification.

3. Results

Two samples of pleural fluid from the oryx were initially sent to a local laboratory for bacteriology and *Mycoplasma* genus PCR search, but no pathogens were identified. Due to the ongoing CCPP outbreak in the sand gazelles and to the conspicuous macroscopic lesions, another sample of pleural fluid was sent to CIRAD-CMAEE, OIE/FAO reference laboratory for CCPP. At CIRAD, DNA was extracted using the DNeasy blood and tissue kit (Qiagen) and tested by real-time PCR for the detection of *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp) (Lorenzon et al., 2008) providing a positive result. Subsequently, a pure mycoplasma culture was isolated from the same sample after four days of incubation in a modified CCPP medium (World Organization for Animal Health, 2009). The Mccp isolate was then genotyped using a multilocus sequence scheme (Manso-Silván et al., 2011). A new sequence type was identified, which differed by a single nucleotide polymorphism in locus 03 as compared to sequence type 1-010. This sequence type, previously identified in East Africa, but also in Qatar, was identical to the Mccp strain isolated from a sand gazelle in the adjacent pen during the CCPP outbreak giving a very strong indication that CCPP was transmitted from the sand gazelles to the oryx.

4. Discussion

This case shows for the first time that members of the hippotraginae family, here the Arabian oryx, can be affected by CCPP. In addition, genotyping shows that the oryx was most probably contaminated, at a distance, by sand gazelles housed in an adjacent pen separated by a double chain linked fence.

Some wild ungulates have recently been reported to be highly susceptible to CCPP (Arif et al., 2007). It was

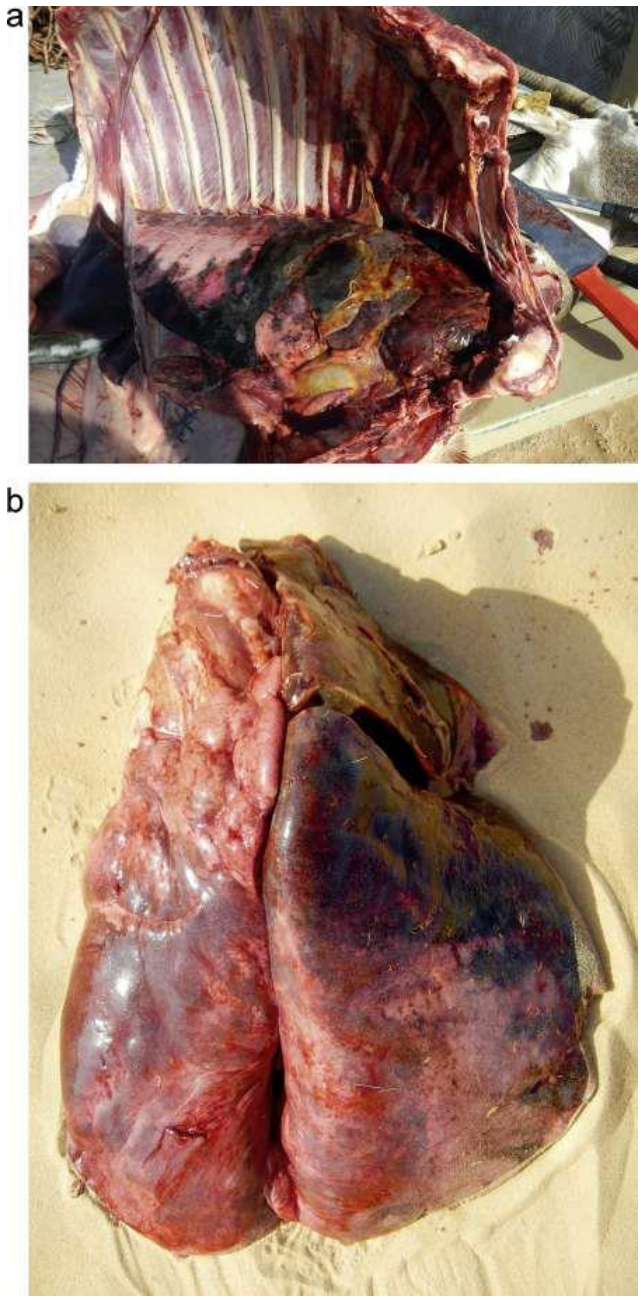

Fig. 2. (A) Thoracic cavity of an Arabian oryx affected by CCPP. (B) Lungs of an Arabian oryx affected by CCPP.

discovered in the caprinae subfamily: Nubian ibex (*Capra ibex nubiana*), Laristan mouflon (*Ovis orientalis laristanica*) (Arif et al., 2007) and Tibetan antelopes (*Pantholops hodgsonii*) (Yu et al., 2013) and was also suspected in the markhors (*Capra falconeri*) (Ostrowski et al., 2011) but could not be confirmed in that species yet. CCPP was also detected in gerenuk (*Litocranius walleri)* (Arif et al., 2007) and in sand gazelles (*Gazella subgutturosa marica*) (Nicholas et al., 2008) both from the antilopinae subfamily. The Arabian oryx must be added to the list of CCPP-susceptible species. Further studies may be needed to determine precisely which species/families are CCPP-sensitive and to understand whether this susceptibility may be caused by a recent evolution of the infectious agent or more likely by increased exposure between infected and naïve, susceptible animals but what is now certain is that CCPP should be considered a real threat to wild ungulates, both in their natural habitat and in captivity. Intensive breeding programs followed by re-introduction plans in the Middle East allowed this emblematic species to recover part of its territories with a total reintroduced population over 1000 animals (International Union for Conservation of Nature and Natural Resources, 2011). Re-introduction of animals into the wild relies heavily on captive stocks where genetic and veterinary management are crucial. The last Middle East Arabian oryx disease survey (Lignereux and Al Kharusi, 2013) revealed that 94% of the Arabian oryx collections surveyed in the region are in direct contact with other ungulate species and may therefore be threatened by CCPP. For animals kept in captivity, the risk may be linked to animal movements between zoos or reserves. Such movements may even cause an introduction into CCPP-free countries. This risk calls for renewed efforts directed to a better detection and control of CCPP.

Although mycoplasmas are theoretically very fragile wall-less bacteria, this CCPP case shows that "at a distance" transmission is possible even in the Emirates' environment. In September air temperature ranges from 28 °C at night to 42 °C during the day. No precipitation was recorded in September 2013 Although gazelles and oryx, especially males, tend to rub their horns on the mesh and will occasionally travel along the fence, nose-to-nose contact was impossible due to the behavioural habits of these species and, most importantly, to the presence of the double fence. During this case, CCPP did not affect the 13 other oryx that were housed in the same pen and no clinical signs were detected within the seven months following this case. The low animal density in the enclosure may have reduced the transmission risk, notably since the affected animal was reluctant to move and did not mix with the others.

Since Mccp isolation is very fastidious (Nicholas and Churchward, 2011) PCR and real-time PCR methods are the preferred detection techniques being both rapid and specific. Local diagnostic laboratories should have the capability to perform these tests and should be structured as part of an emergency prevention system for CCPP in the region.

Prevention of CCPP introduction in ungulate collections must rely on the existence of buffer zones around pens housing susceptible animals and on the application of strict biosafety measures. These must include quarantine procedures prior to introducing any domestic or wild ruminants to a given population (bio-exclusion) (Saegerman et al., 2012). In addition, vaccination of susceptible animals must be considered, as it may prove the best strategy to reduce the contamination risk. CCPP vaccines are currently based on saponin-adjuvanted inactivated Mccp antigen (Rurangirwa et al., 1991). Correctly vaccinated animals will develop an antibody rise that can be monitored using a specific competition ELISA (Peyraud et al., 2014) hence verifying the vaccine was appropriate and the animal responded adequately.

Increased surveillance and control strategies will be required to limit the expansion of the disease in wild

ungulate species, preventing its introduction to CCPP-free countries and avoiding losses in endangered species.

Disclaimer

The content of this article is the sole responsibility of the authors. The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official position of any agency, group or organization.

References

Arif, A., Schulz, J., Thiaucourt, F., Taha, A., Hammer, S., 2007. Contagious caprine pleuropneumonia outbreak in captive wild ungulates at Al Wabra Wildlife Preservation, State of Qatar. J. Zoo Wildl. Med. 38, 93–96.

Henderson, D., 1974. The Arabian oryx: a desert tragedy. Natl. Parks Conserv. Mag. 48, 15–21.

International Union for Conservation of Nature and Natural Resources, 2011. Oryx leucoryx 2011. http://www.iucnredlist.org/details/15569/0.

Lignereux, L., Al Kharusi, Y., 2013.In: Middle East Arabian oryx disease survey report 2011. . https://www.arabianoryx.org/En/SitePages/Downloads.aspx.

Lorenzon, S., Manso-Silván, L., Thiaucourt, F., 2008. Specific real-time PCR assays for the detection and quantification of *Mycoplasma mycoides* subsp. mycoides SC and *Mycoplasma capricolum* subsp. capripneumoniae. Mol. Cell. Probes 22, 324–328.

MacOwan, K., Minette, J., 1976. A mycoplasma from acute contagious caprine pleuropneumonia in Kenya. Trop. Anim. Health and Prod. 8, 91–95.

Manso-Silván, L., Dupuy, V., Chu, Y., Thiaucourt, F., 2011. Multi-locus sequence analysis of *Mycoplasma capricolum* subsp Capripneumoniae for the molecular epidemiology of Contagious caprine pleuropneumonia. Vet. Res. 42, 1–10.

Nicholas, R., Ayling, R., McAuliffe, L., 2008. Contagious caprine pleuropneumonia. In: Mycoplasma Diseases of Ruminants. CABI, , pp. 116–129.

Nicholas, R., Churchward, C., 2011. Contagious caprine pleuropneumonia: new aspects of an old disease. Transbound. Emerg. Dis. 59, 189–196.

Ostrowski, S., Thiaucourt, F., Amirbekov, M., Mahmadshoev, A., Manso-Silván, L., Dupuy, V., Vahobov, D., Ziyoev, O., Michel, S., 2011. Fatal outbreak of *Mycoplasma capricolum* pneumonia in endangered markhors. Emerg. Infect. Dis. 17, 2338–2341.

Peyraud, A., Poumarat, F., Tardy, F., Manso-Silván, L., Hamroev, K., Tilloev, T., Amirbekov, M., Tounkara, K., Bodjo, C., Wesonga, H., Gacheri Nkando, I., Jenberie, S., Yami, M., Cardinale, E., Meenowa, D., Jaumally, M., Yaqub, T., Shabbir, M., Mukhtar, N., Halimi, M., Ziay, M., Schauwers, W., Noori, H., Rajabi, A., Ostrowski, S., Thiaucourt, F., 2014. An international collaborative study to determine the prevalence of contagious caprine pleuropneumonia by monoclonal antibody-based cELISA. BMC Vet. Res. 10, 48.

Rurangirwa, F., McGuire, T., Mbai, L., Ndung'u, L., Wambugu, A., 1991. Preliminary field test of lyophilised contagious caprine pleuropneumonia vaccine. Res. Vet. Sci. 50, 240–241.

Saegerman, C., Dal Pozzo, F., Humblet, M.-F., 2012. Reducing hazards for humans from animals: emerging and re-emerging zoonoses. Ital. J. Public Health 9, 13–24.

World Organization for Animal Health, 2009. Contagious Caprine pleuropneumonia. http://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/CONTAGIOUS_CAPRINE_PLEURO_FINAL.pdf.

Yu, Z., Wang, T., Sun, H., Xia, Z., Zhang, K., Chu, D., Xu, Y., Xin, Y., Xu, W., Cheng, K., Zheng, X., Huang, G., Zhao, Y., Yang, S., Gao, Y., Xia, X., 2013. Contagious caprine pleuropneumonia in endangered Tibetan antelope, China, 2012. Emerg. Infect. Dis. 19, 2051–2053.

ARTICLE 5

Unexpected field observations and transmission dynamics of contagious caprine pleuropneumonia in a sand gazelle herd

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Lignereux Louis, Chaber Anne-Lise, Saegerman Claude, Manso-Silvan Lucia, Peyraud Armelle, Apolloni Andrea and François Thiaucourt

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Unexpected field observations and transmission dynamics of contagious caprine pleuropneumonia in a sand gazelle herd

Louis Lignereux[a,*,1], Anne-Lise Chaber[b,1], Claude Saegerman[a], Lucía Manso-Silván[c], Armelle Peyraud[c], Andrea Apolloni[c,d], François Thiaucourt[c]

[a] Faculty of Veterinary Medicine, University of Liège, Unit of Epidemiology and risk analysis applied to veterinary sciences (UREAR-ULg), Fundamental and Applied Research for Animal and Health (FARAH) Center, Quartier Vallée 2, Avenue de Cureghem 7A, Bât. B42, B-4000, Liège, (Sart-Tilman), Belgium
[b] School of Animal & Veterinary Sciences, One Health & Wildlife Population Health, University of Adelaide, Leske Building G13, Roseworthy, SA 5371, Australia
[c] Centre de coopération internationale en recherche agronomique pour le développement (CIRAD), Institut National de Recherche Agronomique (INRA), AnimalS, health, Territories, Risks and Ecosystems (ASTRE), TA A-117/E Campus international de Baillarguet, 34398, Montpellier cedex 05, France
[d] Institut Sénégalais de Recherches Agricoles, Laboratoire National de l'Elevage et de Recherche Vétérinaire, Parc Scientifique de Hann, Dakar, Senegal

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ABSTRACT

Contagious caprine pleuropneumonia (CCPP), caused by *Mycoplasma capricolum* subsp. *capripneumoniae*, has long been considered a goat-specific disease. Since 2007 there has been growing evidence that this disease can affect wild ungulates either kept in captivity or in the wild. In 2013, a large collection of sand gazelles (*Gazella marica*) held in the United Arab Emirates suffered heavy losses due to a CCPP epizootic confirmed by PCR and isolation. Animals displayed typical lesions, with unilateral pneumonia and profuse pleurisy. An initial antibiotic treatment consisting of tylosin administered in drinking water did not improve the animals' condition and vaccination failed to stop the spread to contiguous pens. A treatment with tetracycline mixed in feed pellets finally succeeded to stop the evolution of the disease. A subsequent vaccine trial, performed on naïve animals, showed that only a reference CCPP vaccine produced according to OIE standards induced a sero-conversion by CCPP competition ELISA, while the commercially available vaccines did not. A SEIRD compartment transmission model was developed to better understand the dynamics of the disease. The parameters were initially set as per expert opinion and then adjusted to fit the observed mortality data. The basic reproductive number R_0 was estimated to be between 2.3–2.7, while the final mortality rate reached up to 70% in some pens. Transmission of infectious droplets from an external source, through a distance of at least the 50 m separating the pens from the perimeter fence, remains the most plausible explanation for the contamination of this stock of gazelles.

1. Introduction

Contagious caprine pleuropneumonia (CCPP) is caused by *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp), a wall-less bacteria that is vulnerable to the environment and requires close animal contact to transmit and induces lesions of pneumonia and pleurisy resulting in respiratory distress and often death in acute cases (Thiaucourt and Bölske, 1996). The disease was first described by Thomas in Algeria (Thomas, 1873) and then by Hutcheon, who reported its introduction to South Africa through a shipment of goats from Turkey (Hutcheon, 1881). Long considered restricted to Africa and the Middle-East, CCPP is now known to occur in China (Chu et al., 2011) and Tajikistan (Amirbekov et al., 2010). Hence, CCPP must be present in a region spanning from Tunisia-Niger to China and, consequently, represents a threat to hundreds of millions of goats. The distribution of CCPP outside this area, notably west of Niger, north of Turkey-Tajikistan and south of Tanzania, is still uncertain.

CCPP was long considered a goat-specific disease, though this strict host specificity was put in question when Mccp was isolated from sheep in Kenya (Litamoi et al., 1990) and Uganda (Bölske et al., 1995, 1996). However, sheep were less susceptible than goats and it remains unclear whether they may play a role in the epidemiology of CCPP, as asymptomatic carriers, or if they only become infected when in contact with infected goats. Since 2007 there has been a growing number of reports

* Corresponding author.
E-mail addresses: l_louis77@hotmail.com (L. Lignereux), alchaber@hotmail.com (A.-L. Chaber), claude.saegerman@uliege.be (C. Saegerman), lucia.manso-silvan@cirad.fr (L. Manso-Silván), armelle.peyraud@cirad.fr (A. Peyraud), andrea.apolloni@cirad.fr (A. Apolloni), francois.thiaucourt@cirad.fr (F. Thiaucourt).
[1] These authors contributed equally.

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of CCPP in wild ungulates, either held in captivity or free ranging. The number of affected species is expanding progressively: wild goat (*Capra aegagrus*), Laristan mouflon (*Ovis orientalis laristanica*), Nubian ibex (*Capra ibex nubiana*), gerenuk (*Litocranius walleri*) (Arif et al., 2007), Tibetan antelope (*Pantholops hodgsonii*) (Yu et al., 2013) and Arabian oryx (*Oryx leucoryx*) (Chaber et al., 2014).

On the 27th of April 2013, sand gazelles (*Gazella marica*) started to show symptoms of CCPP in a large collection held in the United Arab Emirates, where climatic conditions are harsh and at first glance unsuitable for environmental mycoplasma survival. While the origin of the infection remains unclear as it was not possible to determine whether the outbreak started from a gazelle in the latent carrier stage or was transmitted from outside the collection. The course of the disease was precisely recorded by mean of daily mortality and its spread through the various pens could be deduced from observations made during this unfortunate outbreak. Even though the enclosures set up was not designed to study infectious diseases, it was observed, in disagreement with what was thought before, that CCPP could be transmitted in the absence of close animal contact, at a distance of at least 80 m in this case. The role of wind, temperature, humidity and rain in the transmission dynamics is discussed here. Furthermore, these observations were successfully used to validate the parameters of an *in-silico* transmission model, which allowed us to estimate the basic reproductive number (R_0) and the case fatality rate of CCPP in those herds. The analysis of the various control measures that were implemented, consisting of antibiotic treatments and vaccination, were used to issue some general recommendations, both for the early response to an outbreak and for its prevention. After the outbreak was under control, the effect of the vaccines used during the outbreak was investigated more in depth by mean of a vaccine trial based on serological conversion confirming our concerns.

2. Materials and methods

2.1. Case report and outbreak investigation

2.1.1. Housing facilities and population affected by the outbreak

At the beginning of the outbreak, the herd consisted of 3355 sand gazelles (*Gazella marica*) held in a compound as described in Fig. 1. There were four groups of pens (numbered I to IV), separated from each other by a distance of 50 to 80 m. In each group, each pen (numbered .1 to .4) had a size of 100 × 200 m, and contained up to five 10 × 15 m shade structures, where animals could shelter. Feeders and water troughs were located away from the fence. All the pens were contiguous to at least another pen, separated by a wire fence. A perimeter fence around the whole compound delimited a buffer zone of 50 m between all the groups of pens and the outside. Most of the pens initially housed large numbers of animals, ranging from 250 to 400, except for pen I.1 and pen I.2, which housed 39 and 19 animals respectively. Sexes were separated and no births were recorded. Pens IV.3 and IV.4 housed Arabian oryx. About 70 days after the onset of the outbreak, for vaccination management purposes, the herd in pen II.1 was merged with that in pen II.4 and, similarly, II.2 with II.3, III.1 with III.4 and III.2 with III.3. No animals had been introduced in the herd within the 12 months preceding the outbreak. A traditional sheep and goat farm, locally called “Ezba”(Chaber and Saegerman, 2016), housing 50 animals, was situated 270 m south-east of the compound, with the closest pen being II.1. Wild gazelle’s tracks and free roaming camels could be seen occasionally along the perimeter fence.

2.1.2. Clinical and pathological data

Mortality was recorded daily, including an indication of the possible cause of death, and 70 dead animals were randomly selected for necropsy during the outbreak, which made it possible to follow the course of the disease through macroscopical observation.

2.1.3. Daily climatic data

Wind direction and speed, temperature and humidity data were recorded by automated surface observing systems (ASOS) at two local airports: Al Ain airport, located 61 km to the east, and Al Dafrah airport, located 54 km to the west. These data are accessible online: https://mesonet.agron.iastate.edu/sites/dyn_windrose.phtml?station = OMAL&network = AE_ASOS.

2.1.4. Mccp culture, isolation and genotyping

Lung samples were collected aseptically for pathogen identification on four different gazelles. The samples were frozen and stored at -20 °C, and then shipped to CIRAD-ASTRE (Montpellier, France), the OIE/FAO reference laboratory for CCPP. DNA was extracted from lung samples with the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) and analyzed by real-time PCR (Lorenzon et al., 2008). Cultivation of mycoplasmas was performed using modified Hayflick’s medium supplemented with horse serum (25%) and sodium pyruvate (0.2%) (Thiaucourt et al., 1996). Plates were incubated at 37 °C under anaerobic conditions. *Mycoplasma* colonies were cloned to ensure purity and then grown in liquid medium for identification of Mccp by real-time PCR. One of these clones was then typed by multi-locus sequence analysis (MLSA), based on the concatenation of 8 locus sequences according to (Manso-Silván et al., 2011).

2.2. Control measures and vaccine trial

2.2.1. Antibiotics and vaccines used during the outbreak

The sequence of antibiotic treatments and vaccinations is displayed in Table suppl. 1. Tylosin tartrate powder (ADWIA/Egypt) was administered at a dose of 10 mg/kg of body weight (BW) single in day (SID) for 14 days dissolved in drinking water. Oxytetracyclin-HCL (Oxivet 20%®, Centrovet Ltd/Chile) was given at 10 mg/kg BW, SID for 14 days mixed with the feed (pellets). Two commercially available CCPP vaccines were used during the outbreak. Pulmovac® (VETAL Animal Health Products, Adiyaman, Turkey) batches 12/PU/11 and 13/PU/03 were initially used, then Caprivax® batch 18/013 (KEVEVAPI, Nairobi, Kenya). All the animals in each pen were vaccinated to obtain 100% immunization coverage.

2.2.2. Serological assessment of CCPP vaccines

After the outbreak was considered over, one hundred male sand gazelles that were unexposed to the disease were virtually divided in five groups of 20 animals (Table suppl. 2) in a pen situated in the northern part of the facility (Fig. 1) where evidence of CCPP was never found. Group A did not receive any treatment, and was used as negative control. Group B was vaccinated first with “Pulmovac®” batch 13/PU/03, and three months later with “Jovaplasm C®” batch JP1812 (JOVAC, Jordan), a third commercially available vaccine. Group C, used as positive control, received one dose of a reference vaccine produced at CIRAD according to the OIE guidelines (ref. OIE terrestrial manual), containing 0.15 mg of purified Mccp antigen and 3 mg saponin (Sigma S4521) per dose. Group D received an injection of “Caprivax®” batch 18/013 and a triple dose of the same vaccine three months later. Finally, group E received two injections of “Caprivax®” one month apart. It must be noted that the number of gazelles decreased over time due to traumatic injuries following fights.

Blood samples were harvested monthly from all animals, coded blindly, and sent to CIRAD for analysis. Serology was performed using the CCPP competition ELISA (cELISA) kit (IDEXX, Montpellier, France). The test was performed according to the manufacturer’s instructions and under ISO17025 accreditation by the French committee of accreditation (N° 1–2207 rev. 9). The validity conditions, notably homogeneity of variances and covariance matrixes, were first-tested (Howell, 1998) and a two-factor ANOVA with repeated measures on one factor was used to compare the seroconversion kinetics (Petrie and Watson, 2006). In addition, an in-house indirect ELISA was performed

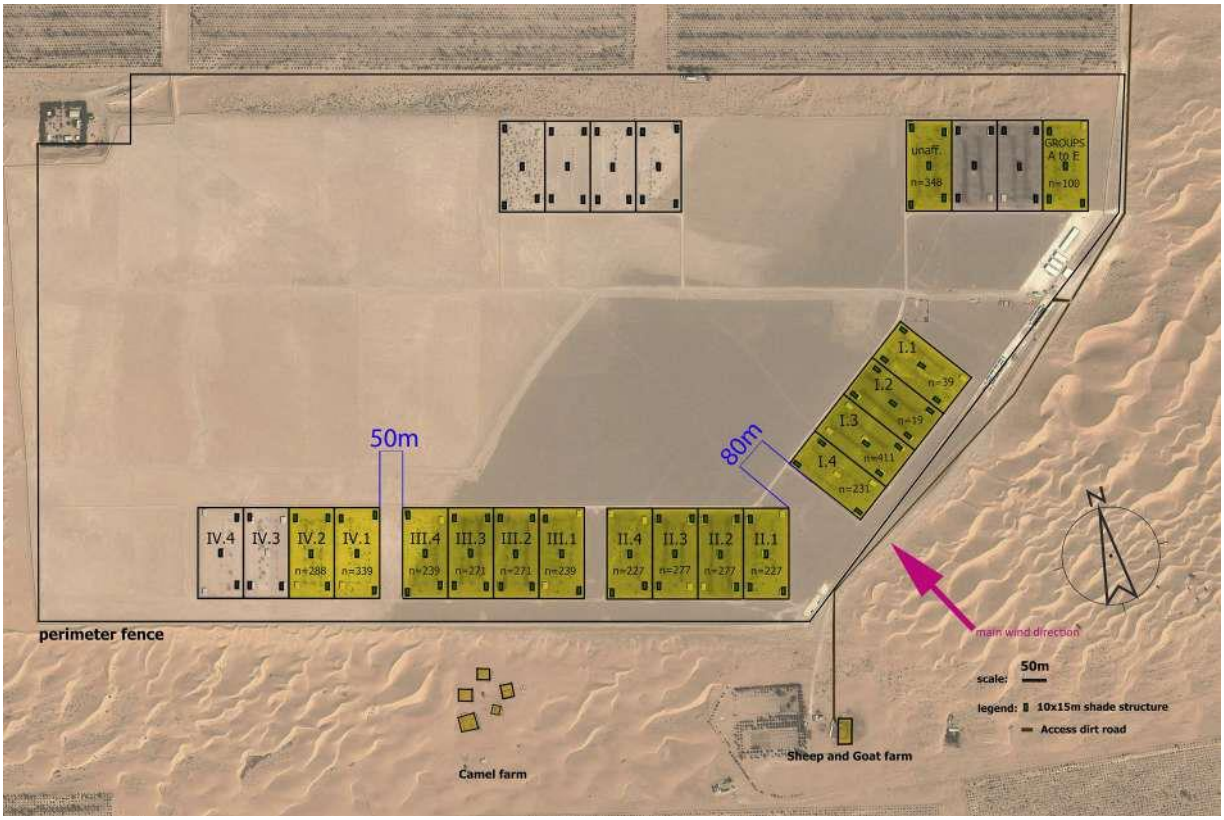

Fig. 1. Map of the compound.
Each pen has a size of 100 × 200 m. Groups of 4 pens are separated from each other and from the perimeter fence by at least 50 m. A private sheep and goat farm is located 270 m away from the compound in a South-Southeast direction, coinciding with the predominant wind direction.

to evaluate and compare the global quantity of Mccp-recognizing antibodies one month after vaccination. Shortly, Greiner plates ref. 655,061 coated overnight at + 4 °C with an inactivated, purified Mccp antigen (0.03 µg/ml) were incubated one hour at 37 °C with goat sera diluted 1/1300, then 30 min in DAKO™ P0160 conjugate (1/1500), and finally 20 min in tetramethylbenzidine substrate. After adding the stop solution, the optical density (OD) readings were performed at 450 nm. Each step was followed by plate washes. All diluents and solutions were obtained from the IDEXX cELISA kit. The mean OD between groups of animals was assessed using a two-sample *t*-test with unequal variances (Welch's test) (Dagnelie, 1998). For all tests, P values < 0.05 were considered significant.

2.3. Development of a compartmental model

A compartmental deterministic model was developed to estimate the basic reproductive number (R_0), as a measure of the contagiousness of the disease, and the case fatality rate (p). The pen II.2 was chosen for these estimations because it was the first where cases were recorded. Consequently, the gazelles in this pen could be considered a naïve population and the disease spread through direct contact within the pen. As the disease progressed to other pens the dynamics certainly became more complicated, involving interactions among animals in different pens. The within-pen spread of CCPP was described using a "SEIRD" model, a modified version of the SEIR (Chowell and Nishiura, 2014; Baguelin et al., 2013; Woolhouse et al., 1996), where S, E, I, R and D are non-overlapping epidemiological compartments representing the number of susceptible (S), exposed (E, infected but not infectious yet), infectious (I), recovered (R) and infection related dead (D) animals respectively. N is the total number of animals alive each day. A pictorial representation of the model is shown in Fig. 2.

The temporal evolution of the disease spread is described through

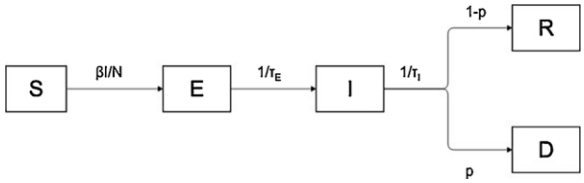

Fig. 2. SEIRD compartmental model for CCPP.
The model comprises 5 compartments, namely, susceptible (S), exposed (E = infected but not infectious), infectious (I), recovered (R) and infection-related dead animals (D). β represents the probability, by unit of time, that a susceptible animal becomes infected and switches to the exposed compartment. Exposed animals then spend τ_E days in a latent stage before becoming infectious, and remain τ_I days in this stage. At the end of the infection stage, animals either recover, with a probability "1-p", or die, with probability "p", which represents the case fatality rate.

the set of equations:

$$\frac{dS}{dt} = -\beta\frac{SI}{N}$$

$$\frac{dE}{dt} = \beta\frac{SI}{N} - \frac{1}{\tau_E}E$$

$$\frac{dI}{dt} = \frac{1}{\tau_E}E - \frac{1}{\tau_I}I$$

$$\frac{dR}{dt} = (1-p)\frac{1}{\tau_I}I$$

$$\frac{dD}{dt} = p\frac{1}{\tau_I}I$$

$$N = S + E + I + R$$

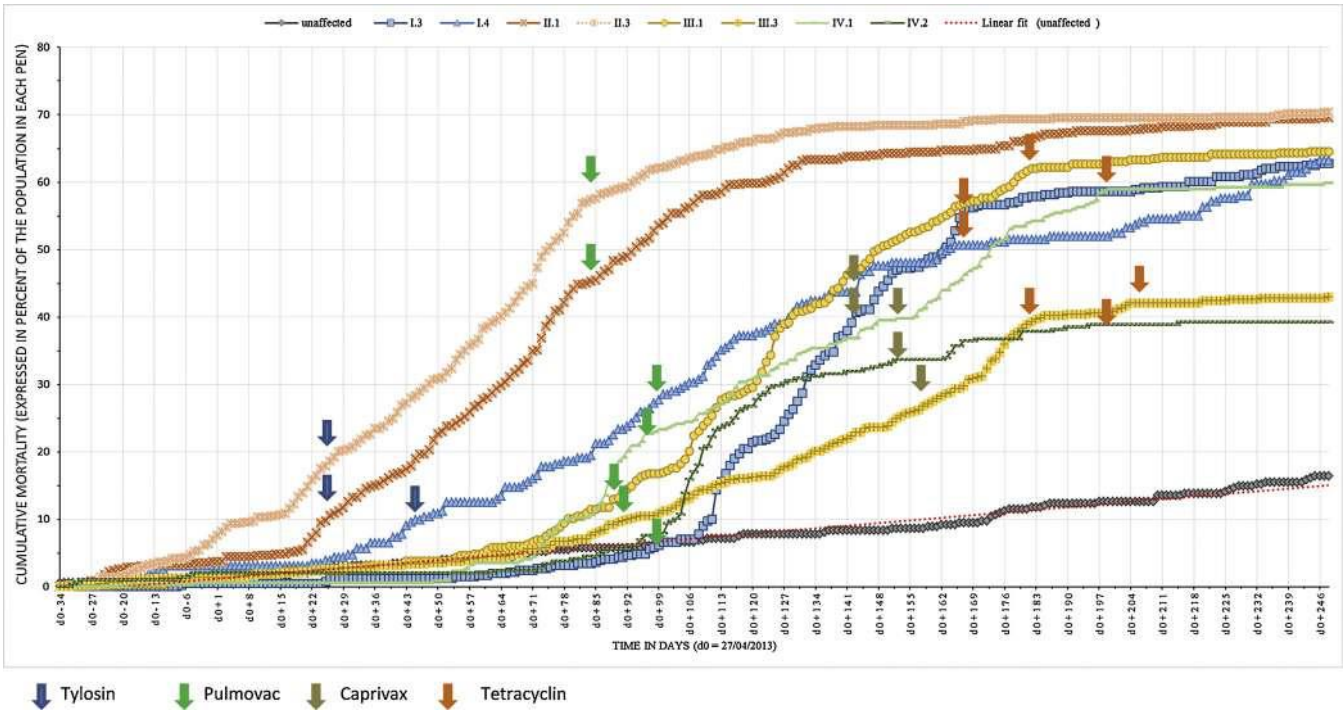

Fig. 3. Cumulative death rate and interventions in the various pens.
This graph represents the cumulated death rate in each pen according to the number of days after the initial identification of CCPP losses. All causes of death contribute and the data from a pen unaffected by CCPP provides a reference for this background death rate. For convenience, the data from the pens that were grouped at day 70 were cumulated. The date and type of interventions in the pens are indicated by arrows pointing towards the respective curves.

Animals in the infectious stage can infect susceptible ones with a probability β per unit of time. The probability β, or transmission contact rate, takes into consideration many factors related to the transmission of the mycoplasma in the local environment. Exposed animals spend τ_E days in a latent stage before becoming infectious and then remain τ_I days in this stage. At the end of the infection stage animals either recover, with a probability "1-p", or die, with a probability "p", which represents the case fatality rate. A time step of one day was used and the disease progression was simulated for 100 days. The population was considered constant except for infection-induced deaths. This assumption was supported by the fact that during the first 80 days of the infection, the mortality rate in pen II.2 (first infected) was almost 15 times higher than that in pen IV.I (last infected)

The basic reproductive number R_0 was then evaluated using two approaches:

- As the largest eigenvalue of the Next Generation matrix. $R_0 = \beta\tau_I I$ (Diekmann et al., 2009)
- Using the final size relation: $R_0 = -\frac{\ln(s_f) - ln(s_0)}{s_f - s_0}$ (Keeling and Rohani, 2008), where s_0 and s_f indicate the fraction of susceptible animals at the beginning and at the end of the epizootic respectively, with the final fraction of susceptible animals estimated as $s_f = 1 - \frac{Total\ Number\ of\ Deaths}{pN}$

This model depends on four parameters: the contact rate β, the latent τ_E and infectious τ_I periods, and the case fatality rate p. All of them, except the latent period τ_E whose value was fixed at 7 days as per expert opinion, were estimated through the calibration of the model. We used Monte Carlo Markov Chains (MCMC) methods (Ferguson et al., 1999; Spiegelhalter et al., 2002), with a Poissonian likelihood, to fit the model to the daily number of deaths in pen II.2. Metropolis-Hastings sampling was used to explore the parameters space, checking convergence by using 100 chains of 1000 iterations (after a 100 burn in period), starting from several different initial values of the parameters set. The calibration was performed using the R-package FitR (https://github.com/sbfnk/fitR) developed by Funk and Camacho at the London School of Hygiene and Tropical Medicine, where a uninformative uniform prior distribution was considered for all parameters.

A single infectious period was assumed in our model, thus excluding differences in contagiousness between asymptomatic and symptomatic phases. To strengthen the results from our model, two other models were considered. In one model (called 'SEIIRD') the infectious stage and its period were partitioned in two infectious stages, asymptomatic and symptomatic, with different transmission probabilities ($\beta_A < \beta_S$). In the other model (called 'SEIRD with constant mortality'), a constant mortality rate was considered during the infectious stage, indicating that animals could die at any moment. The two models were calibrated with the same set of data and R_0 was evaluated accordingly. The lower Deviance Information Criterion (DIC) (Ferguson et al., 1999; Spiegelhalter et al., 2002) was used as a measure of model adequacy, to compare the three models.

3. Results

3.1. Outbreak

3.1.1. Onset of a CCPP outbreak in the sand gazelle herd

On the 27th of April 2013, "salivating gazelles" and a sudden mortality increase were observed in pen II.2. Two days later, the first three necropsies presenting CCPP-compatible lesions were performed in this same pen and initiated a closer follow-up of the pens. Biosecurity measures were immediately implemented. These notably included the disinfection of boots and wheels of vehicles with 1/200 diluted Virkon-S (DuPont). Healthy pens were visited first and contaminated pens last. Gazelle carcasses were disposed of in individual plastic bags and incinerated. Nonetheless, CCPP spread to adjacent pens (II.1 and II.3) and then to groups of pens I and III (Figure suppl. 1).

Initially, animals presented good body condition, with or without sero-hemorrhagic epistaxis and salivation, and did not show overt

disease signs. At a later stage, animals were reluctant to move and their breathing was labored and painful. Typical CCPP lesions, confined to the thoracic cavity, were observed at post-mortem examinations.

The administration of tylosin in the water was not followed by any improvement in the animal's health and the initial vaccination with "pulmovac" did not prevent the spread of CCPP to neighboring pens. Only the treatment with oxytetracyclin-HCL in the feed yielded some improvement and induced a marked bending of the mortality curve, notably in pen III.3 after day 179. At that date the cumulative mortality was reaching 40% and it did not increase much after that date. However a second treatment was given on day 205 as mortality seemed to resume in that pen. The overall evolution of the disease mortality is illustrated in Fig. 3.

3.1.2. Atmospheric conditions preceding and during the outbreak

The climate in the UAE was arid and from April to October, period when the outbreak happened, air temperature varied from 20 °C at night to over 48 °C during daytime, while the average humidity ranged from 20% during the day to 75% at night. Most of the animals tended to congregate under shade structures at daytime, thus favoring close animal contacts. Some rain was recorded between the 22nd and the 29th of April 2013, 5 days before and 2 days after the first observation of symptoms respectively and no precipitations were observed for 198 consecutive days from May 2nd to November 15th. After the rainfall in April, a pungent ammoniac smell was detected in the enclosures due to manure degradation. A strong prevailing wind, locally called "Alkaus", which blows from the South-South East, was recorded at over 12 m/sec on April 5th and 6th 2013.

3.1.3. Laboratory diagnosis and molecular typing

Out of four samples tested at CIRAD, only one yielded a positive result by direct Mccp-specific real-time PCR following DNA extraction. However, given the strict specificity of this test, this was sufficient to confirm the presence of Mccp. Isolation trials were positive for two of the samples and the identity of the mycoplasma cultures was confirmed by PCR. The molecular typing of these isolates by MLSA resulted in the identification of a new type, only differing from previously defined MLSA types by a single nucleotide polymorphism.

3.2. Experimental assessment of the seroconversion induced by CCPP vaccines

As clinical observations had cast a reasonable doubt on the efficacy of the commercially available CCPP vaccines, a comparative efficacy study was performed in the northern pens of the compound, where gazelles had stayed unaffected. The outcome of the vaccination was monitored by serology. Before vaccination, all gazelles displayed low percentage of inhibition (PI) values (PI $<$ 35) by the cELISA test. After vaccination, none of the locally available CCPP vaccines induced any seroconversion (Fig. 4A). By contrast, the reference vaccine batch, produced according to the OIE manual of standards, induced a sharp seroconversion, which started to decline only after two months. This seroconversion differed significantly from that of the other vaccines (ANOVA *P*-value = 0.01). After five months, 11 gazelles out of 15 remaining in this group had titers above the cut-off, which is set at 55 PI for this test in goats.

These results were confirmed by an in-house indirect ELISA, which measured the global quantity of antibodies recognizing the Mccp antigens coated on the plate. Using this second type of ELISA, only the OIE reference vaccine induced a sharp seroconversion (mean OD = 2.1) one month after vaccination. This value was significantly higher than the value obtained before vaccination (Wilcoxon signed-rank test; P-value = 0.001). Group B, vaccinated with "Pulmovac", showed a significant (Wilcoxon signed-rank test; P-value = 0.002) but very slight seroconversion (mean OD = 0.128). Groups D and E, vaccinated with "Caprivax" did not show a significant OD increase (Wilcoxon signed-rank test; P-value ≥ 0.22). (Fig. 4B)

3.3. R0 estimates from SEIRD model outcomes

The total population at the beginning of the epizootic in pen II.2 consisted of 277 gazelles, of which around 7% were considered in the latent phase. The latent time τ_E was fixed to 7 days, as from expert opinion. The other parameters were calibrated by MCMC procedure using a Poissonian Likelihood for the data. Results are shown in Fig. 5, where black dots correspond to data and red shades correspond to the average and confidence intervals of the model. Table Suppl. 3 shows the estimated parameters of the model that yield the best agreement between model and data. The case fatality rate "p" was estimated at around 59% (95% CI: 54–70). Using the estimated parameter values, R_0 was estimated to have an average value of 2.32 (95% CI: 1.86–2.79), when evaluated through the Next Generation matrix (Diekmann et al., 2009), and a slightly higher value, 2.68 (95% CI: 2.07 to 3.43), when evaluated using the final size relation (Keeling and Rohani, 2008). The comparison of the three models as seen in Table Suppl. 4 through the estimation of the DIC, showed that the simplest SEIRD model was the most adequate to describe the disease propagation in pen II.2.

4. Discussion

The susceptibility of *Gazella marica* to CCPP infection has been confirmed, with animals showing typical symptoms and lesions and final cumulated mortality rates reaching 70% in the absence of antibiotic treatment. This is very similar to what is observed in goats (Thiaucourt and Bölske, 1996; Nicholas and Churchward, 2011).

The onset of a CCPP outbreak in the sand gazelle stock was quite a surprise as this herd was supposedly well isolated from surrounding susceptible animals. No ungulates were introduced in the compound since the arrival of Arabian oryx a year before. These oryx had been kept for two years in enclosures where no history of disease had been reported. It is therefore unlikely that these animals were the source of the infection. A contamination through fodder is also very unlikely, as alfalfa hay was imported from CCPP-free countries.

CCPP is enzootic in the UAE, and more generally in the Arabic Peninsula (El-Deeb et al., 2017) and the first pen that became contaminated was located about 270 m away, leeward a goat farm located south from the gazelles. Unfortunately, it was not possible to obtain epidemiological data to determine if there had been an incidence of CCPP in this farm just before the outbreak in the gazelle herd. Transmission from an external source, through a distance of at least the 50 m separating the pens from the perimeter fence remains nevertheless the most plausible explanation. Although the daytime atmospheric conditions were very harsh, the nighttime conditions (20 °C and 75% relative humidity) were propitious to mycoplasma survival, which may have allowed the dissemination of cough droplets by the wind over relatively long distances. Such indirect transmission had been strongly suspected for contagious bovine pleuropneumonia, another pulmonary mycoplasma disease affecting cattle (Giovannini et al., 2000). Much longer aerosol transmissions, 5–10 km, were experimentally recorded for *Mycoplasma hyopneumoniae* (Otake et al., 2010). In addition, the rain that occurred just before the outbreak had induced a peak of ammonia production from the dampened dejections, which may have acted as predisposing factor. Climatic factors have often been associated to the onset of CCPP outbreaks. In North Africa, CCPP appeared more often in the winter (Castelet, 1906; Curasson, 1942); in Oman, CCPP outbreaks seemed to be more frequent in January, when the lowest temperatures and highest pluviometry are recorded, and in July, when the highest temperatures are registered (Jones and Wood, 1988). A long distance transmission was confirmed during this outbreak, since CCPP spread centrifugally from one group of pens to another, separated by up to 80 m, in spite of the biosecurity measures implemented. However, the pens located in the Northern part of the compound (400 m away from

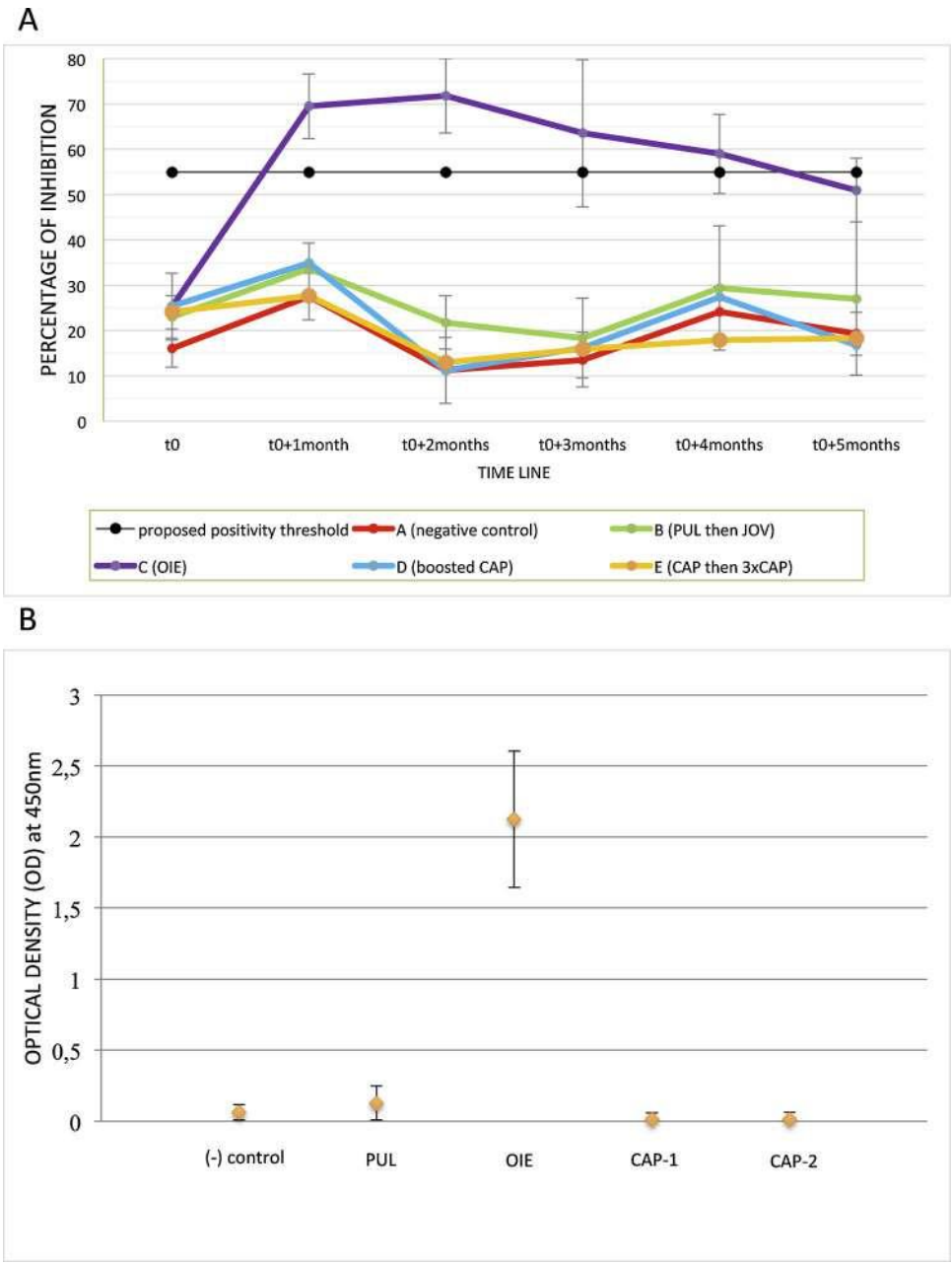

Fig. 4. serological results after vaccination of naïve sand gazelles.

A: Mean percentage of inhibition (PI) values obtained by CCPP competition ELISA on gazelles vaccinated either with commercial vaccines or with a reference vaccine. Bars indicate the minimum and maximum values obtained. The PI obtained on the unvaccinated, negative control remained very low (< 35) throughout the assay and did not significantly differ from the results obtained with the commercial vaccine. The PI values obtained on the positive control group rose sharply after a single injection and remained above the 55 PI cut-off (threshold established for goats) for more than 3 months.

B: Indirect CCPP ELISA results, expressed in optical density (OD), with sera collected one month after the first vaccine injection. The untreated control group is shown as "(-) control". The names of the vaccines were Pulmovac (PUL); Caprivax (CAP-1 and CAP-2) reference batch produced by CIRAD according to the manual of standards of the OIE (OIE). The reference OIE vaccine batch induced a significant and strong seroconversion. Pulmovac induced a significant but very slight sero-conversion while Caprivax did not induce any seroconversion.

the infected pens) were never affected.

The MLSA type of the Mccp strain isolated from the gazelles was closely related to the genetic types of strains isolated in Qatar and East Africa (Manso-Silván et al., 2011). Unfortunately, no information regarding Mccp strains circulating in neighboring herds was available to establish a possible epidemiological link.

The use of tylosin in drinking water had apparently no effect on the course of the disease, though this and other macrolides are considered active against mycoplasmas. Tylosin is usually administered parenterally, which was considered completely impractical for a collection of wild ungulates, but it is also administered orally to calves at a dose of 10–20 mg/kg BW twice daily for 7–14 days (www.biovet.com/products). Many factors could explain the failure of this initial treatment. First, the quality of the antibiotics commercially available is not routinely and independently controlled by a regulatory body of inspection. The way the tylosin powder was dissolved into the 5000 L water tanks may not have ensured a homogeneous distribution of to all animals. Besides, tylosin tartrate is a weak base with a pKa value of 7.1 (Gingerich et al., 1977). The low pH adult ruminant stomach may not favor its bioavailability, which has been shown to be quite low (25%) in poultry (Ji et al., 2014). All these factors put together, it is very unlikely that the animals received an appropriate dose ensuring a clinical cure. On the other hand, oxytetracyclin mixed with the food pellets yielded better results, as the mortality decreased very rapidly following this treatment. In some pens the treatment had to be repeated after 30–40 days, which indicated that Mccp was still circulating in the gazelle herd after the initial treatment.

Appart from a batch of Pulmovac, none of the CCPP vaccines that were commercially available in the UAE and used at the time of the outbreak seemed to be effective. In fact, they were used in an emergency situation that was far from ideal and the clinical observations were not sufficient to prove their lack of immunogenicity. However, the serological testing results were consistent with a lack of protection: the three commercial CCPP vaccines did not induce any detectable seroconversion by cELISA, while the reference control vaccine did, as expected (Peyraud et al., 2014). The absence of seroconversion using a

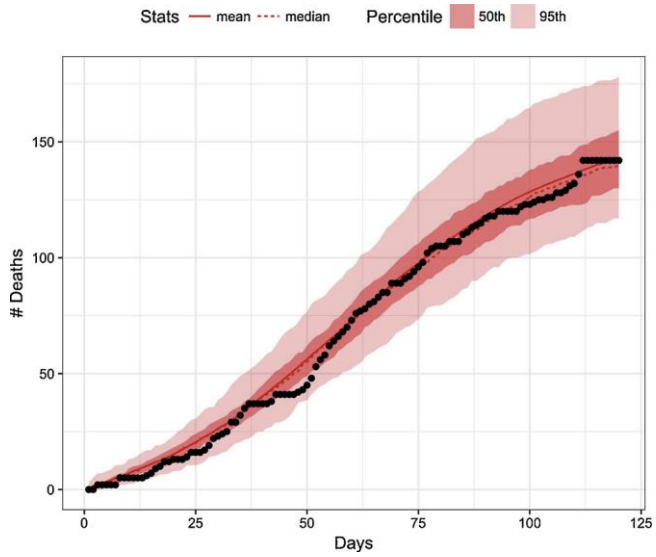

Fig. 5. Comparison of the MCMC model output with observed data. The cumulative number of deaths in pen II.2 is represented by the black dots. The red line is the mean value predicted by the model, the dashed red line is the median, and the shaded areas correspond to 50% and 95% confidence intervals for the values estimated by the model.

monoclonal antibody (MAb)-based assay may have been attributed to a modification of the MAb-recognized epitope during the vaccine production process. This possibility was ruled out by the use of an indirect ELISA, which confirmed the absence of seroconversion induced by the commercial vaccines. It can be argued that cell-mediated immunity, which was not measured here, is involved in the protection against the disease, but all CCPP vaccines are to this date supposedly inactivated vaccines and produced on the same model following the work from (Rurangirwa et al., 1987). They should therefore induce a similar humoral response detected by the tests used in this work, no matter the type of immunity required to protect against the disease.

Such results are of great concern for the protection of CCPP susceptible wildlife, but also for the subsistence of commercial goat herds, which use vaccines that are neither cheap nor effective.

To our knowledge, this is the first time that a SEIRD mathematical model is developed to describe the CCPP transmission dynamics and that model parameters are calibrated with observed data. The basic reproductive number R_0 was established between 2.3 and 2.7, with an average case fatality rate of around 60%. These values pertain to the epidemiological situation of the gazelle herd under study and they represent some kind of “maximum value” for CCPP, as the animals were permanently in very close contact. In practical terms, it means that CCPP is not very contagious but that an infected animal has a high probability of dying in the absence of antibiotic treatment. By comparison, R_0 values are much higher for viral diseases such as foot and mouth disease with an R_0 around 4.5 (Heffernan et al., 2005) or peste des petits ruminants with an R_0 certainly superior to 4 (Kivaria et al., 2013). Determining R_0 makes it possible to define the percentage of animals (q) that should be protected to prevent the dissemination of the disease by using the relation $q = 1 - \frac{1}{R_0}$. In the case of CCPP, q should be around 64% to stop the epizootic spread, if we assume that the CCPP vaccines are 100% efficient. Obviously, the commercial vaccines tested here did not induce such level of protection. Another efficient way to stop disease transmission is to reduce the contact rate β. This may be achieved by various means including the culling of symptomatic animals, their isolation in quarantine, or even their treatment with appropriate antibiotics to reduce mycoplasma shedding. All these measures can naturally complement the effect of vaccination and lead to a $R_0 < 1$, which would lead to the extinction of the epizootic disease situation.

5. Conclusion

CCPP may be transmitted at a distance of more than 50–80 m and that swift action must be taken to prevent dramatic losses, especially in flocks of endangered species kept in captivity. The most efficient strategy may be to vaccinate the animals with quality-assured CCPP vaccines injected simultaneously with an appropriate effective antibiotic treatment, as previously shown (Hunter, 2012). We strongly recommend that CCPP vaccine batches be validated by cELISA showing a clear seroconversion in vaccinated animals. Affected groups of animals can quite easily be treated with antibiotics by mixing tetracyclin to feed pellets. However such treatments may lead to the emergence of antimicrobial resistance, not only in the mycoplasmas but also in the rest of the bacterial flora. Preventive vaccination should always be preferred to antibiotic treatment, which should be restricted to emergency situations. This can only be advocated when good quality vaccines are available commercially, which did not seem to be the case in 2013.

From a practical point of view CCPP-free countries should pay a close attention to animals imported in zoos, as these may represent a risk of CCPP introduction. Further studies may be needed to establish if the increased detection of CCPP in wildlife results from raised awareness and improved diagnostic capabilities or if it is the result of an evolution of Mccp strains and a host switch, similar to what has been observed for *M. gallisepticum* in finches (Delaney et al., 2012).

Competing interests

None.

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Appendix A. Supplementary data

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References

Amirbekov, M., Murvatulloev, S., Ferrari, G., 2010. Contagious caprine pleuropneumonia detected for the first time in Tajikistan. EMPRES Transbound. Anim. Dis. Bull. FAO Anim. Prod. Health Div. 35, 20–22.

Arif, A., Schulz, J., Thiaucourt, F., Taha, A., Hammer, S., 2007. Contagious caprine pleuropneumonia outbreak in captive wild ungulates at Al Wabra wildlife preservation, State of Qatar. J. Zoo Wildl. Med. 38, 93–96.

Baguelin, M., Flasche, S., Camacho, A., Demiris, N., Miller, E., Edmunds, W.J., 2013. Assessing optimal target populations for influenza vaccination programmes: an evidence synthesis and modelling study. PLoS Med. 10, e1001527.

Bölske, G., Johansson, K., Heinonen, R., Panvuga, P., Twinamasiko, E., 1995. Contagious caprine pleuropneumonia in Uganda and isolation of Mycoplasma capricolum subspecies capripneumoniae from goats and sheep. Vet. Rec. 137, 594.

Bölske, G., Mattsson, J.G., Bascuñana, C.R., Bergström, K., Wesonga, H., Johansson, K.-E., 1996. Diagnosis of contagious caprine pleuropneumonia by detection and identification of Mycoplasma capricolum subsp. capripneumoniae by PCR and restriction enzyme analysis. J. Clin. Microbiol. 34, 785–791.

Castelet, 1906. La pleuropneumonie de la chèvre en Algérie. Prog. Vét. 27, 324–329.
Chaber, A., Saegerman, C., 2016. Biosecurity measures applied in the United Arab Emirates–a comparative study between Livestock and wildlife sectors. Transbound. Emerg. Dis.
Chaber, A., Lignereux, L., Al Qassimi, M., Saegerman, C., Manso-Silván, L., Dupuy, V., Thiaucourt, F., 2014. Fatal transmission of contagious caprine pleuropneumonia to an Arabian Oryx (Oryx leucoryx). Vet. Microbiol. 173, 156–159.
Chowell, G., Nishiura, H., 2014. Transmission dynamics and control of Ebola virus disease (EVD): a review. BMC Med. 12, 196.
Chu, Y., Gao, P., Zhao, P., He, Y., Liao, N., Jackman, S., Zhao, Y., Birol, I., Duan, X., Lu, Z., 2011. Genome sequence of Mycoplasma capricolum subsp. capripneumoniae strain M1601. J. Bacteriol. 193, 6098–6099.
Curasson, G., 1942. Pleuropneumonie infectieuse de la chèvre. Traité de pathologie exotique vétérinaire et comparée. Vigot frères, Paris, pp. 346–357.
Dagnelie, P., 1998. Statistique théorique et appliquée. De Boeck, Louvain-la-Neuve.
Delaney, N.F., Balenger, S., Bonneaud, C., Marx, C.J., Hill, G.E., Ferguson-Noel, N., Tsai, P., Rodrigo, A., Edwards, S.V., 2012. Ultrafast evolution and loss of CRISPRs following a host shift in a novel wildlife pathogen, Mycoplasma gallisepticum. PLoS Genet 8, e1002511.
Diekmann, O., Heesterbeek, J., Roberts, M., 2009. The construction of next-generation matrices for compartmental epidemic models. J. R. Soc. Interface rsif20090386.
El-Deeb, W., Almujalli, A.A., Eljalii, I., Elmoslemany, A., Fayez, M., 2017. Contagious caprine pleuropneumonia: The first isolation and molecular characterization of Mycoplasma capricolum subsp. capripneumoniae in the Kingdom of Saudi Arabia. Acta Trop. 168, 74–79.
Ferguson, N., Donnelly, C., Woolhouse, M., Anderson, R., 1999. Estimation of the basic reproduction number of BSE: the intensity of transmission in British cattle. Proc. R. Soc. Lond. B Biol. Sci. 266, 23–32.
Gingerich, D., Baggot, J., Kowalski, J., 1977. Tylosin antimicrobial activity and pharmacokinetics in cows. Can. Vet. J. 18, 96.
Giovannini, A., Bellini, S., Salman, M., Caporale, V., 2000. Spatial risk factors related to outbreaks of contagious bovine pleuropneumonia in northern Italy (1990–1993). Rev. Sci. Tech. Int. Off. Epizoot. 19, 764–772.
Heffernan, J., Smith, R., Wahl, L., 2005. Perspectives on the basic reproductive ratio. J. R. Soc. Interface 2, 281–293.
Howell, D., 1998. De Boeck, Louvain-la-Neuve. Méthodes statistiques en sciences humaines. M. Rogier, V. Yzerbyt, & Y. Bestgen, Trans.
Hunter, A., 2012. Treatment of contagious caprine pleuropneumonia. Vet. Rec. 171 542–542.
Hutcheon, D., 1881. Contagious pleuro-pneumonia in angora goats. Vet. J. 13, 171–180.
Ji, L.-W., Dong, L.-L., Ji, H., Feng, X.-W., Li, D., Ding, R.-L., Jiang, S.-X., 2014. Comparative pharmacokinetics and bioavailability of tylosin tartrate and tylosin phosphate after a single oral and iv administration in chickens. J. Vet. Pharmacol. Ther. 37, 312–315.
Jones, G., Wood, A., 1988. Microbiological and serological studies on caprine pneumonias in Oman. Res. Vet. Sci. 44, 125–131.
Keeling, M.J., Rohani, P., 2008. Modeling Infectious Diseases in Humans and Animals. Princeton University Press, Princeton NJ.
Kivaria, F.M., Kwiatek, O., Kapaga, A.M., Swai, E.S., Libeau, G., Moshy, W., Mbyuzi, A.O., Gladson, J., 2013. The incursion, persistence and spread of peste des petits ruminants in Tanzania: Epidemiological patterns and predictions. Onderstepoort J. Vet. Res. 80, 01–10.
Litamoi, J., Wanyangu, S., Simam, P., 1990. Isolation of Mycoplasma biotype F38 from sheep in Kenya. Trop. Anim. Health Prod. 22, 260–262.
Lorenzon, S., Manso-Silván, L., Thiaucourt, F., 2008. Specific real-time PCR assays for the detection and quantification of Mycoplasma mycoides subsp. mycoides SC and Mycoplasma capricolum subsp. capripneumoniae. Mol. Cell. Probes 22, 324–328.
Manso-Silván, L., Dupuy, V., Chu, Y., Thiaucourt, F., 2011. Multi-locus sequence analysis of Mycoplasma capricolum subsp. Capripneumoniae for the molecular epidemiology of Contagious caprine pleuropneumonia. Vet. Res. 42, 1–10.
Nicholas, R., Churchward, C., 2011. Contagious Caprine Pleuropneumonia: New aspects of an Old disease. Transbound. Emerg. Dis. 59, 189–196.
Otake, S., Dee, S., Corzo, C., Oliveira, S., Deen, J., 2010. Long-distance airborne transport of infectious PRRSV and Mycoplasma hyopneumoniae from a swine population infected with multiple viral variants. Vet. Microbiol. 145, 198–208.
Petrie, A., Watson, P., 2006. Statistics for Veterinary and Animal Science, 2nd ed. Blackwell Publishing, Oxford.
Peyraud, A., Poumarat, F., Tardy, F., Manso-Silván, L., Hamroev, K., Tilloev, T., Amirbekov, M., Tounkara, K., Bodjo, C., Wesonga, H., et al., 2014. An international collaborative study to determine the prevalence of contagious caprine pleuropneumonia by monoclonal antibody-based cELISA. BMC Vet. Res. 10, 48.
Rurangirwa, F., McGuire, T., Kibor, A., Chema, S., 1987. An inactivated vaccine for contagious caprine pleuropneumonia. Vet. Rec. 121, 397–400.
Spiegelhalter, D.J., Best, N.G., Carlin, B.P., Van Der Linde, A., 2002. Bayesian measures of model complexity and fit. J. R. Stat. Soc. Ser. B Stat. Methodol. 64, 583–639.
Thiaucourt, F., Bölske, G., 1996. Contagious caprine pleuropneumonia and other pulmonary mycoplasmoses. Rev. Sci. Tech Int. Epiz 15, 1397–1414.
Thomas, P., 1873. Rapport médical sur le Bou Frida, in: Publication du gouvernement général civil de l'Algérie. Jourdan A., Alger, pp. 35.
Woolhouse, M., Haydon, D., Pearson, A., Kitching, R., 1996. Failure of vaccination to prevent outbreaks of foot-and-mouth disease. Epidemiol. Infect. 116, 363.
Yu, Z., Wang, T., Sun, H., Xia, Z., Zhang, K., Chu, D., Xu, Y., Xin, Y., Xu, W., Cheng, K., Zheng, X., Huang, G., Zhao, Y., Yang, S., Gao, Y., Xia, X., 2013. Contagious caprine pleuropneumonia in endangered tibetan antelope, China, 2012. Emerg. Infect. Dis. 19, 2051–2053.

3.5. Risk assessment

3.5.1. Brucellosis risk assessment

We have observed a very high prevalence of brucellosis due to *B. melitensis* in the source population. Does this mean brucellosis constitutes a risk in the reintroduction project?

In the absence of a management strategy, the likelihood of releasing brucellosis with SHO reintroduction is extreme. Based on the 66% average seroprevalence, there would be an average of 17 individuals that carry the pathogen for every group of 25 reintroduced SHO.

Brucellosis leads to abortion and vaginal discharge associated with the excretion of very large quantities of the bacteria which can persist in organic matter for extended periods, causing environmental contamination, with a risk of transmission to other wildlife, to domestic livestock and to humans exacerbated by the context of shared pastures and possible animal concentration around water points.

A few serological studies indicate that brucellosis is enzootic in livestock and endemic in Chad. For instance, a study (Schelling et al., 2003) reported that brucellosis seroprevalence in Chari-Baguirmi and Kanem districts (600 km and 460 km from OROAFR, respectively, see Erreur ! Source du renvoi introuvable.) was 3.8% among 860 nomadic pastoralists and 7% among 608 cattle in 1999-2000. The disease was not found in 374 goats and 367 sheep. Another study conducted in the Yao and Danamadji districts (Batha region and Moyen Chari regions, 370 km and 750 km from OROAFR, respectively), (Özcelik et al., 2023) reported 0.2% among 966 humans, 0.3% in 1041 animals, with 3.9% apparent seroprevalence in cattle, 7.4% in sheep and 8.4% in goat.

Closer to the release site, (220 km from OROAFR) a study performed in the Abéché region reported a 2.6 % prevalence among 634 cattle (Delafosse et al., 2002).

With both cattle and small ruminants being affected, *B. abortus* and *B. melitensis* are likely prevalent in Chad, but, as in many African countries, bacteriological culture, isolation and typing are unfortunately only rarely performed and there is a shortage of information regarding *Brucella* species, biovars and genotypes circulating in Chad in general and in the Batha region in particular.

For instance, 7212 *Brucella* strains that were genotyped with the common MLVA technique were retrieved (on November 27, 2023) from the publicly available database *Brucella* v4_6_5 hosted at https://microbesgenotyping.i2bc.paris-saclay.fr/databases/public. Only six MLVA allele profiles originated from Chad, and they were all from *B. abortus*. *B.melitensis* has nonetheless been identified in at least one traveller returning from tropical Chad (Badiaga et al., 2005), and one Chadian *B.melitensis* strain was included in a study (Foster et al., 2018).

Given the presence of brucellosis in Chad, the likelihood of introducing a new pathogen into the country through this project is negligible. However, the situation in the OROAFR or the Batha administrative region remains unknown, both among domestic herds and wildlife populations.

Brucellosis in livestock can result in economic losses for local communities and livestock industries. The reintroduction of oryx carrying the disease may pose a risk to domestic animals if there is an overlap in grazing areas.

On the other hand, the impact of brucellosis would have in the released area is extreme.

We observed that brucellosis causes arthrosis in gazelle species (see **Article 1**), a condition that is not compatible with survival in the wild, therefore the impact would potentially be extreme on local vulnerable dorcas gazelles and critically endangered dama gazelles.

The impact of brucellosis in humans, with limited access to health services and treatment, would be very high, causing debilitating disease and risk of abortion.

Furthermore, releasing animals that may carry and transmit diseases raises ethical questions regarding the health and welfare of both the reintroduced animals and local populations and ecosystems.

3.5.2. Foot-and-mouth disease risk assessment

FMD is endemic in the UAE (Eltahir et al., 2024). We have observed that SHO can acutely contract FMD types O and A. However, our understanding of chronic FMD infection in SHO remains limited, prompting the need for further investigation. Our phylogenetic study suggests that the disease has likely recurrently emerged from neighbouring countries, indicating the potential for a recent infection coinciding with a SHO translocation. This raises concerns about viral excretion during transport and release, posing a significant risk of disease transmission in Chad.

In cases of prior exposure, the epidemiological role of SHO remains unclear and warrants in-depth study, particularly concerning carrier status and reservoir function.

The Chadian Animal Disease Surveillance Network (REPIMAT) actively monitors FMD. The disease is enzootic in Chad as well, with serotypes A, O, SAT 1, and SAT 2 described (Ouagal et al., 2018; Abdel-Aziz et al., 2019) specifically in the Batha region where the release site is located (see Erreur ! Source du renvoi introuvable.**).**

The likelihood of releasing FMD during the reintroduction is therefore directly proportional to the likelihood of exposure. However, it is paramount to mitigate the risk of pathogen pollution and the release of

non-African strains, such as strain A from Asia, which do not naturally occur on the African continent (Knowles et al., 2005).

Similarly, the impact of an FMD release is likely equivalent to the impact of the enzootic Chadian FMD. However, SAT1 and 2 have been described in Chad and the possible effects of these two serotypes on released SHO remain unknown.

On the other hand, there is a risk of FMD transmission to local wildlife species in Chad, with unknown impact.

While the presence of FMD in both regions may suggest a shared disease risk, the specific strain, its prevalence, and the potential consequences of reintroducing oryx carrying the disease should be carefully assessed. Therefore, we suggest that a comprehensive disease risk analysis and the implementation of appropriate mitigation measures are essential to ensure the health and safety of both the reintroduced animals and the surrounding ecosystem.

3.5.3. Contagious caprine pleuropneumonia risk assessment

CCPP was isolated in Chad in 1987 and 1994 and it is likely present in the country without clear geographical distribution (Manso-Silván & Thiaucourt, 2019). Therefore, the likelihood of releasing CCPP in Chad is elevated, as well as the likelihood of exposure.

With mortality documented in gazelles' species, the impact of CCPP could be elevated in local gazelles' species.

3.5.4. Bovine tuberculosis risk assessment

Bovine tuberculosis (bTB) is a chronic debilitating zoonotic disease. It has a very broad host range and several wildlife reservoirs have been documented including white-tailed deer (*Odocoileus virginianus*), elk (*Cervus canadensis*), Eurasian badger (*Meles meles*), European wild boar (*Sus scofa*), brushtail possum (*Trichosurus vulpecula*), African buffalo (*Syncerus caffer*) and lechwe antelope (*Kobus leche*) (Fitzgerald & Kaneene, 2013), however, the main host and source of infection for humans is cattle. Infected animals may experience chronic weight loss, respiratory issues, and general debilitation.

The disease is caused by *Mycobacterium bovis*, a Gram-positive acid-base resistant bacteria belonging to the *Mycobacteriun tuberculosis* complex (MTBC), along with *M. tuberculosis, M. africanum, M. microti, M. caprae, M. pinnipedii* and *M. canettii*. (Riojas et al., 2018).

While not officially notified to the WOAH, *M. bovis* is likely present in the UAE, with presence mentioned in official reports from the Abu Dhabi Agriculture Food Safety Authority (ADAFSA), formerly

ADFCA (Abu Dhabi Food Control Authority) (ADFCA, 2010; ADFCA, 2011). Apart from these reports, no published information has been found on *M. bovis* in the UAE.

We have cultured, isolated and genotyped using mycobacterial interspersed repetitive unit - variable-number tandem repeat (MIRU-VNTR) techniques, *M. bovis* from a calf SHO that was positive to the intradermal comparative cervical tuberculin (ICCT) skin test (unpublished data). A large percentage of SHO source population is positive to this same test. This finding requires further research and possibly warrants publication.

On the other hand, bTB is also prevalent in Chad where it was notified to the WOAH (see Table 1). Therefore, it constitutes a risk of exposure too. The bacterium was isolated from zebu carcasses (Diguimbaye-Djaibé et al., 2006). Closer to the release site, a study performed in the Abéché region (220 km from the OROAFR, see Erreur ! Source du renvoi introuvable.) using the ICCT reported a 0.6% prevalence among 848 cattle (Delafosse et al., 2002).

bTB is a concern for several reasons. It is a zoonotic disease with aerosol transmission posing a risk to wildlife researchers, local communities, and anyone who might come into contact with infected animals. It is a risk for local cattle and their herders and, due to the wide range of mammals it can affect, including ungulates like oryx, bTB can potentially impact the reintroduced population's fitness and survival. For instance, bTB had lethal outcomes in Arabian oryx after they were stressed during transportation (Kock et al., 2010). The reintroduced oryx population may interact with native wildlife species, potentially leading to disease transmission within the ecosystem. Through its impact on wildlife health, bTB can have an impact on conservation. Possibly affecting local livestock industries or leading to trade restrictions on livestock products, bTB can have economic implications. Lastly, releasing animals that may carry and transmit diseases raises ethical questions regarding the welfare of both the reintroduced animals and local ecosystems.

3.5.5. Contagious bovine pleuropneumonia (CBPP) risk assessment

The causative agent for this disease is *Mycoplasma mycoides* subsp. *mycoides* (*Mmm*). It can affect cattle, zebu (*Bos indicus*), yaks (*Bos grunniens* and *B. mutus*), American bison (*Bos bison*) and Asian water buffalo (*Bubalis bubalis*). Other ruminants are generally not considered hosts for CBPP (WOAH, 2023) With this in mind, the SHO's susceptibility to this disease, while unknown, is likely limited.

The disease is enzootic in sub-Saharan Africa (Masiga et al., 1998; Di Teodoro et al., 2020) and was persistently notified in Chad (see Table 1). It has not been detected in the UAE but was suspected following incursions with cattle trade from Africa (Lefèvre, 1991) and reintroduced SHO are likely naive to this disease.

3.5.6. Lumpy skin disease, sheeppox, and goatpox risk assessment

These three diseases affect cattle, sheep and goat, respectively with similar clinical signs. They are caused by closely related viruses belonging to the *Poxviridae* family, genus *Capripoxvirus* (Hamdi et al., 2021). Due to an antigenic homology, serological tests cannot differentiate between viruses and definite diagnostic require virus-specific identification techniques.

Sheep and goat pox were reported in the UAE until 2008 and in Chad in 2018 and 2019. Capripox viruses are present in neighbouring countries: Yemen, Oman (Kitching et al., 1986), Saudi Arabia (Kasem et al., 2018) , Sudan (Khalafalla et al., 1993), Nigeria (Limon et al., 2020). They are likely still present in Chad and the UAE (Babiuk et al., 2008; Hamdi et al., 2021). Capripox virus infection has been documented in closely related Arabian oryx in Saudi Arabia (Greth et al., 1992), and the SHO source population has been affected by goatpox (see Figure 6), as evidenced by PCR, associated with mortality (unpublished information).

Figure 6: Scimitar-horned oryx presenting scars from past goatpox infection

3.5.7. Ticks and tick-borne pathogens

While hard tick species occur in the UAE (Perveen et al., 2021 and Perveen, 2021), as well as tick-borne pathogens such as *Anaplasma phagocytophilum*, *Babesia/Theileria* spp (El Tigani-Asil et al., 2021), the SHO population is devoid of external parasites due to ectocide treatment and surveillance. Also no clinical signs suggesting tick-borne infections have been observed. This population is therefore considered naive to tick-borne pathogens.

Several tick species have been documented in Chad (Walker et al., 2003), possibly vectors for several infectious diseases (Mucheka et al., 2023), all posing a risk of exposure to the reintroduced SHO.

3.5.7.1. Babesiosis risk assessment

Also known as redwater disease, it is caused by Piroplasmids protozoa of the genus *Babesia*, which are transmitted by hard tick species. Various species of *Babesia* can infect livestock, with each species having a preferred tick vector (Antunes et al., 2017). Upon invading the host's red blood cells, *Babesia* sp. triggers clinical signs such as fever, anaemia, diarrhoea and hemoglobinuria (Ristic, 1981). The negative impact of babesiosis on wildlife and conservation actions has been documented, with fatal cases for instance in sable antelope (*Hippotragus niger*) or black rhinoceros (*Diceros bicornis*) (Penzhorn, 2006).

While *Babesia* sp. has not been documented in Chad, its presence has been reported in neighbouring countries like Nigeria (Dipeolu & Amoo, 1984) and Sudan (Springer et al., 2020).

3.5.7.2. Theileriosis risk assessment

Different diseases are caused by Piroplasmid protozoan from the *Theileria* genus. The associated clinical signs include high fever, enlarged lymph nodes, dyspnea, anaemia, and possible high mortality. The UAE reported an outbreak in 2013 (see Table 1).

Theileria parva causes the **East Coast fever**, an acute disease of cattle, distributed mainly in east and southern Africa and transmitted by *Rhipicephalus appendiculatus* (Morrison, 2022).

T. annulata is the causal agent of **tropical theileriosis or Mediterranean theileriosis**, which is widely distributed in north Africa, the Mediterranean coastal area, the Middle East, India, countries of the southern former USSR, and Asia. It is transmitted by several species of ticks of the genus *Hyalomma* (Morrison, 2024)). It can cause mortality of up to 90%, but strains vary in their pathogenicity. It has not been reported in Chad, but it was reported in Nigeria (Mamman et al., 2021).

The *T. orientalis* group causes other theilerioses of cattle, in this group. *T. velifera* has been documented in Chad (Uilenberg, 1970).

Other *Theileria* species will affect different hosts: for instance, *T. lestoquardi* causes the malignant ovine theileriosis, affecting sheep and goats (WOAH, 2023).

3.5.7.3. Anaplasmosis risk assessment

Caused by bacteria of the order Rickettsiales belonging to the genus *Anaplasma*. This disease is transmitted by up to 17 species of tick vectors. Among them, the bacterium *A. marginale* causes bovine erythrocytic anaplamosis, while other species such as *A. phagocytophilum, A. platys* or *A. centrale* have different preferred hosts (Tabor, 2022). In young cattle, the disease is usually subclinical, but it induces anaemia, jaundice, and, in severe cases, death in older animals (Aubry & Geale, 2011).

Although *Anaplasma* sp. has not been directly reported in Chad, its presence has been documented in neighbouring countries (Cossu et al., 2023), leading to suspicions of its occurrence in Chad.

Additionally, cases affecting cattle have been notified by the UAE in 2014, 2022 and 2023 (see Table 1).

3.5.7.4. Crimean-Congo Haemorrhagic Fever risk assessment

Caused by the *Orthonairovirus haemorrhagiae* a ribonucleic acid (RNA) virus of the genus *Orthonairovirus,*in the family Nairoviridae, within the Bunyavirales order (ICTV, 2024) this disease is zoonotic and lethal, with no vaccine available yet.

Positive serology results indicating exposure to the Crimean-Congo haemorrhagic fever (CCHF) have been documented in camel, cattle, sheep and goats from the UAE (Khan et al., 1997). Human cases of CCHF were reported in the UAE (Perveen & Khan, 2022), although the disease is not considered endemic in this country (Al Dabal et al., 2016).

While not directly reported in Chad, its principal tick vector *Hyalomma* spp. has been documented there and the disease is likely present there (Messina et al., 2015; Temur et al., 2021).

3.5.7.5. Heartwater or Cowdriosis risk assessment

This disease is caused by the protozoan parasite *Ehrlichia ruminantium*, transmitted by ticks of the genus *Amblyomma*. It primarily affects ruminants and can cause high mortality.

Heartwater, present in Africa and reported in Chad in 2013 and 2021, has been observed affecting various wildlife species (Peter et al., 2002). However, SHO was reported to be resistant to the infection in a literature review (Oberem & Bezuidenhout, 1987), though this claim relies on unpublished data for this claim and requires further verification.

3.5.8. Insect-borne diseases

3.5.8.1. Rift valley Fever (RVF) risk assessment

Rift Valley fever is a viral disease affecting humans, sheep, goats, cattle, water buffalo and wildlife species (Rostal et al., 2017). Species and individual susceptibility and clinical signs might vary and include fever, anorexia, reluctance to move, abortion and death. Morbidity and mortality are higher in neonates.

It is caused by a segmented negative-sense RNA virus belonging to the genus *Phlebovirus,* family *Phenuiviridae* in the order of Bunyavirales. Only one serotype exists butthere are different strains. It is vector-borne by mosquitoes from the *Aedes* and *Culex* families. Outbreaks usually follow heavy rainfalls, with severity increasing with the length of relative drought, due to higher number of naive individuals.

Historically, the disease has been limited to the African continent. While only sporadically notified, it is present in Chad (Ringot et al., 2004). A study (Özcelik et al., 2023) showed that RVF seroprevalence was 28.1% among 966 humans and 10.2% among 1041 livestock in the Yao (in the Batha region) and Danamadji (in the Moyen Chari region) rural health districts (see Erreur ! Source du renvoi introuvable.).

There have been incursions of RVF in the Middle East, in Saudi Arabia (Balkhy & Memish, 2003) associated with fatal cases, but the disease is considered not endemic in this region.

According to the WOAH report from May 20, 2019 (WAHIS, 2019), based on RVF-specific PCR, 69 SHO were thought to suffer from RVF among the 171 released and 42 reportedly died of it in September 2018.

3.5.8.2. Trypanosomiasis risk assessment

Numerous *Trypanosoma* species cause a range of diseases, some with potential implications for both humans and animals (Wilkowsky, 2022). Human African Trypanosomiasis (HAT or sleeping sickness) falls into the former category, while Animal African Trypanosomosis (AAT or nagana) is specific to animals. *Trypanosoma* are protozoa and most of the species rely on the tsetse fly (*glossina*) as their biological vector, mechanical transmission has been associated with Tabanids and Stomoxys. *T. brucei* had been responsible for the death of all Southern white rhinoceroses (*Ceratotherium simum simum*) during a high-profile reintroduction in Kenya (Kock et al., 2010).

In Chad, various *Trypanosoma* species have been identified, with a more comprehensive understanding of those causing HAT. Their distribution tends to be concentrated in the eastern and southern regions of the country (Vourchakbé et al., 2022). On the other hand, information about the species responsible for AAT is more scattered. *T. vivax,* for instance, has been documented in the Lake Chad area (Delafosse et al., 2006), while *T. evansi*, linked to "Sura" in camels, has been reported in eastern Chad (Delafosse & Doutoum, 2004) and notified several times in the UAE (see Table 1).

3.6. Risk management

3.6.1. Brucellosis risk management

3.6.1.1. Likelihood reduction

The likelihood to release brucellosis in the OROAFR can be reduced in different ways.

The management of the risk of releasing brucellosis involved simultaneous approaches within the UAE collection: mass vaccination with the Rev. 1 vaccine, and animal testing for brucellosis associated with isolation. Faced with the unknown, these two strategies necessitated preliminary studies, such as evaluating the safety and effectiveness of live *Brucella* vaccines and studying parameters for brucellosis screening tests in this species. In **Article 6**, we lay the groundwork for controlling this outbreak and mitigating the risk of disease transmission in Chad. In this study, studied the immune responses, both humoral and cellular, to the administration of one dose of *B.melitensis* Rev. 1 vaccine administered via subcutaneous or conjunctival routes in young SHO. Our focus is on vaccine safety and the long-term test results post-vaccination, essential for informed decision-making in disease management strategies.

Another approach aimed at obtaining *Brucella*-free animals, offering a faster initiation of the reintroduction under favourable conditions, and introducing new bloodlines to increase the genetic diversity of the UAE population. This involved the procurement of animals from *Brucella*-free countries or collections within the UAE.

As seen in the introduction, large populations of SHO are available in zoological institutions and hunting ranches across the USA, mainly in Texas where BTV is enzootic. The disease is also present in the UAE (Frölich et al., 2005). In this context and apart for the possible different serotypes found in these two countries, translocating from the USA to the UAE does not pose a major risk of releasing or exposing to the disease. However, the shipment transited through western Europe, where strict regulations were in place to prevent BTV introduction, creating a risk of release in this part of the world. We have investigated this risk and the results were published in **Appendix 1**.

3.6.1.2. Impact reduction

Limit the impact of brucellosis on domestic livestock: after an active brucellosis surveillance plan, that includes the investigation of circulating *Brucella* species and strain a vaccination campaign associated with animal identification and monitoring can be suggested in livestock.

Limit its impact on public health, through awareness campaigns aiming at reducing the contact with aborted material or preventing transmission from milk, but also improved access to health system.

Mitigate the impact of brucellosis on wildlife by preventing the risk of release. As released SHO are expected to share pastures and potentially interact with local wildlife, safeguarding the latter from potential brucellosis becomes challenging. Although active brucellosis surveillance in local wildlife could assess the situation and detect new cases, capturing wildlife induces significant stress, possibly leading to lethality. It's crucial to evaluate the cost/benefit ratio before undertaking such measures.

There are opportunities to assess the prevalence of the pathogen in both livestock and humans. This includes studying the circulating *Brucellae* species and their various strains, while also establishing reference values for genotyping, phylogenetic analysis, and pathogen tracing purposes.

ARTICLE 6

Comparison of immune responses to *Brucella melitensis* Rev.1 conjunctival or subcutaneous vaccinations in sexually immature scimitar-horned oryx (*Oryx dammah*)

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Lignereux Louis, Pesci Maria-Elena and Claude Saegerman

Comparison of immune responses to *Brucella melitensis* Rev.1 conjunctival or subcutaneous vaccinations in sexually immature endangered scimitar-horned oryx (*Oryx dammah*)

Louis LIGNEREUX[1]; Maria Elena PESCI[2]; Claude SAEGERMAN[1]

[1]Faculty of Veterinary Medicine, Research Unit in Epidemiology and Risk Analysis Applied to Veterinary Sciences (UREAR-ULiège), Fundamental and Applied Research for Animals and Health (FARAH) Center, University of Liège, Liège, Belgium.; claude.saegerman@uliege.be
[2]Terrestrial Biodiversity, Environment Agency- Abu Dhabi, Abu Dhabi, United Arab Emirates. elenapesci@hotmail.com

Corresponding author: Louis LIGNEREUX l_louis77@hotmail.com

Abstract

A single dose of 1-2 10^9 CFU of the *Brucella melitensis* Rev.1 vaccine strain was administered either via the subcutaneous or the conjunctival route to two groups of ten sexually immature scimitar-horned oryx (*Oryx dammah*) (SHO). A third group of ten was kept unvaccinated as control. These groups were housed together and bred, producing offspring. No clinical signs were observed during the week following administration. The rose Bengal test (RBT), a lateral flow assay (LFA) and the brucellin skin test (BST) were performed before and throughout the experiment to assess the humoral and cellular immune responses after vaccination. These responses were rapid and strong. The cellular response was durable and similar in both groups, with 80% (12 out of 15) of SHO vaccinated using either route still positive 184 weeks post-vaccination (PV). The conjunctival route provided a shorter serological response, with all animals RBT seronegative 12 weeks PV, instead of 57% still positive 74 weeks PV with the subcutaneous route. The level of LFA positivity remained elevated and not different in the two groups until 30 weeks PV where it decreased quicker in the group vaccinated conjunctivally. Eventually, all SHO returned to LFA negativity 74 weeks PV. No reaction to RBT and BST was observed either in the control group or the offspring. Consistent with results in young goats, those findings provide a first step for the strategic management of brucellosis outbreaks in captive SHO.

Keywords

immune response; wildlife; prevention; randomised controlled trial

1. **Introduction**

Brucellosis, also known as Malta fever, is a debilitating zoonotic disease always associated with an animal reservoir in humans. It is caused by facultative intracellular Gram-negative bacteria. Among 12 known *Brucella* species (Kurmanov et al., 2022), *Brucella melitensis* causes ovine and caprine brucellosis and is the main cause for human brucellosis (Young, 1995). Since its development in 1957 by Elberg et al., the attenuated live *B. melitensis* Rev.1 strain has been the central component to manage outbreaks in domestic ruminant species when prevalence is over 2% (Fensterbank et al., 1987). This vaccine provides a long-lasting immunity, but vaccine-induced abortions in sexually mature females (Jiménez et al., 1989), and pseudo-horizontal transmission to the offspring through colostrum and milk limits its use to younger, sexually immature, animals when pregnancy status is unknown. Because the subcutaneous administration induces a persistent serological reaction, hindering discrimination between vaccinated and infected individuals, the conjunctival route has been developed: it provides a protection almost as good in young ewes (Blasco, 2010), with a serological response usually disappearing within 4 months (Fensterbank et al., 1985).

Brucellosis outbreak management in wildlife aims at preventing spillover to domestic species and ultimately to humans (Godfroid et al., 2013). Culling might be an option for domestic species, but it is usually not socially acceptable in wildlife due to conservation status (Ponsart et al., 2019). Vaccines might then be a realistic option for brucellosis control, but no *Brucella* vaccines are currently registered for use in wildlife (Godfroid et al., 2010). Their safety and efficacy must be assessed in the targeted species beforehand. In preparation for controlling a *B. melitensis* biovar 1 outbreak (Lignereux et al., 2022a) affecting captive endangered scimitar-horned oryx (*Oryx dammah*) (SHO), this study aims to observe the possible side effects and assess the humoral and the cell-mediated immune responses following the single administration of a standard dose of *B. melitensis* Rev.1 vaccine by two different routes to sexually immature SHO of both sexes. This is the First documented use of this vaccine in this species.

2. MATERIALS AND METHODS

2.1. Animals and vaccines

In this experiment, we conducted a randomized controlled trial on 30 sexually immature and apparently healthy SHO. They originated from a parent group undergoing biannual active brucellosis serological surveillance with the rose Bengal test (RBT). This group was considered *Brucella*-free. The parent and the trialed groups were held in separate enclosures in, but isolated within, the collection affected by *B. melitensis* (Lignereux et al., 2022a). Physical isolation, with a buffer zone and strict biosecurity measures applied to the staff and to the equipment limited the risk of transmitting brucellosis.

On April 17, 2017, the 30 SHO were driven into a mechanical restrain device (Tamer junior®, Fauna Research, USA). They were aged, sexed and identified with an ear tag and a subcutaneous microchip. Blood was collected via jugular venipuncture using plain vacuum tubes with clot activator and separation gel. The first steps of the brucellin skin test (BST) (see below), with the injection of brucellin, were performed. After processing all 30 animals, the data was collated in a spreadsheet, and the SHO were randomly distributed into three experimental groups of ten animals each. One group would receive one single drop of OCUREV® (CZ Veterinaria, Spain) batch #142477 (exp 9/2015) in the right lower conjunctival sac while one millilitre of CZV REV-1® (CZ Veterinaria, Spain) batch #153295 (exp 11/2016) would be injected subcutaneously in the pre-scapular area in the second group. According to the manufacturer's leaflets, each dose contains 1 to 2 x 109 colony forming units (CFU) of *B. melitensis*, strain Rev.1. This corresponds to the standard dose in small ruminants. The control group would be left unvaccinated.

The 30 SHO were recaptured three days after. The freeze-dried vaccines were reconstituted. The BST was read, and the Rev.1 vaccines were administered according to the schedule.

The three groups were housed together in the same enclosure for the entire experiment to ensure identical conditions and possible vaccine strain circulation.

Eventually, they reached sexual maturity and bred. Part of the animals were removed between weeks 30 and 74 for population management (brucellosis unrelated) reason. On October 25, 2020, 184 weeks post-vaccination (PV), during the last capture of the experimental groups, the offspring were tested with RBT and BST.

2.2. Clinical examination

Throughout the week following vaccination, we conducted daily monitoring of each animal for any clinical signs, such as discomfort or abnormal behavior, with particular attention to the sites of vaccine administration.

2.3. Tests and schedule

2.3.1. **Serological tests**

The humoral immune response was assessed with the RBT and a lateral flow assay (LFA) on serum. The RBT (Bengatest®, Synbiotics, France) is a rapid buffered agglutination test. To perform the test, we mixed 30 µl of an inactivated and concentrated solution of *B. abortus* strain 99 (Weybridge) stained with rose Bengal with an equal volume of serum on a clean, single-use microscope slide following the protocol outlined in (WOAH, 2022). We observed the agglutination pattern after four minutes of gentle agitation and considered the test positive when agglutination occurred. Separately, we assigned scores ranging from 0/6 (negative) to 6/6 (very strong positive) to semi-quantify each result. The characteristics for interpretation of each score are given in Appendix 1

In addition to the RBT, we employed a LFA (Antigen Rapid Bovine *Brucella* Ab Test Kit, RB2301DD, Bionote, South Korea). It is a chromatographic immunoassay based on the deposition of an invisible band of *B. abortus* 1119-3 lipopolysaccharide (LPS) on a nitrocellulose membrane. Sera were used and the test was performed following the manufacturer's instructions. We scored for each result, with the interpretation detailed in Appendix 1.

2.3.2. Allergic test

The cell-mediated immune response was evaluated with the brucellin skin test (BST) (Saegerman et al., 1999). In the middle on the left side of the neck, a 5x5cm skin area was clipped and its skinfold measured with a vernier calliper to the nearest ½ mm. Then we injected 0.1ml of Brucellergene OCB® (Synbiotics, France), intradermally in the center of the area with insulin syringe and needle. This is equivalent to 200 units of *B. melitensis* B115 rough brucellin protein extract.

After three days, the same veterinarian measured the same skinfold. Both measurements were reported. A reaction was considered positive if the skinfold thickness increased by more than 1mm (i.e., equal or greater than 1.5mm), which is modified from (Saegerman et al., 1999).

2.3.3. Testing schedule

We tested serologically and intradermally all animals three days before vaccination.

We performed the RBT after 2, 4, 12, 30, 74 and 184 weeks, and the LFA at 2, 4, 12 and 30 weeks PV. We conducted the BST at 4, 12, 30, 74 and 184 weeks PV. The specific vaccination and testing timeline is provided in Supplementary material 1.

2.4. Statistical Analysis

The Agresti-Coull (modified Wald) method was used to calculate the 95% confidence intervals (CI) for the percentages of positivity while the binomial method was used to calculate the 95% CI for the means.

Homogeneity of the three groups was evaluated with the sex and age distributions, with a Chi-squared test and a one-way ANOVA, respectively.

For each pair of experimental conditions (control vs. conjunctival route; control vs. subcutaneous route; conjunctival route vs. subcutaneous route) at each sampling time point and for each of the three tests, we compared the mean percentage of SHO that tested positive and the mean score of the reactions, using a mixed effect analysis (Prism®, Graphpad, USA) because the number of animals was not the same over the length of the experiment. We applied the Tukey method (Lee and Lee, 2018) to correct the confidence intervals for multiple comparisons.

All *p*-values <.05 were considered significant.

3. Results and discussion

3.1. Group homogeneity

Group homogeneity was assessed with data presented in Supplementary material 2. The estimated age spanned from two months (59 days), to approximately 12 months old (366 days), with an average estimated age of 127.4 days. The three groups were homogenous, with age and sex distributed similarly in the three groups ($F(2, 27) = 0.5511$, $p = .5827$), ($\chi 2(2$ d.f., $N=30) = 0.2679$, $p = .8747$), respectively.

3.2. Immune responses to vaccines

We did not observe visible clinical signs or abnormal behavior during the week following the vaccination.

All 30 animals tested negative for all three tests before vaccination (see Figure 1) and none of the control animals showed a positive reaction to either the RBT or the BST during the entire experiment, this demonstrates no evidence of false positive reactions, infection from the environment, or transmission of the vaccine strain from vaccinated to unvaccinated individuals. This last point aligns with observations made in vaccinated pregnant ewes and goats (Zundel et al., 1992) but differs from those in Alpine ibex (*Capra ibex*) vaccinated ocularly, where in-contact control ibex did seroconvert (Ponsart et al., 2019). Additionally, the repeated intradermal injection of brucellin during the multiple BST did not lead to any measurable response in this group, which is in agreement with similar findings in cattle (Fensterbank et al., 1977).

Also, we found that the intensity of the reaction to brucellin does not decrease over time after repeated testings, which contrasts with the situation in cattle (Saegerman et al., 1999).
Although two animals elicited a positive LFA result in the unvaccinated group 12 (scores 2/6 and 3/6) and 30 weeks PV (score 2/6) (see Appendix 2). Because these reactions were transient and not associated with positivity to other tests, this outcome supports our previous suspicion of a lower LFA specificity (Lignereux et al., 2022b).

On the other hand, all vaccinated animals became positive to all tests.
Overall, **the response measured with RBT** was associated with a rapid onset and an elevated score (not statistically different between the vaccinated groups 2 weeks (p = .0558, see Appendix 3) and 4 weeks PV (p = .1002).
Among the 10 animals vaccinated conjunctivally, nine seroconverted after two weeks, with the remaining animal seroconverting after four. This animal returned to negativity almost immediately, but it had longer responses when measured with LFA and BST (see Appendix 2).
The response was short and 0% seropositivity was reached by 12 weeks PV and remained until the end of the experiment.
Other species presented slightly different results: while kids presented similar responses to the conjunctival vaccination, all becoming seropositive at 4 weeks PV, they all returned to seronegativity slightly later, at 16 weeks PV (Fensterbank et al., 1987). The situation in lamb is also different, with fewer animals seroconverting. Only 10% (n=30) (Fensterbank et al., 1982), to 45% (n=33) (Fensterbank et al., 1985) became RBT positive, which was also associated with a quick return to seronegativity starting at 4 weeks PV, and achieved at 10weeks PV.
Conversely, mostly sexually mature Alpine ibex exhibited long-lasting serological reaction with all animals still positive 13 weeks PV (Ponsart et al., 2019). The age at vaccination might explain those differences more than taxonomic relatedness as the duration of the serological response to brucellosis vaccination is shorter in sexually immature animals (Fensterbank et al., 1987).
In contrast, all animals vaccinated subcutaneously remained positive until 30 weeks PV, apart from one animal found seronegative 12 weeks PV (see Appendix 2). The difference with the group vaccinated conjunctivally was significant 12 and 30 weeks PV (p < .0001, see Appendix 3). At 74 weeks PV, 57.14% (95%CI [100, 77%]) of the group vaccinated subcutaneously were still RBT-positive, but the humoral response faded away and no animal tested positive 184 weeks PV.
This duration following the subcutaneous administration is longer in SHO than in domestic small ruminants. In lambs and kids, the percentage of RBT-positivity started to decline as soon as 4 and 8 weeks PV, respectively. Interestingly, the percentage of positivity plateaued at approximately 50%

until the end of the experiments, which happened 64 weeks in lambs (Fensterbank et al., 1982) and 32 weeks in kids (Fensterbank et al., 1987).

In comparison, **the immune response following conjunctival vaccination measured with the LFA** started as early , with all animals testing positive to LFA 2 weeks PV, but lasted longer than with the RBT: Nine animals (out of 10) remained positive 12 weeks PV instead of zero with the RBT, and the percentage of animals positive to LFA decreased to 30% (95CI [64.6, 0]) 30 weeks PV, returning to 0% 74 weeks PV. On the other hand, the response to the subcutaneous vaccination followed an evolution with LFA like the one obtained with RBT.

The mean LFA score was similar in both vaccinated groups until 12 weeks PV (identical 2 weeks PV and not different 4 and 12 weeks PV, p>.9999), but different 30 weeks PV (p = .0173), and not different 74 weeks PV (p = .1513) (see Appendix 3).

The percentage of positivity followed this evolution and was only significantly different between the vaccinated groups 30 weeks PV (p=.0034) (see Appendix 3).

To the best of our knowledge, no previous studies have used this test to evaluate the immune response following Rev.1 vaccination in other species.

It is noteworthy that the manufacturer of the LFA produces a similar rapid test, “Rapid GS.brucella Ab”, labelled for caprine and ovine brucellosis detection, which may have different levels of sensitivity and specificity.

Lastly, **the cellular response measured by the BST** is also rapid as it happened within 4 weeks PV in all vaccinated animals and persistent, lasting at least until the end of the experiment: five out of seven SHO that received the subcutaneous form and seven out of eight that received the ocular form were still positive to the BST 184 weeks PV (3 ½ years).

There was no significant difference at any time point between the two groups for both the percentage of positive animals (4, 12 and 30 weeks PV p=.5951; 74 weeks PV p=.3358 and .7564 184 week PV, see Appendix 3) and regarding the skinfold thickness difference (4 weeks PV, p=.2671; 12, 30 and 74 weeks PV p>.9999 and .1634 weeks 184 PV).

To the best of our knowledge, no information is currently available in domestic or wildlife species regarding such an extended duration of immune response, however (Pardon et al., 1989) reported that out of 50 ewes vaccinated with Rev.1 only four (8%) were positive to the BST performed on the eyelid three years PV.

No information regarding the cellular immunity provided by Rev.1 vaccine and the protection against brucellosis is available in SHO, but previous study has suggested that cellular immune response correlates with immunity in adult goats vaccinated subcutaneously with Rev.1 (Brinley Morgan et al., 1966).

It's worth noting that two individuals vaccinated conjunctivally presented fluctuating BST results: after testing positive 4 weeks PV, then turned negative either 12 or 74 weeks PV (see Appendix 2) but tested positive during the following testing. This finding raises concerns regarding the sensitivity of the BST.

3.3. Absence of positive test in non-vaccinated offspring

The offspring did not show any positive reactions in RBT (n=19) nor in the BST (n=17, excluding the two youngest animals aged 54 days). This observation is preliminary and might indicate an absence of transmission of the vaccine strain from the vaccinated adults to their offspring. Specific study associated with bacteriological investigation should confirm this observation.

3.4. Limitations of the study

Several logistical and technical constraints posed some limitations: No live bacteria count in vaccine doses was performed and we relied solely on information from the manufacturer.

As a result of logistical constraints, we utilized vaccines and reagents beyond the expiration dates indicated on the packaging. The subcutaneous and the conjunctival vaccines were five and 19 months expired, respectively. All tests and vaccines were stored in the dark at 6°C before use. Nevertheless, we observed immune responses with both vaccines that were consistent with findings reported in the literature from fresh batches, which tends to confirm the excellent stability of the Rev.1 vaccine claimed by Milward (1995) who suggested that this stability exceeds 5 - 10 years.

Brucellin had been commercially unavailable for a long time. We employed a batch that expired over four years before we used it for the second BST (see Supplementary material 1). However, the results conformed to expectations, indicating that the freeze-dried purified protein extract, which is supplied in a sealed vial devoid of oxygen and kept in obscurity at 6°C. is expected to have excellent stability (M.Zygmunt, INRAE - France, personal communication Dec 2022).

At the start of the experiment, the LFA was two months expired. While it can be kept at room temperature, we stored it at 6°C which could have extended its shelf life.

We do not recommend using tests and vaccines past their expiry dates to reduce variation, but based on their expected stability when properly stored, and the results we have obtained, we believe that their performance was not impacted.

4. Conclusion

Both ocular and subcutaneous Rev.1 *Brucella* vaccines induced no adverse reaction in young SHO. but a rapid onset of immune responses and a persistent effect on the cellular response, lasting at least 3 ½ years. The conjunctival route gives a shorter-lived humoral immunity than the subcutaneous route, providing a benefit in the event of outbreak control with a test and isolation strategy. Furthermore, neither the control group nor the offspring responded to the vaccination of the animals in contact.

While assessing the efficacy of the vaccines and ensuring the absence of vaccine strain circulation are imperative, this preliminary study suggests that both vaccines could be beneficial in a brucellosis outbreak control strategy for this species, albeit with potential limitations in distinguishing between vaccinated and infected SHO.

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Ethical statement

All animals experiment has been carried out according to the Technical Guidance Document for Scientific Research Permitting in Abu Dhabi Emirate EAD-TMBS-TG-02, 2016 (Environment Agency Abu Dhabi, 2016).

Data availability statement

The data that support the findings of this study are available from the corresponding author upon request.

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References

Blasco, J.M., 2010. Control and eradication strategies for *Brucella melitensis* infection in sheep and goats. Prilozi 31, 145–165.

Brinley Morgan, W.J., Littlejohn, A.I., Mackinnon, D.J., Lawson, J.R., 1966. The degree of protection given by living vaccines against experimental infection with *Brucella melitensis* in goats. Bull. World Health Organ. 34, 33–40.

Environment Agency Abu Dhabi, 2016. Technical guidance document for scientific research permitting in Abu Dhabi Emirate [WWW Document]. URL https://www.ead.gov.ae/-

/media/Project/EAD/EAD/Documents/ResearchHub/Research-Project-Technical-Guidance-Document.pdf (accessed 6.14.23).
Fensterbank, R., Doutre, M.P., Sagna, F., 1977. Étude de la brucellose bovine dans un village de Basse-Casamance (Sénégal). II. Diagnostic allergique. Rev. D'élevage Médecine Vét. Pays Trop. 30, 353–358. https://doi.org/10.19182/remvt.8072
Fensterbank, R., Pardon, P., Marly, J., 1985. Vaccination of ewes by a single conjunctival administration of *Brucella melitensis* Rev. 1 vaccine. Ann. Rech. Vét. 16, 351–356.
Fensterbank, R., Pardon, P., Marly, J., 1982. Comparison between subcutaneous and conjunctival route of vaccination with Rev. 1 strain against *Brucella melitensis* infection in ewes. Ann. Rech. Vét. 13, 295–301.
Fensterbank, R., Verger, J.M., Grayon, M., 1987. Conjunctival vaccination of young goats with *Brucella melitensis* strain Rev 1. Ann. Rech. Vét. 18, 397–403.
Godfroid, J., Garin-Bastuji, B., Saegerman, C., Blasco, J.M., 2013. Brucellosis in terrestrial wildlife. Rev. Sci. Tech. OIE 32, 27–42. https://doi.org/10.20506/rst.32.1.2180
Godfroid, J., Nielsen, K., Saegerman, C., 2010. Diagnosis of brucellosis in livestock and wildlife. Croat. Med. J. 51, 296–305. http://dx.doi.org/10.3325/cmj.2010.51.296
Jiménez, de B.M., Marin, C.M., Barberán, M., Blasco, J.M., 1989. Responses of ewes to *B. melitensis* Rev1 vaccine administered by subcutaneous or conjunctival routes at different stages of pregnancy. Ann. Rech. Veterinaires Ann. Vet. Res. 20, 205–213.
Kurmanov, B., Zincke, D., Su, W., Hadfield, T.L., Aikimbayev, A., Karibayev, T., Berdikulov, M., Orynbayev, M., Nikolich, M.P., Blackburn, J.K., 2022. Assays for identification and differentiation of *Brucella* species: a review. Microorganisms 10, 1584. https://doi.org/10.3390/microorganisms10081584
Lee, S., Lee, D.K., 2018. What is the proper way to apply the multiple comparison test? Korean J. Anesthesiol. 71, 353–360. https://doi.org/10.4097/kja.d.18.00242
Lignereux, L., Chaber, A.-L., Fretin, D., Godfroid, J., Saegerman, C., 2022a. *Brucella melitensis* biovar 1 isolation in a captive wildlife population in the United Arab Emirates. First isolation in the scimitar-horned Oryx (*Oryx dammah*). Vet. Microbiol. 266, 109360. https://doi.org/10.1016/j.vetmic.2022.109360
Lignereux, L., Chaber, A.-L., Nemery, Q., Godfroid, J., Saegerman, C., 2022b. Brucellosis seroprevalence in captive scimitar-horned oryx (*Oryx dammah*) in the United Arab Emirates and associated risk factors. Emerg. Anim. Species 5, 100016. https://doi.org/10.1016/j.eas.2022.100016
Milward, F.W., 1995. Production and quality control of vaccines for brucellosis with a specific emphasis on Rev1 vaccine, in: FAO/WHO/OIE Round Table on the Use of Rev.1 Vaccine in Small Ruminants and Cattle. CNEVA - Alfort, France, CNEVA - Alfort, France, pp. 53–60.
Pardon, P., Sanchis, R., Molenat, G., Bouchard, N., Schmitt, D., Marly, J., Durand, M., 1989. Persistance des réactions sérologiques et allergiques consécutives à la vaccination par les vaccins H.38 ou Rev.1 de brebis en zone d'enzootie brucellique (*Brucella melitensis*). Ann. Rech. Vét. 20, 65.
Ponsart, C., Riou, M., Locatelli, Y., Jacques, I., Fadeau, A., Jay, M., Simon, R., Perrot, L., Freddi, L., Breton, S., Chaumeil, T., Blanc, B., Ortiz, K., Vion, C., Rioult, D., Quéméré, E., Sarradin, P., Chollet, J.-Y., Garin-Bastuji, B., Rossi, S., 2019. *Brucella melitensis* Rev.1 vaccination generates a higher shedding risk of the vaccine strain in Alpine ibex (*Capra ibex*) compared to the domestic goat (*Capra hircus*). Vet. Res. 50, 100. https://doi.org/10.1186/s13567-019-0717-0
Saegerman, C., Vo, T.-K.O., Waele, L.D., Gilson, D., Bastin, A., Dubray, G., Flanagan, P., Limet, J.N., Letesson, J.-J., Godfroid, J., 1999. Diagnosis of bovine brucellosis by skin test: conditions for the test and evaluation of its performance. Vet. Rec. 145, 214–218. https://doi.org/10.1136/vr.145.8.214
WOAH, 2022. Manual of diagnostic tests and vaccines for terrestrial animals 2022. Chap 3.1.4 Brucellosis (infection with *Brucella abortus, B.melitensis* and *B. suis*).

Young, E.J., 1995. An overview of human brucellosis. Clin. Infect. Dis. 21, 283–290. https://doi.org/10.1093/clinids/21.2.283

Zundel, E., Verger, J., Grayon, M., Michel, R., 1992. Conjunctival vaccination of pregnant ewes and goats with *Brucella melitensis* Rev 1 vaccine: safety and serological responses. Ann. Rech. Vét. 23, 177–188.

Captions

Figure 1: temporal evolution of the immune responses of scimitar-horned oryx following the administration of *Brucella melitensis* Rev-1 ocularly (⊖); subcutaneously (△), and in the control group (✳)
Diagrams A, **B** and **C** show the evolution of the percentage of SHO positive to the tests. Diagrams **E** and **F** show the evolution of the mean score attributed to the rose Bengal test (RBT) and to the lateral flow assay (LFA) Antigen rapid *B. Brucella* test, respectively. Diagram **G** shows the evolution of the mean difference in skinfold thickness (in mm) during the brucellin skin test (BST). Diagrams **A** and **D** represent the results obtained with the RBT; diagrams **B** and **E** with the LFA, diagrams **C** and **F** with the BST.
The time is shown in weeks after vaccination. The error bars represent the 95% confidence intervals.

Appendix 1: score chart for the rose Bengal test (RBT) and the lateral flow assay (LFA) Anigen rapid *B. Brucella* test

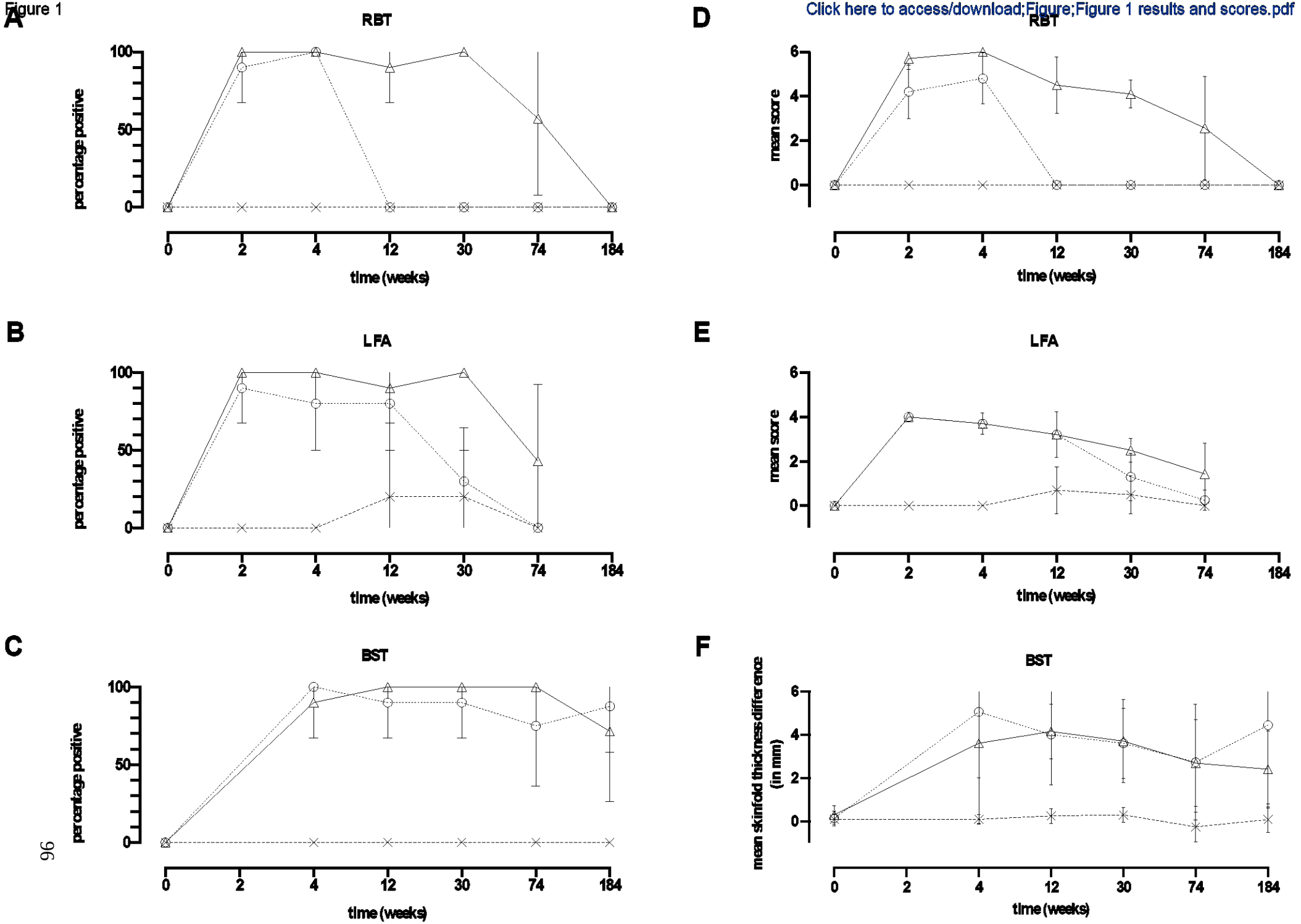
Appendix 2: individual temporal evolutions of test outcomes in each experimental group
Each row (**A**, **B** and **C**) presents the evolution of score according to time for each test: rose Bengal test (RBT), lateral flow assay (LFA) and brucellin skin test (BST). Graphs on the left present the results in the control group, in the middle are the results obtained after conjunctival administration of the *B.melitensis* Rev1. vaccine, and on the right the results after subcutaneous vaccination. The results from individuals that reacted oddly appear with different symbols.

Appendix 3: *p* values for each pairwise comparison performed at each time point for the percentage of positivity and for the mean score (or mean skinfold thickness increase) obtained with rose Bengal test (RBT), lateral flow assay (LFA) and brucellin skin test (BST)

Supplementary material 1: injection and testing schedule
The commercial names are provided. The batch numbers and expiration dates of vaccines and tests are recorded at the time of performance. "Missing" is indicated whenever this information is not available. "PV" means "post-vaccination.

Supplementary material 2: age and sex distribution in each experimental group.

Figure 1

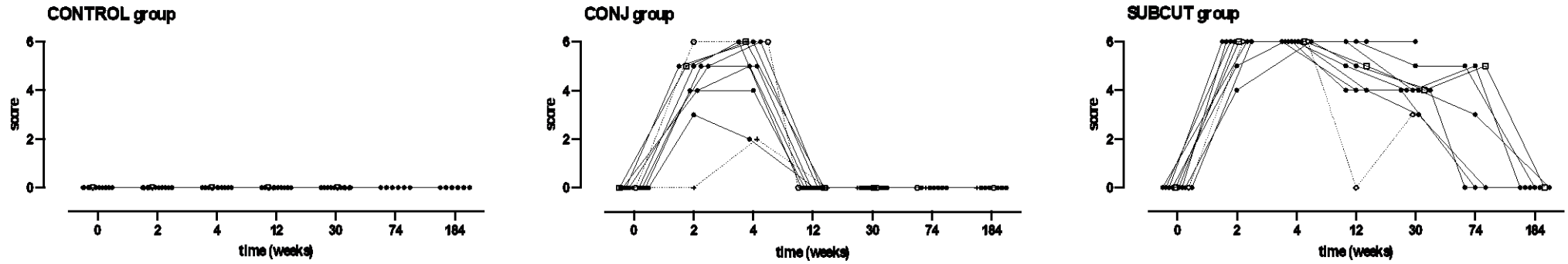


serological test	parameters for interpretation	category	score
RBT	no agglutination seen after 4 minutes + longer agitation + carefull examination under bright light. Viscosity change is sometime observed.	negative	0/6
	very small aggregates that require longer agitation and carefull inspection under bright light	positive_very_weak	2/6
	agglutination requires longer agitation and carefull inspection under bright light	positive_weak	3/6
	clear agglutination at reading at 4 minutes with gentle agitation	positive	4/6
	clear agglutination within 4 minutes without further agitation	positive_strong	5/6
	clear agglutination as soon as both serum and reagent are mixed together	positive_very_strong	6/6
LFA	no line at test band when read is done 20min after sample deposit. Control line positive	negative	0/6
	angle. Some people won't see it. Won't show on a photography. Control line positive	positive_very_very_weak	1/6
	Usually seen by several people. Won't show on a photography. Control line positive	positive_very_weak	2/6
	not so contrasty line appears at test band when reading is done 20 minutes after sample deposit. Control line positive	positive_weak	3/6
	clear and contrasty line appears at test band when reading is done 20 minutes after sample deposit. Control line positive	positive	4/6
	clear and strong line appears before the liquid reaches the control band, control line positive	positive_strong	5/6
	clear and strong line appears as soon as the liquid reach the test band, usually within 3 minutes, control line positive	positive_very_strong	6/6

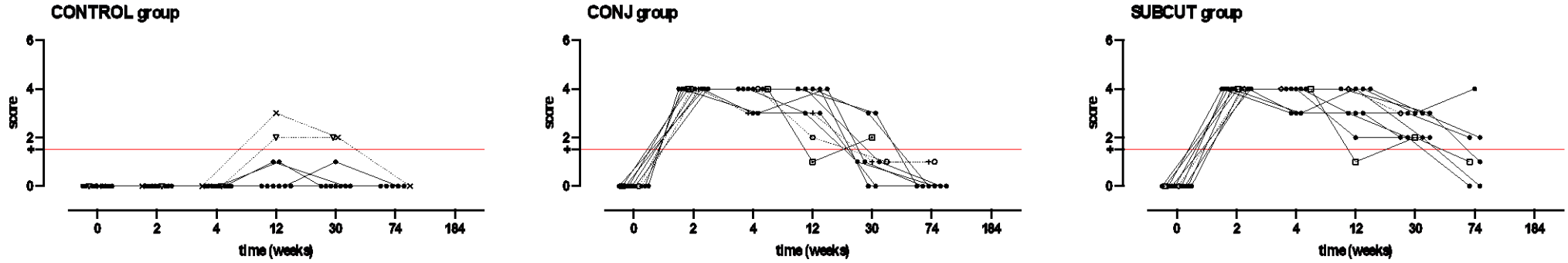
Appendix 2

Click here to access/download;Figure;Appendix 2 showing odd reactions.pdf

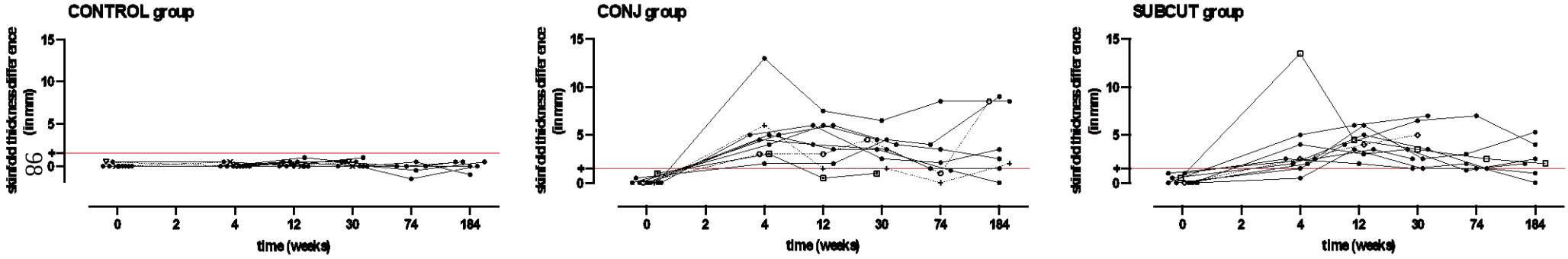
A - RBT



B - LFA



C - BST



Click here to access/download;Figure;Appendix 3 p values for each pairwise comparison.xlsx

pairs compared	sampling size in both groups		RBT		LFA		BST	
			percent of positivity	mean score	percent of positivity	mean score	percent of positivity	mean thickness difference
Week 0								
CONTROL vs. CONJ	10	10	=	=	=	=	=	>0.9999
CONTROL vs. SUBCUT	10	10	=	=	=	=	=	>0.9999
CONJ vs. SUBCUT	10	10	=	=	=	=	=	>0.9999
Week 2								
CONTROL vs. CONJ	10	10	<0.0001	<0.0001	<0.0001	<0.0001	x	x
CONTROL vs. SUBCUT	10	10	<0.0001	<0.0001	<0.0001	<0.0001	x	x
CONJ vs. SUBCUT	10	10	0.5951	0.0558	0.5951	identical	x	x
Week 4								
CONTROL vs. CONJ	10	10	<0.0001	<0.0001	0.0005	<0.0001	<0.0001	<0.0001
CONTROL vs. SUBCUT	10	10	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0002
CONJ vs. SUBCUT	10	10	identical	0.1002	0.3358	>0.9999	0.5951	0.2671
Week 12								
CONTROL vs. CONJ	10	10	=	=	0.0136	0.0001	<0.0001	<0.0001
CONTROL vs. SUBCUT	10	10	<0.0001	<0.0001	0.0017	0.0001	<0.0001	<0.0001
CONJ vs. SUBCUT	10	10	<0.0001	<0.0001	0.822	>0.9999	0.5951	>0.9999
Week 30								
CONTROL vs. CONJ	10	10	=	=	0.8754	0.1796	<0.0001	0.0004
CONTROL vs. SUBCUT	10	10	<0.0001	<0.0001	0.0005	<0.0001	<0.0001	0.0003
CONJ vs. SUBCUT	10	10	<0.0001	<0.0001	0.0034	0.0173	0.5951	>0.9999
Week 74								
CONTROL vs. CONJ	6	8	=	=	=	0.3358	0.0062	0.014
CONTROL vs. SUBCUT	6	7	0.0674	0.0778	0.1655	0.0787	<0.0001	0.0126
CONJ vs. SUBCUT	8	7	0.0674	0.0778	0.1655	0.1513	0.3358	>0.9999
Week 184								
CONTROL vs. CONJ	6	8	=	=	x	x	0.0005	0.0001
CONTROL vs. SUBCUT	6	7	=	=	x	x	0.0193	0.0673
CONJ vs. SUBCUT	8	7	=	=	x	x	0.7564	0.1634

CONTROL was the unvaccinated group; CONJ and SUBCUT were the groups vaccinated by conjunctival or subcutaneous route, respectively

mixed statistical model in Prism = : identical value in both groups

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

		TEST		
	***BRUCELLA* VACCINE**	**Rose Bengal Test**	**Lateral Flow Assay**	**Brucellin Skin Test**
week post vaccination		Synbiotics Bengatest	Anigen Bionote B. *Brucella* rapid test	Synbiotics Brucellergen OCB
0	CZV Rev1, 153295 (exp 11/2016) CZV Ocurev, 142477 (exp 9/2015)	15BGT80 (exp 5/11/2018)	2301058 (exp 17/2/2017)	162058 (exp 3/2019)
2		15BGT80 (exp 5/11/2018)	2301058 (exp 17/2/2017)	not done
4		15BGT80 (exp 5/11/2018)	2301058 (exp 17/2/2017)	10001 (exp 4/2013)
12		15BGT80 (exp 5/11/2018)	missing	162058 (exp 3/2019)
30		missing	missing	162058 (exp 3/2019)
74		missing	not done	missing
184		18ZBAB011 (exp 3/7/2021)	not done	920115 (exp 5/2022)

Supplementary material 1

	AGE (in days)		**SEX**		sampling size
vaccination route	mean	standard deviation	male	female	
none	148.6	51.18	5	5	10
conjunctival	176.0	94.72	4	6	10
subcutaneous	186.7	97.11	5	5	10

Supplementary material 2

3.6.2. Foot-and-mouth disease risk management

3.6.2.1. Likelihood reduction

To reduce the likelihood to release or to expose to FMD, one could suggest creating buffer zones or separation areas between the reintroduced oryx and areas where FMD-prone livestock are present, and implementing controlled access to these zones. This zoning and separation strategy has been implemented in different countries, for instance in Botswana with the buffalo fence, at the cost of heavy impact on natural process such as wildlife migration interruption.

Biosecurity measures can be implemented before the SHO are released. It is part of the project to maintain the SHO in a quarantine pen, after they are transported during the dry season and before they are released, at the beginning of the rainy season. Domestic livestock is kept at a distance. A contingency plan for managing an outbreak should be developed for the case where FMD is detected, including rapid response measures, isolation of affected individuals, and disease control strategies.

Given the airborne transmission and the high contagiousness of the FMD over long distances, the effects of a buffer zone and quarantine to minimise the spread of the disease once potentially infected animals are in Chad could be discussed. The released SHO are expected to evolve naturally in the OROAFR, along with domestic species. This option of buffer zone seems therefore unpracticable, and not appropriate in the current context of the OROAFR.

Consequently, preventing the arrival of FMD-infected SHO in Chad seems the most feasible measure.

The level of FMD viral excretion and its duration for the different serotypes are unknown in this species, as well as the effects of the vaccine on these parameters. For instance, FMD vaccines decrease both the excretion and its duration in sheep, but the impact of a single dose administration on transmission might be limited (Orsel et al., 2007). By decreasing viral shedding, FMD vaccination could prove interesting, but further studies are needed to address this knowledge gap.

It could be tempting to perform FMD-specific pre-transport serological testing , but the benefit of this approach can also be discussed: seropositive results might only reflect past exposure. In the absence of data regarding the possible carrier status of the SHO, it is impossible to take a decision based solely on seropositivity.

On the other hand, acutely infected SHO might seroconvert few days post-infection, possibly after showing typical clinical signs. In this case, infected SHO might be falsely seronegative at the time of testing and seroconvert later.

A pre-transport clinical examination might prove a better option to detect the infection, in a timely and cost-effective manner. Associated with a pre-transport quarantine and strict biosecurity measures during transport, this approach could effectively reduce the risk of releasing FMD.

Regarding this point, our **Article 4** highlights that the source population has been affected by a diversity of FMD serotypes and lineages. Also, there has been an apparent absence of outbreaks outside major circulation events in the region. Both are clues in agreement with repeated new exposures rather than re-emergence possibly due to chronic carriers. While we suggest that the SHO may not serve as a reservoir for FMD, this assertion requires verification. We suggest performing pre-movement retro-pharyngeal scraping and multi-strain FMD-specific PCR analysis to minimize the risk of transporting carrier SHO.

To mitigate the risk of FMD infection in the Scimitar-Horned Oryx (SHO) prior to transport, the implementation of a quarantine coupled with stringent biosecurity measures may be effective. However, the group of animals quarantined within the source population (in pen D, as shown in Figure 1 in Article 4) was affected by the third outbreak, despite being physically separated from the rest of the collection and following strict biosecurity protocols. Therefore, the quarantine facility should be relocated to a site distant from any potential sources of airborne transmission. The Deleikha Holding Facility (refer to the "problem description" chapter) could be considered as an alternative location.

3.6.2.2. Impact reduction

Several options are presented below.

Considering the context of the FMD control program in the UAE and the impending reintroduction to Chad, strain-specific FMD vaccination could prove beneficial by enhancing the acquired herd's immunity.

However, given the apparently limited susceptibility of the SHO to the virus without obvious clinical disease, the benefit of FMD vaccination in this species warrants discussion.

Moreover, there is no approved vaccine for wildlife. Vaccine safety is apparently good in Arabian oryx, with short-lived imunity levels compatible with protection (Kilgallon et al., 2008), but information regarding FMD vaccine use in SHO is lacking. FMD vaccines typically provide immunity for a limited period. Given the logistics of vaccination in wild populations, maintaining a sufficient level of immunity over time would be challenging.

Administering vaccines to wild animals can be logistically challenging. Capturing and handling oryx for vaccination can be stressful and may have negative health and behavioural consequences. It may not be feasible to vaccinate a significant portion of a wild population. For instance, in cattle, FMD vaccination campaigns are costly and labour-intensive, involving a two-injection primo-vaccination followed by bi-annual boosters. This complicates further the decision-making process.

Furthermore, FMD vaccine safety and efficacy in wild SHO populations would need to be thoroughly tested. The response to vaccines can vary among species, and the vaccine's effectiveness in oryx would need to be determined.

Also, the use of vaccines in wildlife should consider ethical and ecological implications. It may alter disease dynamics in the ecosystem, affect other species, and raise ethical questions regarding the potential impacts on individual animals.

3.6.3. Contagious caprine pleuropneumonia risk management

3.6.3.1. Likelihood reduction

Close contact with CCPP-infected individuals is the main contamination pathway. While SHO might share pastures with livestock and wildlife, it is unknown whether this might lead to close contact between susceptible species or not. However, it seems sensible to prevent the release of CCPP.

To reduce the likelihood of transporting CCPP-affected SHO pre-movement preventive health screening, based on serological testing could be performed. A multi-species ELISA (c-ELISA) exists, with the aim to detect past exposure and possible carrier state. However, the epidemiological role of the SHO is unknown. Furthermore, the sensitivity and specificity of the test are unknown in SHO, which requires a preliminary validation step.

The SHO are quarantined in a pre-release pen for a few months before they are released. This quarantine is important by possibly allowing the expression of clinical signs, (respiratory in the case of CCPP), facilitating the clinical detection of SHO possibly affected by the disease. The isolation of the suspected cases, or their death, would decrease the risk of releasing the disease. While definite diagnostic might prove challenging in a timely manner at this location, a contingency plan should be put in place in case the disease is detected at this point.

3.6.3.2. Impact reduction

Several options are presented below.

While CCPP vaccines are safe in goats, no specific vaccine for wildlife, including the SHO, currently exists. If proven effective and safe for SHO, **vaccination** could be a viable option to mitigate the impact of CCPP exposure on the SHO population. However, potential challenges, particularly the use of short-lived vaccines in released wildlife, should be carefully considered. In **Article 5**, we expressed concerns about the efficacy of commercial vaccines due to inadequate quality control, recommending pre-testing vaccine batches.

CCPP control program in local goats, based on mass vaccination, would reduce the impact on goats, as well as the transmission to SHO likelihood.

Implementing a serological screening plan in local goats, ideally coupled with diagnostic methods utilising specific molecular detection and bacteriological investigation (such as isolation, culture, and genotyping), could significantly benefit local herds. This comprehensive approach would aim at minimizing the impact of a CCPP outbreak through early detection and treatment. Additionally, the identification of problematic goats within the local herds would further diminish the risk of transmitting the disease to the SHO.

3.6.4. Bovine tuberculosis risk management

should incorporate an analysis that assesses the specific bTB strains, its prevalence, and the potential impacts in both the source and destination regions. Appropriate mitigation measures should be developed and implemented based on this analysis, including strict biosecurity protocols, pre-release testing, and response plans for disease detection. According to (Delafosse et al., 2002) the constrained survival conditions for the pathogen, coupled with the extensive nature of livestock farming practices featuring low animal densities, likely contribute to the restricted dissemination of the infection

3.6.5. Lumpy skin disease and Sheep and Goat pox risk management

Both inactivated and live attenuated vaccine formulations exist based on different capripox virus strains. Live attenuated ones provide long-term, cross-protection against the three diseases in targeted species, with potential side effects. While they might be potentially useful to mitigate both risks of release and exposure during the reintroduction project, information regarding their safety and effectiveness is lacking in SHO as in most wildlife species susceptible to the infection.

3.6.6. Rift Valley Fever risk management

While further epidemiological investigation might be required, mitigation strategies could include pre-reintroduction vaccination. Attenuated live vaccines and killed vaccines have been developed for livestock, but they are not licensed for use in wildlife. Attenuated vaccines provide longer-term immunity, but they might induce abortion and possible RNA reassociation with wild strain. Their efficacy and safety should be assessed before implementation. Vector control by draining standing water has also proved helpful in outbreak management, as well as control of animal movement, but these options might not be possible to put in place.

3.6.7. Riks management of arthropod-borne infections

Active surveillance within the recipient area is recommended for both the vectors and the various pathogens they transmit.

The effective risk management of arthropod-borne infections should consider the proper timing for SHO release, aligned with the specific biological cycles of vectors. For instance, releasing the SHO during the season with the lowest tick burden could be a prudent strategy, aiming at gradually enhancing the population's natural immunity.

As part of this approach, limiting contact between livestock and wildlife species might be contemplated, but its implementation and efficacy are questionable.

In the future, effective tick infestation control and risk of pathogen transmission mitigation might involve anti-tick vaccine formulations (Contreras et al., 2019; Rodríguez et al., 2022), but there is currently a large gap in the knowledge regarding their effects on wildlife species.

However, the complexity of tick biology, their host range, their predators, the impact of human activities on tick populations require a One Health approach to help with appropriate decision-making (Machtinger et al., 2024).

3.7. Risk communication

The risks should be communicated to the team responsible for managing the source population, with a particular emphasis on zoonotic diseases to protect the staff, and highly contagious infectious diseases, such as FMD, to contain the diseases and avoid internal and external transmission with biosecurity measures.

The risks should also be communicated to senior management and project partners through written reports, as well as verbally during meetings and workshops. During these discussions, options for risk management should be presented, and active participation should be encouraged to foster understanding, gather feedback, and promote responsible decision-making.

Peer-reviewed publications serve as a valuable form of communication, providing long-term access to information, enhancing credibility and trustworthiness, and facilitating dissemination to a wider audience. This form of communication supports evidence-based decision-making.

For notifiable diseases, it is a legal requirement to report diagnosed cases to official entities.

Chapter 4 - General discussion, perspectives, limitations and conclusion

4.1. General discussion

Most parts of the Arabian Peninsula are under arid climates with low fodder production, largely unsuitable for cattle and small ruminant production. Historically, there has been an important livestock trade through the Red Sea to provide meat and animal products to the Middle East, largely from the horn of Africa. Infectious disease screening has not been part of this trade until recently, partially explaining why the statuses of the UAE and Chad regarding livestock infectious diseases are somewhat comparable, but there is also an important livestock trade from Asia (Iran/Pakistan/India) explaining the emergence of Asian lineages.

In this study, we have confirmed the presence of at least three infectious diseases with potential impact during the reintroduction of the SHO to Chad.

Brucella melitensis biovar 2 was cultured and isolated in the source population, where it causes neonatal death and orchitis in SHO, as well as arthritis in gazelles' species. Two isolates provided the same genotyping profile through Multi Locus Variable Number of Tandem Repeat Analysis (MLVA-VNTR) using 15 markers. Their profiles were compared to the global database, the phylogenetic analysis indicated they belonged to the African cluster, and they formed a smaller cluster with strains located in UAE, Oman, and Qatar.

The initial steps of disease control were put in place as soon as January 2013, with serological testing and isolation of the negative animals. The disease had a 67% overall seroprevalence and was present in all tested enclosures. Repeat testing has been performed twice a year since then. Because the prevalence was so elevated, the option to control the outbreak through vaccination has been implemented. Facing a lack of knowledge regarding live *Brucella* vaccine safety and effectiveness, we conducted a vaccine trial based on *B. melitensis* Rev.1 vaccine. Our results indicate an apparent vaccine safety and that both the cellular and the humoral immune responses are somewhat comparable to the response in small ruminants, with a long-term allergic reaction associated with a short serological immunity. Vaccine effectiveness should be evaluated.

We have also discovered that SHO is susceptible to FMD. This was expected, but we have observed three different outbreaks in the population raising concerns regarding the status as a maintenance host.

Based on viral culture and isolation techniques, and genotyping techniques using the capsidal viral protein (VP1) sequence, our results demonstrate that the three FMD outbreaks were caused by different viruses: from two different serotypes (A and O) and the two O serotypes outbreaks were caused by different genotypes, belonging to the main lineages circulating in the region at this time, namely O/ME-SA/PanAsia-2^{ANT-10}, and O/ME-SA/Ind-2001d. The A serotype belonged to the A/ASIA/Iran-05^{FAR-11} lineage. This indicates three different episodes, rather than a re-emerging occurrence. Also, no seropositivity was observed in the SHO before the first outbreak, and the seroconversion happened during this outbreak. While systematic

research is required to confirm this, these observations tend to indicate that SHO does not constitute a maintenance host for FMD.

Nevertheless, the SHO can indeed be acutely infected by FMD, and with over 70% of the population affected within weeks, the disease spreads like a bushfire in this species. The SHO is likely to spread the disease during this acute phase, therefore it is important to contain the viral excretion as well as limit the risk of transporting FMD acutely infected SHO. Pre-transport vaccination might be an option, but a cost-benefit ratio of vaccination program in this species should be evaluated to fill this knowledge gap. Clinical examination before transport and retropharyngeal scraping (probang) associated with FMD-specific PCR might be easily implemented to reduce the risk.

Because being a reservoir depends on the interactions between hosts and pathogens in a certain context, being a reservoir in the UAE context does not necessarily translate into being a reservoir in a natural set-up, where intraspecific interactions are different, but also interactions with other wildlife, livestock, and humans, and where predators and reduced fitness possibly linked to disease might exert natural selection pressure.

However, based on the brucellosis transmission pathways, notably with horizontal and vertical transmission, and long environment persistence, we posit that the SHO exhibits characteristics indicative of potential brucellosis reservoir status. However, comprehensive investigations are warranted to assess the suitability of the entire captive population and the specific predisposition of this species towards serving as a reservoir.

We have also observed a single case of CCPP in the Arabian oryx, as well as in the SHO source population, calling for further investigation. For instance, we suggest carrying CCPP surveillance in these species, based on multi-species serological tests, such as the c-ELISA we used in our study, with the inclusion of a preliminary test validation in this species. Based on observations we made, CCPP constitutes a lethal risk for gazelle species, with 70% mortality in high-density captive sand gazelle. Furthermore, the disease spread over the 80m wide corridors between the enclosures, a distance much longer than previously expected, when the disease was thought to be transmitted only through close contact. Furthermore, the commercial vaccines available at the time did not provide the anticipated protection calling for better quality control in the manufacturing process, and the testing of vaccine batches before their implementation in national disease control plans.

This outbreak gave another example of pathogen's movement between the African continent and the Middle-East: upon culture and isolation of *Mycoplasma capricolum capripneumoniae*, we obtained a genotype based on a multilocus sequence scheme, which compared with available profiles proved closely related to eastern African strains.

Tuberculosis due to *Mycobaterium bovis* is another important concern in the source population that will require further investigation in many aspects: pathogen characterization, screening tests evaluation, and control strategy assessment.

4.2. Limitations of the study

4.2.1. Limitations related to epidemiological information

Limitations are:

- Limited information regarding the basic epidemiological knowledge on infectious animal diseases in Chad. The Chadian national veterinary laboratory located in Farcha near N'Djamena has co-authored numerous publications with international collaborators, such as CIRAD, etc…, but recent or regularly updated information on diseases' prevalence and incidence, circulating pathogens strains and genotypes is needed.
-Lack of knowledge regarding the epidemiological roles of the SHO for most infectious diseases, encompassing susceptibility or carrier function.
-Lack of information regarding test parameters (sensitivity and specificity) for disease screening in SHO.
-Lack of knowledge regarding animal vaccines' safety and effectiveness in SHO.

4.2.2. Limitations related to physical constraints

Limitations are:

-Staffing: The screening, diagnostics, and vaccination campaign faced significant challenges due to the limited staffing, with only one or two veterinary surgeons available in the source population at the time. This proved overwhelming given the substantial population of over 18,000 animals.
- Climatic window: The arid climate of the UAE, where temperatures can reach up to 50°C, permits diurnal work only during the cooler months between October - November and March - April. This seasonal limitation hinders the ability to maximize the time dedicated to animal care.
- CITES: While CITES was established with good intentions, its application and execution may appear to burden well-intentioned individuals with unwarranted paperwork and administrative tasks. In the event of an outbreak affecting CITES-listed species that necessitates the international shipment of diagnostic samples, the current regulations mandate the acquisition of a CITES export permit, along with a corresponding CITES import permit in the country where the laboratory is situated. In the best-case scenario, this process is time-consuming and may lead to delays as the outbreak progresses, potentially resulting in the loss of animals. In the worst-case scenario, the local CITES authority collaborates with the international disease notification authority, and the latter may be hesitant to allow the dissemination of infectious disease data without explicit consent, which may not be readily obtained.
- Possible passive reluctance to the active unwillingness to notify infectious diseases from governments

- Laboratory capacity in UAE: At the time of the study, laboratory capacity in the UAE was constrained. The most advanced veterinary laboratories could conduct certain PCRs or serological tests using commercial kits, but often with inadequate quality controls or utilizing obscure materials and methods. The available PCRs were primarily diagnostic and not consistently designed for pathogen genotyping. Pathogen culture and isolation were generally confined to standard bacterial culture, with viral culture being nearly non-existent. This, associated with the two previous points, proved a challenge.

4.3. Perspectives

This project provides a unique opportunity to advance veterinary knowledge in both the source and recipient countries. Through integration within a One Health framework, surveillance of pathogens and their potential vectors can guide prioritization of actions and development of evidence-based control strategies, benefiting local human, livestock, and wildlife populations. This collaboration also fosters numerous opportunities for research and international cooperation.

Given the presumed naivety of the current reintroduced population of SHO to certain arthropod-borne infections, it is crucial to assess their susceptibility and epidemiological role. Despite their extinction, the SHO likely had interactions with these diseases in the past, highlighting the need for vigilance regarding potential exposure and the subsequent impact on the population.

In the recipient area, recognizing the logistical challenges of capturing wild, endangered species, the disease surveillance efforts should prioritize species with easier handling and lower stress susceptibility, such as domestic livestock. However, acknowledging the broader ecological context, wildlife should also be included in Chad's disease monitoring program whenever possible. Sampling opportunities, such as during conservation-related captures, should be maximized. Implementing distant wildlife health monitoring should be considered as viable options for infectious disease surveillance. Regular health monitoring, coupled with external indicators, will be essential for ensuring the ongoing health and well-being of both wildlife and domestic animals in the recipient area.

4.4. Conclusion

The central inquiries surrounding the risk of release in this project are as follows: Considering that these pathogens already exist in Chad, why should extensive efforts be made to prevent the transport of potential carriers? Can a conservation initiative ethically release pathogens already present in the recipient country and potentially in the ecosystem where the SHO will be reintroduced?

A deliberation is necessary to determine the prioritization between two distinct approaches: the first one involves the creation of a disease-free population of SHO, which potentially demands substantial investment in time, effort, and resources. While this approach aims to mitigate infectious risks upon release, it raises concerns about exposing the population to pathogens once reintroduced into the natural environment. The second approach consists in addressing biodiversity loss and safeguarding natural ecosystems. This approach prioritizes seizing the opportune moment for reintroduction, aligning decision-makers, staff, budget, and animal availability. However, it may risk compromising disease control measures due to time constraints and resource limitations.

This discussion, which includes various stakeholders and experts as highlighted in the introduction, will lead to informed and responsible decision-making. Ultimately, the authority to decide on the implementation of mitigation measures and reintroduction rests with the stakeholders. Informed by comprehensive risk assessments and transparent communication, stakeholders play a pivotal role in shaping the trajectory of this conservation endeavour.

Figure 7: Reintroduced scimitar-horned oyrx in the Ouadi Rimé - Ouadi Achim Faunal Reserve

APPENDIX 1

Orbivirus screening from imported captive oryx in the United Arab Emirates stresses the importance of pre-import and transit measures

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Martinelle Ludovic, Haegeman Andy, Lignereux Louis, Chaber Anne-Lise, Dal Pozzo Fabiana, De Leeuw Ilse, De Clercq Kris and Claude Saegerman

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MDPI

Communication

Orbivirus Screening from Imported Captive Oryx in the United Arab Emirates Stresses the Importance of Pre-Import and Transit Measures

Ludovic Martinelle [1,*], Andy Haegeman [2], Louis Lignereux [1,3], Anne-Lise Chaber [3], Fabiana Dal Pozzo [1], Ilse De Leeuw [2], Kris De Clercq [2] and Claude Saegerman [1]

1 CARE-FEPEX Experimental Station, Fundamental and Applied Research for Animals & Health (FARAH) Center, Faculty of Veterinary Medicine, University of Liege, 4000 Liege, Belgium; l_louis77@hotmail.com (L.L.); fdalpozzo@uliege.be (F.D.P.); claude.saegerman@uliege.be (C.S.)
2 Sciensano, Infectious Diseases in Animals, Exotic and Particular Diseases, 1050 Brussels, Belgium; andy.haegeman@sciensano.be (A.H.); ilse.deleeuw@sciensano.be (I.D.L.); kris.declercq@sciensano.be (K.D.C.)
3 School of Animal and Veterinary Sciences, University of Adelaide, Adelaide, SA 5005, Australia; anne-lise.chaber@adelaide.edu.au
* Correspondence: lmartinelle@uliege.be; Tel.: +32-4-366-40-39

Abstract: From 1975 to 2021, the United Arab Emirates (UAE) imported more than 1300 live Arabian oryxes (AOs) and scimitar-horned oryxes (SHOs) for conservation programs. The objective of this study was to estimate the prevalence of orbiviruses Bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) in AOs and SHOs from captive herds in the UAE. Between October 2014 and April 2015, 16 AOs and 13 SHOs originating from Texas (USA) and 195 out of about 4000 SHOs from two locations in the UAE were blood sampled to be tested by indirect enzyme-linked immunosorbent assay (ELISA) and real-time reverse transcriptase polymerase chain reaction (RT-qPCR) assays. Eight imported AOs (50% CI [24.7–75.4%]) and eight imported SHOs (61.5% CI [31.6–86.1%]) were found BTV seropositive, in contrast with three out of 195 SHOs (1.5% CI [0.3–4.4%]) from the Emirates. BTV-2 genome was detected in 6/16 of the Arabian Oryx, and amongst those, one out of six was seronegative. None of the tested samples was found positive for EHDV. Our results illustrate the wide local variation regarding BTV seroprevalence in domestic and wild ruminants in the Arabian Peninsula. These results stress the need for pre-import risk assessment when considering translocation of wild ruminant species susceptible to orbiviruses not only in the country of destination but also where transit happens.

Keywords: bluetongue; vector-borne disease; orbivirus; arboviruses; oryx; biosecurity

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1. Introduction

Bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) are vector-borne RNA viruses belonging to the genus *Orbivirus*, family *Reoviridae* that are the causative agents of bluetongue disease (BT) and epizootic hemorrhagic disease (EHD), respectively [1]. Both viruses are transmitted to host species by the bite of hematophagous midges of the genus *Culicoides* (Diptera: Ceratopogonidae) [2]. All ruminants are susceptible to infection with BTV; clinical disease is most often observed in sheep, whereas cattle (and goats) are considered reservoir species. In wildlife, a serious disease develops in white-tailed deer (*Odocoileus virginianus*) and pronghorn antelope (*Antilocapra americana)* [3–5]. EHDV severe infections, by contrast, are mostly limited to white-tailed deer despite some sporadic serious cases in other ruminant species. In susceptible livestock and other wildlife, the disease is considered generally subclinical [6]. When present, clinical manifestations of both diseases are quite similar, from a mild non-specific clinical picture including hyperthermia, weakness, depression, and anorexia to a fulminant hemorrhagic disease syndrome [6,7], possibly leading to dramatic economic losses [8]. In addition to the affected host species,

virulence depends on the virus strains, serotypes, and geographical origin [9]. So far, up to 36 BTV serotypes have been described, including historical serotypes (BTV-1 to BTV-24) and the more recent non-virulent BTV-25 to BTV-36 [10,11]. There are currently at least seven EHDV serotypes [12,13].

The Arabian oryx (AO, *Oryx leucoryx*) and the scimitar-horned oryx (SHO, *Oryx dammah*) are two of six surviving species within the subfamily Hippotraginae [14]. These antelopes were endemic to the Arabian Peninsula and North Africa, respectively. Massive poaching and destruction of the habitat of the AOs and SHOs led to the extinction of these species in the wild in the 1970s for the AO and in the late 1980s or early 1990s for the SHO [15,16]. Reintroduction and conservation programs of the AO and SHO in the Middle East significantly rely on the use of captive-bred animals to be released into their former ranges [14]. The import of semi-wild animals in the United Arab Emirates (UAE) from game ranches in the United States of America (USA) is not uncommon, and Texas is one of the states providing AOs and SHOs to the UAE for conservation purposes. Based on the CITES database (https://trade.cites.org/en/cites_trade/#, accessed on 14 October 2021) and prioritizing the numbers reported by the exporters, there were 5814 SHOs and AOs live-traded globally between 1975 and 2021. The UAE was the main importer (1325 oryx), preceding Qatar (1066 oryx), Oman (635 oryx), Saudi Arabia (621 oryx), and Jordan (226 oryx). The UAE was also the largest exporter (2758 oryx). The second largest exporter was the USA, and out of the 1469 oryx exported by this country, 1000 were addressed to the UAE. Oryx species, therefore, represent the majority of the 1898 CITES-listed live Bovidae that have been shipped from the USA to the UAE since 1975. Over the last ten years (2011–2021), 195 oryx (76 SHOs and 119 AOs) were officially shipped on five different occasions from the USA to the UAE.

In the aftermath of the unexpected 2006–2012 BTV-8 and 2007–2010 BTV-1 epizootics, by 2015, Western Europe was facing the re-emergence of BTV-8 in France [17]. BTV-8 is believed to have caused greater economic damage than any previous single serotype BTV outbreak [8]. Indeed, it showed an increased severity toward cattle [18] and displayed a noticeable ability to be vertically transmitted [19]. In addition, BTV-1, 2, 4, 9, and 16 have been continuously present in parts of the Mediterranean Basin, including several EU member states, since at least 1998 [20]. These BTV strains originated from the Near and Middle East, where they have been identified since the 1960s and have persisted since [21].

BTV-11, 13, and 17 were regularly reported in Texas [22]. BTV-2 has been considered enzootic in the southeastern US since the early 1980s and, more recently, was isolated in California [23,24]. Comparing BTV serotypes possibly found in Europe, southwestern USA, and the Middle East, BTV-2 happens to be potentially present in all three areas. Significant levels of EHDV antibodies were also recorded in wild ruminants both in the Arabian Peninsula [14] and Texas (serotypes 1 and 2 [25]).

In the current study, we investigated the prevalence of BTV and EHDV in AOs and SHOs imported from Texas to the UAE with a stopover in the European Union in 2013 and 2015 and compared them to the prevalence found in indigenous captive animals. Viruses that were detected were further characterized.

2. Results

All results are summarized in Table 1, sorted by species and origin. Test results are expressed as the number of positive animals/tested animals.

2.1. Serology

2.1.1. Indigenous Animals

Of the 176 SHOs tested with iELISA at the Abu Dhabi location (site A), three were found to be BTV seropositive (1.7%; 95% CI: 0.3–4.9) and none out of the 19 (0%; 95% CI: 0–17.7) from the Dubai collection (site B).

Table 1. Indirect ELISA (iELISA), competitive ELISA (cELISA), and RT-qPCR results according to the species and origin of the tested animals.

			Positive Animals/Tested Animals (% of Positive Animals; 95% CI)			
Origin of the Animals	**Species**	**Nb of Sampled Animals/Total nb of Animals**	**BTV iELISA**	**RT-qPCR pan-BTV**	**RT-qPCR BTV-2**	**EHDV cELISA**
Indigenous, site A	Scimitar Horned Oryx	176/4000	3/176 (1.7%; 0.3–4.9)	0/3	0/3	0/176
Indigenous, site B	Scimitar Horned Oryx	19/50	0/19	NA	NA	0/19
Imported (Texas)	Scimitar Horned Oryx	13/42	8/13 (61.5%; 31.6–86.1)	0/13	0/13	0/13
Imported (Texas)	Arabian Oryx	16/39	8/16 (50%; 24.7–75.4)	6/16 (38%; 18–61)	6/16	0/16

Only seropositive samples from indigenous animals were tested by RT-qPCR.

2.1.2. Imported Animals

Eight out of the sixteen AOs (50%; 95% CI: 24.7–75.4) and eight out of the thirteen SHOs (61.5%; 95% CI: 31.6–86.1) from the USA were found BTV seropositive.

None of the tested samples could be found positive for EHDV.

2.2. Pan-BTV RT-qPCR and Serotype-Specific RT-qPCR

No positive samples were detected in indigenous animals. The BTV genome was detected in 6/16 of the AOs from Texas (38%; 95% CI: 18–61), and amongst those, one out of six was seronegative. Overall, the Cp values were high (33–39). No viral genome could be detected in the SHO samples.

All six AO samples positive by RT-qPCR were found positive for BTV-2 by serotype-specific RT-qPCR. Among those, two samples were confirmed to be BTV-2 by sequencing, including the seronegative sample. As none of the animals could be found seropositive against EHDV, no further EHDV genome detection was carried out.

3. Discussion

The low seroprevalence and viral RNA detection for BTV, as well as the absence of EHDV detection, we observed in the animals from the two locations within the UAE somewhat contrasts the rather elevated seroprevalences reported by Frölich et al. (2005) in AOs (up to 48 and 50% for BTV and EHDV, respectively). These discrepancies might originate from the different natural habitats affecting the local *Culicoides* populations and dynamics. Indeed, Frölich et al. (2005) also reported negative sample collections from specific locations for both viruses. Our results suggest that the UAE's local natural conditions where the two collections were realized are inadequate for the survival or transmission of orbiviruses.

On the other hand, 55% of the animals imported from Texas overall were BTV seropositive (respectively, 50% and 61.5% for AO and SHO). In the current study, BTV antibodies were detected using an indirect ELISA kit advised by the manufacturer to be used on milk [26,27]. The preliminary results suggested better correspondence with cELISA on serum when compared to homologous blood samples on dried blood spots tested with cELISA and sELISA [28].

Despite the quite high seroprevalence in both AO and SHO from Texas, the BTV genome was found in 6/16 AOs but 0/13 SHO. BTV RNA is known for being detectable for months following infection in ruminants, up to 213 days in cattle [29], and up to 89 days at the least in sheep [30], although the BTV-25 genome could be detected for 19–25 months in goats [31]. In addition, in red deer, it could be detected for up to 112 days [32], but the length of RNAemia in AO and SHO remains unknown. The positive low-level viral RNA

detection in AO suggests a potential outbreak several weeks or months earlier. In the SHO, by contrast, the negative PCR results are likely related to an older infection.

A BTV-2 PCR-positive sample happened to be seronegative. The iELISA used in the current study detects circulating antibodies targeting VP7. The lack of anti-VP7 antibodies following BTV vaccination or infection was previously reported in cattle and was additionally only poorly correlated to clinical and virological protection [33,34]. A weak positive PCR result and the absence of detectable VP7 antibodies could also be related to a very early stage of infection; however, given the serological and virological status of the other AO, this hypothesis is unconvincing. BTV-2 is not considered the most prevalent serotype in Texas. Neutralizing antibodies to BTV-2 were observed nonetheless in Texas in 1991–1992 [35]. Previous exposures to other BTV serotypes cannot be excluded as BTV-13, BTV-11, and BTV-17 are considered enzootic in Texas [36]. Moreover, BTV-12 and BTV-3 were isolated in Texas in 2008 and 2014, respectively [37].

No positive EHDV samples could be found either from USA or UAE. Previous reports showed an EHDV seroprevalence in white-tailed deer widespread throughout Texas, with up to 100% of the tested animals being positive [35]. In 2014, EHDV-2 was successfully isolated in Eld's deer (*Panolia eldii*) [38]. In Texas, EHDV-2 is the most prevalent serotype, followed by EHDV-1 and the more recent identification of EHDV-6 [39]. In the Middle East, outbreaks of EHDV-6 were reported in 2006, along with EHDV-7 outbreaks in Israel. In 2015, clinical EHDV-6 outbreaks were also reported in Israel [40]. As for BTV, EHDV seroprevalence demonstrated large local variations within a considered country [14].

Since BTV is prevalent in certain areas of the UAE, a transmission occurring between landing and testing cannot be totally ruled out. This case demonstrates that import health permits are not requested by countries where a stopover is carried out, possibly posing a risk of vector-borne disease transmission. Usually on long distance, flight between continents freight is routed to local hubs to be sorted to the final destination (P. Lignereux, personal communication). This implies to take the animals off and reload them in a second plane. Freight transfer only takes a couple of hours with limited contact of the animals with the environment. European Palearctic *Culicoides* species were proven to be competent orbivirus vectors with some species displaying a strong endophilic behavior [41]. Therefore, importation of ruminants infected with exotic BTV serotypes threatens not only the importing country but any European country where at risk animals would stopover [42].

Animal exportation screening is usually solely based upon the importer's requirements. At the time of sampling, the requirements to import live ruminants into the UAE were brucellosis and Foot-and-Mouth Disease testing. The reason was mainly that most livestock would arrive by road from neighboring countries. This is of major concern, especially in cases where animals might transit in a third-party country. These results stress the need for pre-import risk assessment, precaution, and the implementation of biosecurity measures when considering the translocation of wild ruminant species susceptible to BTV and EHDV.

Therefore, we would suggest avoiding stopovers of live ruminant shipments in countries where competent orbiviruses vectors were reported or at minimum vector control in resting areas inside airports.

In addition, we recommend *Culicoides* biological surveys to be carried out in the different natural habitats and BTV and EHDV virus surveys in all susceptible species within the UAE.

4. Materials and Methods

4.1. Sample Origin

One hundred and ninety-five SHOs from two locations in the UAE (Figure 1) were sampled ("indigenous animals"). In addition, 16 AOs and 13 SHOs imported from the USA ("imported animals") were blood sampled once they arrived in the UAE.

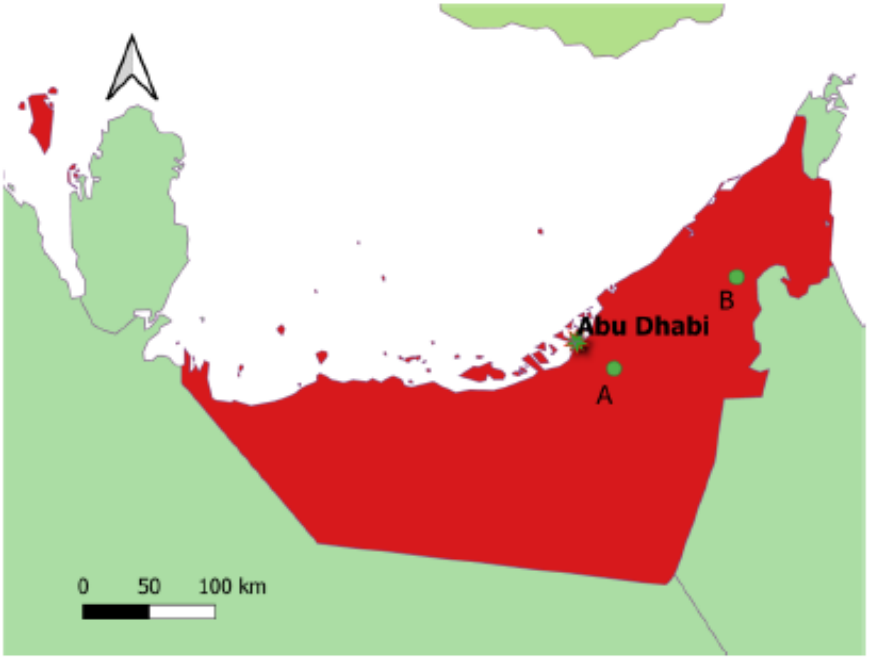

Figure 1. Indigenous animals sampling locations. A total of 176 out of 4000 SHOs were sampled in January and May at site A (high animal density, 5.4 km^2). Nineteen out of about fifty SHOs were sampled in January 2015 at site B (semi-wild desert area, 225 km^2).

4.1.1. Indigenous Animals

For herd management and disease screening purposes, 176 SHOs were bled in January and May 2013 amongst approximately 4000 other SHOs in a high-density wildlife collection housing over 13,000 wild ungulates, located 45 km inland (24.22295, 54.76194, site A) and east of Abu Dhabi. The overall setup is approximately 5.4 km^2 and was already described in [43].

Another 19 SHOs were bled out of approximately 50 individuals in a remote and desert semi-wild location, 225 km^2, in Dubai emirate (24.89347, 55.66370, site B) in January 2015. Locations were about 120 km apart.

4.1.2. Imported Animals

To improve the genetic diversity of the local AO and SHO populations for conservation purposes, 39 adult AOs and 42 SHOs coming from different wildlife ranches and zoological parks in the USA were gathered by an animal broker located in Texas where they underwent brucellosis and bovine tuberculosis testing required by the importer. They were flown in individual crates and transited through the European Union without being subjected to any European health requirements prior to their transit. The AOs landed on 13 October 2013 and the SHOs on 15 April 2015. They were then quarantined in a wildlife facility remotely located in the desert (24.06453, 55.04956). Sixteen AOs and thirteen SHOs were physically restrained with a chute system 15 and 5 days post-arrival, respectively. At that time, they underwent clinical examination and were blood-sampled. None of the sampled animals displayed clinical signs evocative of an *orbivirus* infection.

4.2. Dry Blood Spots (DBS) for Virological and Serological Testing

Blood was drawn from the jugular vein of the animals and placed into 9 mL ethylenediamine tetraacetic acid (EDTA)-coated tubes. Blood samples were then stored at −20 °C and processed within 6 months. Blood was allowed to thaw at 6 °C overnight and then placed in a dry bath set at 56 °C for 30 min to deactivate any potential Foot-and-Mouth disease virus. With a pipettor, 80 µL of blood were dispensed on Whatman protein saver cards and left out to dry at room temperature (RT) according to an adapted protocol described

elsewhere [44,45]. Dry blood spots were subsequently punched out in paper discs with a 6 mm diameter punch in the middle of the deposit and diluted in 250 µL PBS and Tween 20 0.05%. Samples were then gently vortexed and left overnight at 4 °C. The obtained eluates were reported to be equivalent to a 1/25 dilution of the corresponding serum [46]. Samples were slightly stirred before using the required amount of supernatant.

4.3. Serology

4.3.1. Epizootic Hemorrhagic Disease Virus Competitive Enzyme-Linked Immunosorbent Assay

Oryx dried blood paper discs were tested to detect antibodies against EHDV VP7 protein by competitive ELISA (LSIVet Ruminant EHDV Serum ELISA Kit, LSI, Lissieu, France) following manufacturer's recommendations [47]. Percentage of inhibition (% inh) of each sample was interpreted as follows: % inh < 55 = negative, 55 < % inh < 60 = doubtful and % inh > 60 = positive.

4.3.2. Bluetongue Virus Indirect Enzyme-Linked Immunosorbent Assay

Antibodies against BTV VP7 protein were tested by indirect ELISA (iELISA, ID Screen®Bluetongue Milk Indirect, ID Vet, Grabels, France) according to the manufacturer's instructions with minor modifications. Briefly, 50 mL of supernatant and 50 mL 'wash solution' were added to the wells of a BTV-VP7-coated microtiter plate. After incubation for 45 min at RT, plates were washed and incubated with 100 mL anti-ruminant peroxidase conjugate for 30 min at RT. After washing, wells were incubated for 15 min at RT with 100 mL TMB substrate. Color development was stopped by the addition of 100 mL 0.5 M H_2SO_4. S/P% ($OD_{sample}/OD_{positive\ control} \times 100\%$) was calculated using optical density values measured at 450 nm. S/P% $\geq$ 50% was considered as positive [27]. In addition to positive and negative controls from the kit, highly positive BTV-8 cattle serum and negative cattle serum from previous experiments [48] were included on each plate.

4.4. RT-qPCR pan-BTV (S5)

Pan-BTV RT-qPCR was performed on all imported animals (AO n = 16, SHO n = 13), whereas only seropositive samples from animals already in the UAE were tested. The detection of the BTV genome in eluted oryx samples was carried out using a pan-BTV RT-qPCR consisting of a triplex RT-qPCR (RT-qPCR) targeting segment 5, internal control (IC), and external control (EC), as described by [49]. Prior to being used on oryx samples, the RT-qPCR protocol was validated on cattle dry blood spots of known infectious status from previous experiments [50]. The RT-qPCR was performed on a LightCycler- 480 (Roche Diagnostics, Mannheim, Germany). For this assay, crossing-point values (Cp values) < 40.0 were classified as positive, Cp values >40.0 and <45.0 were classified as doubtful, and Cp values > 45.0 were considered as negative (Neg) [49].

4.5. Serotype-Specific RT-qPCR

As BTV-2 was reported in all three areas of interest (Europe, southwestern USA, and the Middle East), we focused serotype-specific detection on that particular serotype. In-house serotype-specific real-time RT-qPCRs for BTV-2 targeting segment 2 of the viral genome were carried out (cycling profile and mix set-up) similarly to the BTV serotype real-time RT-qPCRs described by Vandenbussche et al., 2009 with the extra addition of 1U Taq Platinum (Invitrogen, Merelbeke, Belgium) to the enzyme mix [51]. The following primers/probe were used, with the final concentration between brackets and LNA nucleotide with "+": BTV-2 (forward ATGATGTTTCCAAGATTCCTGAGATG (1 µM); Reverse CATTTCGT-GTTGGCATATATTTGAGTG (0.75 µM); Probe 6'FAM-CT+CA+TC+TT+TGATATCG+TAAGC-BHQ1 (0.25 µM)).

4.6. Cloning/Plasmid/Preparation Sequencing

Each BTV sequence amplified by serotype-specific RT-qPCR was prepared for further sequencing. A total of 4 µL of purified serotype-specific real-time RT-qPCR fragment was ligated into the pCR2.1-Topo vector using TOPO TA Cloning (Invitrogen, Merelbeke, Belgium). Following blue/white screening on X-gal containing kanamycin (50 µg/mL) LB plates, plasmids were purified using QIAprep Spin Miniprep Kit (Qiagen, Venlo, Netherlands) according to the manufacturer's instructions. Insert verification was carried out by Eco RI digestion and gel electrophoresis.

The purified plasmids were sequenced using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). The sequence reactions were purified by precipitation with 80% ethanol and centrifugation at 12,000× *g* for 15 min at 4 °C. After washing with 70% ethanol, the pellet was air-dried and dissolved subsequently in a 25 µL template suppression reagent (Applied Biosystems Foster City, CA, USA). Next, the purified product was denatured by incubation for 2 min at 94 °C and was subsequently analyzed on the ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The obtained sequences were identified and compared with publicly available sequences and with the obtained reference sequence using the "blast" engine at "http://www.ncbi.nlm.nih.gov/BLAST/ (accessed on 8 February 2017)" [52].

4.7. Statistical Analysis

The seroprevalences and their 95% confidence intervals (CI) were calculated using the binomial "exact" method in the online calculator Epitools [53].

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References

1. Ahmed, S.; Mahmoud, M.A.E.; Viarouge, C.; Sailleau, C.; Zientara, S.; Breard, E. Presence of bluetongue and epizootic hemorrhagic disease viruses in Egypt in 2016 and 2017. *Infect. Genet. Evol.* **2019**, *73*, 221–226. [CrossRef]
2. Mellor, P.S.; Boorman, J.; Baylis, M. Culicoides biting midges: Their role as arbovirus vectors. *Ann. Rev. Entomol.* **2000**, *45*, 307–340. [CrossRef]
3. Johnson, D.J.; Ostlund, E.N.; Stallknecht, D.E.; Goekjian, V.H.; Jenkins-Moore, M.; Harris, S.C. First report of bluetongue virus serotype 1 isolated from a white-tailed deer in the United States. *J. Vet. Diagn. Investig.* **2006**, *18*, 398–401. [CrossRef]
4. Parsonson, I.M. Pathology and pathogenesis of bluetongue infections. *Curr. Top. Microbiol. Immunol.* **1990**, *162*, 119–141. [CrossRef]
5. Drolet, B.S.; Reister-Hendricks, L.M.; Podell, B.K.; Breitenbach, J.E.; McVey, D.S.; van Rijn, P.A.; Bowen, R.A. European bluetongue serotype 8: Disease threat assessment for U.S. sheep. *Vector Borne Zoonotic Dis.* **2016**, *16*, 400–407. [CrossRef]
6. Maclachlan, N.J.; Zientara, S.; Savini, G.; Daniels, P.W. Epizootic haemorrhagic disease. *Rev. Sci. Tech.* **2015**, *34*, 341–351. [CrossRef]
7. Maclachlan, N.J.; Drew, C.P.; Darpel, K.E.; Worwa, G. The pathology and pathogenesis of bluetongue. *J. Comp. Pathol.* **2009**, *141*, 1–16. [CrossRef]

8. Wilson, A.J.; Mellor, P.S. *Bluetongue* in Europe: Past, present and future. *Philos. Trans. R. Soc. B Biol. Sci.* **2009**, *364*, 2669–2681. [CrossRef]
9. Dahiya, S.; Prasad, G.; Kovi, R.C. VP2 gene based phylogenetic relationship of Indian isolates of *Bluetongue virus* serotype 1 and other serotypes from different parts of the world. *DNA Seq.* **2004**, *15*, 351–361. [CrossRef]
10. Ries, C.; Beer, M.; Hoffmann, B. BlueTYPE—A low density TaqMan-RT-qPCR array for the identification of all 24 classical *Bluetongue virus* serotypes. *J. Virol. Methods* **2020**, *282*, 113881. [CrossRef]
11. Ries, C.; Vogtlin, A.; Hussy, D.; Jandt, T.; Gobet, H.; Hilbe, M.; Burgener, C.; Schweizer, L.; Hafliger-Speiser, S.; Beer, M.; et al. Putative novel atypical BTV serotype '36' identified in small ruminants in Switzerland. *Viruses* **2021**, *13*, 721. [CrossRef]
12. Anthony, S.J.; Maan, S.; Maan, N.; Kgosana, L.; Bachanek-Bankowska, K.; Batten, C.; Darpel, K.E.; Sutton, G.; Attoui, H.; Mertens, P.P. Genetic and phylogenetic analysis of the outer-coat proteins VP2 and VP5 of epizootic haemorrhagic disease virus (EHDV): Comparison of genetic and serological data to characterise the EHDV serogroup. *Virus Res.* **2009**, *145*, 200–210. [CrossRef]
13. Mendiola, S.Y.; Mills, M.K.; Maki, E.; Drolet, B.S.; Wilson, W.C.; Berghaus, R.; Stallknecht, D.E.; Breitenbach, J.; McVey, D.S.; Ruder, M.G. EHDV-2 infection prevalence varies in *Culicoides sonorensis* after feeding on infected white-tailed deer over the course of viremia. *Viruses* **2019**, *11*, 371. [CrossRef]
14. Frolich, K.; Hamblin, C.; Jung, S.; Ostrowski, S.; Mwanzia, J.; Streich, W.J.; Anderson, J.; Armstrong, R.M.; Anajariyah, S. Serologic surveillance for selected viral agents in captive and free-ranging populations of Arabian oryx (Oryx leucoryx) from Saudi Arabia and the United Arab Emirates. *J. Wildl. Dis.* **2005**, *41*, 67–79. [CrossRef]
15. Henderson, D.S. Were they the last arabian oryx? *Oryx* **1974**, *12*, 347–350. [CrossRef]
16. Woodfine, T.; Gilbert, T. The fall and rise of the scimitar-horned oryx: A case study of ex-situ conservation and reintroduction in practice. In *Antelope Conservation: From Diagnosis to Action*, 1st ed.; Bro-Jorgensen, J., Mallon, D.P., Eds.; Wiley-Blackwell: Hoboken, NJ, USA, 2016; p. 376. [CrossRef]
17. Courtejoie, N.; Durand, B.; Bournez, L.; Gorlier, A.; Breard, E.; Sailleau, C.; Vitour, D.; Zientara, S.; Baurier, F.; Gourmelen, C.; et al. Circulation of bluetongue virus 8 in French cattle, before and after the re-emergence in 2015. *Transbound. Emerg. Dis.* **2018**, *65*, 281–284. [CrossRef]
18. Caporale, M.; Di Gialleonorado, L.; Janowicz, A.; Wilkie, G.; Shaw, A.; Savini, G.; Van Rijn, P.A.; Mertens, P.; Di Ventura, M.; Palmarini, M. Virus and host factors affecting the clinical outcome of bluetongue virus infection. *J. Virol.* **2014**, *88*, 10399–10411. [CrossRef]
19. Haegeman, A.; Vandaele, L.; De Leeuw, I.; Oliveira, A.P.; Nauwynck, H.; Van Soom, A.; De Clercq, K. Failure to remove bluetongue serotype 8 virus (BTV-8) from in vitro produced and in vivo derived bovine embryos and subsequent transmission of to recipient cows after embryo transfer. *Front. Vet. Sci.* **2019**, *6*, 432. [CrossRef]
20. Saegerman, C.; Berkvens, D.; Mellor, P.S. *Bluetongue* epidemiology in the European Union. *Emerg. Infect. Dis.* **2008**, *14*, 539–544. [CrossRef]
21. Shimshony, A. *Bluetongue* in Israel—A brief historical overview. *Vet. Ital.* **2004**, *40*, 116–118.
22. United States Department of Agriculture. *Bluetongue Standard Operating Procedures: Overview of Etiology and Ecology*; US Department of Agriculture: Riverdale, MD, USA, 2016.
23. Gaudreault, N.N.; Mayo, C.E.; Jasperson, D.C.; Crossley, B.M.; Breitmeyer, R.E.; Johnson, D.J.; Ostlund, E.N.; MacLachlan, N.J.; Wilson, W.C. Whole genome sequencing and phylogenetic analysis of *Bluetongue virus* serotype 2 strains isolated in the Americas including a novel strain from the western United States. *J. Vet. Diagn. Investig* **2014**, *26*, 553–557. [CrossRef]
24. Maclachlan, N.J.; Wilson, W.C.; Crossley, B.M.; Mayo, C.E.; Jasperson, D.C.; Breitmeyer, R.E.; Whiteford, A.M. Novel serotype of bluetongue virus, western North America. *Emerg. Infect. Dis.* **2013**, *19*, 665–666. [CrossRef]
25. Stevens, G.; McCluskey, B.; King, A.; O'Hearn, E.; Mayr, G. Review of the 2012 Epizootic hemorrhagic disease outbreak in domestic ruminants in the United States. *PLoS ONE* **2015**, *10*, e0133359. [CrossRef]
26. Lignereux, L.; Chaber, A.L.; Saegerman, C.; Heath, L.; Knowles, N.J.; Wadsworth, J.; Mioulet, V.; King, D.P. Foot-and-mouth disease outbreaks in captive scimitar-horned oryx (Oryx dammah). *Transbound. Emerg. Dis.* **2020**, *67*, 1716–1724. [CrossRef]
27. Sun, D.; Cho, Y.I.; Comyn, P.; Yoon, K.J. Use of blood collected onto and dried on filter paper for diagnosing pregnancy in cattle. *Vet. J.* **2013**, *198*, 494–497. [CrossRef]
28. Yoon, K.J.; Cho, Y.I.; Kim, W.I.; Pittman, J.S.; Brinkman, M.; Winton, C. Experimental and field trial of TEGO™ Animal Blood Collection kit for PRRS testing. In Proceedings of the Annual Meeting of American Association of Swine Veterinarians, Omaha, NE, USA, 6–9 March 2010; pp. 211–214.
29. Fenollar, F.; Raoult, D. Diagnosis of rickettsial diseases using samples dried on blotting paper. *Clin. Diagn. Lab Immunol.* **1999**, *6*, 483–488. [CrossRef]
30. Mejri, S.; Dhaou, S.B.; Jemli, M.; Breard, E.; Sailleau, C.; Sghaier, S.; Zouari, M.; Lorusso, A.; Savini, G.; Zientara, S.; et al. Epizootic haemorrhagic disease virus circulation in Tunisia. *Vet. Ital.* **2018**, *54*, 87–90. [CrossRef]
31. Kramps, J.A.; van Maanen, K.; Mars, M.H.; Popma, J.K.; van Rijn, P.A. Validation of a commercial ELISA for the detection of bluetongue virus (BTV)-specific antibodies in individual milk samples of Dutch dairy cows. *Vet. Microbiol.* **2008**, *130*, 80–87. [CrossRef]
32. Martinelle, L.; Dal Pozzo, F.; Sarradin, P.; De Leeuw, I.; De Clercq, K.; Thys, C.; Ziant, D.; Thiry, E.; Saegerman, C. Two alternative inocula to reproduce bluetongue virus serotype 8 disease in calves. *Vaccine* **2011**, *29*, 3600–3609. [CrossRef]
33. Vandenbussche, F.; Vandemeulebroucke, E.; De Clercq, K. Simultaneous detection of bluetongue virus RNA, internal control GAPDH mRNA, and external control synthetic RNA by multiplex real-time PCR. *Methods Mol. Biol.* **2010**, *630*, 97–108. [CrossRef]

34. Martinelle, L.; Dal Pozzo, F.; Thys, C.; De Leeuw, I.; Van Campe, W.; De Clercq, K.; Thiry, E.; Saegerman, C. Assessment of cross-protection induced by a bluetongue virus (BTV) serotype 8 vaccine towards other BTV serotypes in experimental conditions. *Vet. Res.* **2018**, *49*, 63. [CrossRef] [PubMed]
35. Vandenbussche, F.; De Leeuw, I.; Vandemeulebroucke, E.; De Clercq, K. Emergence of bluetongue serotypes in Europe, part 1: Description and validation of four real-time RT-PCR assays for the serotyping of bluetongue viruses BTV-1, BTV-6, BTV-8 and BTV-11. *Transbound. Emerg. Dis.* **2009**, *56*, 346–354. [CrossRef] [PubMed]
36. Tatusova, T.A.; Madden, T.L. BLAST 2 Sequences, a new tool for comparing protein and nucleotide sequences. *FEMS Microbiol. Lett.* **1999**, *174*, 247–250. [CrossRef]
37. Sergeant, E.S.G. Epitools Epidemiological Calculators. Available online: https://epitools.ausvet.com.au/ (accessed on 22 June 2019).
38. Chaignat, V.; Nitzsche, S.; Scharrer, S.; Feyer, D.; Schwermer, H.; Thur, B. Milk concentration improves *Bluetongue* antibody detection by use of an indirect ELISA. *Vet. Microbiol.* **2010**, *143*, 179–183. [CrossRef] [PubMed]
39. Martinelle, L.; Chaber, A.L.; Dal Pozzo, F.; Lignereux, L.; Saegerman, C. *Dried Blood Spots are Valuable to Perform Bluetongue Virus Diagnostic Tests*; University of Liege: Liège, Belgium, 2021; manuscript in preparation.
40. Zanella, G.; Martinelle, L.; Guyot, H.; Mauroy, A.; De Clercq, K.; Saegerman, C. Clinical pattern characterization of cattle naturally infected by BTV-8. *Transbound. Emerg. Dis.* **2013**, *60*, 231–237. [CrossRef]
41. Katz, J.; Alstad, D.; Gustafson, G.; Evermann, J. Diagnostic analysis of the prolonged bluetongue virus RNA presence found in the blood of naturally infected cattle and experimentally infected sheep. *J. Vet. Diagn. Investig.* **1994**, *6*, 139–142. [CrossRef]
42. Vogtlin, A.; Hofmann, M.A.; Nenniger, C.; Renzullo, S.; Steinrigl, A.; Loitsch, A.; Schwermer, H.; Kaufmann, C.; Thur, B. Long-term infection of goats with bluetongue virus serotype 25. *Vet. Microbiol.* **2013**, *166*, 165–173. [CrossRef]
43. Falconi, C.; Lopez-Olvera, J.R.; Gortazar, C. BTV infection in wild ruminants, with emphasis on red deer: A review. *Vet. Microbiol.* **2011**, *151*, 209–219. [CrossRef]
44. Oura, C.A.; Wood, J.L.; Sanders, A.J.; Bin-Tarif, A.; Henstock, M.; Edwards, L.; Floyd, T.; Simmons, H.; Batten, C.A. Seroconversion, neutralising antibodies and protection in bluetongue serotype 8 vaccinated sheep. *Vaccine* **2009**, *27*, 7326–7330. [CrossRef]
45. De Clercq, K.; De Leeuw, I.; Verheyden, B.; Vandemeulebroucke, E.; Vanbinst, T.; Herr, C.; Meroc, E.; Bertels, G.; Steurbaut, N.; Miry, C.; et al. Transplacental infection and apparently immunotolerance induced by a wild-type bluetongue virus serotype 8 natural infection. *Transbound. Emerg. Dis.* **2008**, *55*, 352–359. [CrossRef]
46. Stallknecht, D.E.; Luttrell, M.P.; Smith, K.E.; Nettles, V.F. Hemorrhagic disease in white-tailed deer in Texas: A case for enzootic stability. *J. Wildl. Dis.* **1996**, *32*, 695–700. [CrossRef]
47. Mayo, C.; McDermott, E.; Kopanke, J.; Stenglein, M.; Lee, J.; Mathiason, C.; Carpenter, M.; Reed, K.; Perkins, T.A. Ecological dynamics impacting bluetongue virus transmission in North America. *Front. Vet. Sci.* **2020**, *7*, 186. [CrossRef] [PubMed]
48. Schirtzinger, E.E.; Jasperson, D.C.; Ostlund, E.N.; Johnson, D.J.; Wilson, W.C. Recent US *Bluetongue virus* serotype 3 isolates found outside of Florida indicate evidence of reassortment with co-circulating endemic serotypes. *J. Gen. Virol.* **2018**, *99*, 157–168. [CrossRef] [PubMed]
49. Gibbs, P.; McVey, D.S. *Report of the Committee on Bluetongue and Related Orbiviruses*; United States Animal Health Association (USAHA): Providence, RI, USA, 2015.
50. Allison, A.B.; Goekjian, V.H.; Potgieter, A.C.; Wilson, W.C.; Johnson, D.J.; Mertens, P.P.; Stallknecht, D.E. Detection of a novel reassortant epizootic hemorrhagic disease virus (EHDV) in the USA containing RNA segments derived from both exotic (EHDV-6) and endemic (EHDV-2) serotypes. *J. Gen. Virol.* **2010**, *91*, 430–439. [CrossRef] [PubMed]
51. Golender, N.; Khinich, Y.; Gorohov, A.; Abramovitz, I.; Bumbarov, V. Epizootic hemorrhagic disease virus serotype 6 outbreak in Israeli cattle in 2015. *J. Vet. Diagn. Investig.* **2017**, *29*, 885–888. [CrossRef]
52. Baldet, T.; Delecolle, J.C.; Cetre-Sossah, C.; Mathieu, B.; Meiswinkel, R.; Gerbier, G. Indoor activity of Culicoides associated with livestock in the bluetongue virus (BTV) affected region of northern France during autumn 2006. *Prev. Vet. Med.* **2008**, *87*, 84–97. [CrossRef]
53. Walton, T.E. The history of bluetongue and a current global overview. *Vet. Ital.* **2004**, *40*, 31–38.

Annexes

Annex 1: Comparison of indicators between the UAE and Chad

Parameter	United Arab Emirates (Source)	Chad (Source)
country size	83,600 km2	1,284,000 km2
Climate	Arid; desert climate, hot and dry	Arid to semi-arid; desert in north
Temperature	Hot throughout the year; extreme heat in summer	Varies by region; hot in north, cooler in south
Yearly average rainfall	100 mm (Weatherbase)	700 mm (Weatherbase)
Yearly average temperature	26°C (Weatherbase)	26°C (Weatherbase)
Human population	9.89 million	17.41 million
Human density	High population density, especially in urban centers	Varied; lower population density in rural areas
GDP	$507.54 billion (2022) (World Bank)	$17.4 billion (2022) (World Bank)
GDP per capita	$51,310 per capita (2022) (World Bank)	$683.89 per capita (2022) (World Bank)
Human Development Index (HDI)	0.891 (very high) (UNDP)	0.404 (low) (UNDP)
Income inequality	Lower levels of income inequality (World Bank)	High levels of income inequality (World Bank)
Poverty rate	Negligible poverty rate due to high standard of living	42.3% (2011) (World Bank)
Unemployment rate	2.6% (2019) (World Bank)	22.4% (2019) (World Bank)
Employment sectors	construction	Predominantly agriculture, with some mining and oil extraction
Healthcare system	Advanced healthcare infrastructure with modern facilities	Limited access to healthcare facilities, particularly in rural areas
Births attended by skilled health staff (in 2015)	99.9% of total (The World Bank, 2024)	24.3% of total (The World Bank, 2024)
Education system	Advanced education system with high-quality schools and universities	Limited access to education, particularly in rural areas
Literacy rate	93.8% (2015) (World Bank)	22.3% (2018) (World Bank)
Natural resources	Oil, natural gas, minerals, fish in coastal areas	Limited water resources; oil, uranium, gold, minerals
Percentage of Agriculture in GDP	0.8% (2022 worldbank)	22.6% (2022 worldbank)
Agricultural resources	Limited agricultural resources due to arid climate, with emphasis on date palms, vegetables, and fruits grown using advanced irrigation techniques	Mainly subsistence agriculture, crops include millet, sorghum, maize, and peanuts
Surface dedicated for agriculture	Crop: limited due to arid climate, with small percentage of land area dedicated to agriculture Pasture: minimal, with emphasis on desert agriculture and advanced irrigation techniques Intensive farming: Limited due to scarcity of arable land and water resources	Crop: 10% of total land area Pasture: 25% of total land area Intensive farming: Negligible
Employment in agriculture (modeled ILO estimate)	1.4% of total employment (The World Bank, 2024)	69% of total employment (The World Bank, 2024)
Domesticated animal density	High, especially in agricultural areas	Moderate to high; nomadic herding prevalent
Number of cattle (in 2018)	103,361 (FAO, 2024)	29,069,601 (FAO, 2024)
Number of goats (in 2018)	2,395,166 (FAO, 2024)	36,534,693 (FAO, 2024)
Number of sheep (in 2018)	2,147,665 (FAO, 2024)	33,230,856 (FAO, 2024)
Number of exported cattle (in 2018)	1,327 (FAO, 2024)	2021)
Number of exported goats (in 2018)	22,842 (FAO, 2024)	110,000 (FAO, 2024)
Number of exported sheep (in 2018)	4,688 (FAO, 2024)	23,796 in 2021 (FAO, 2024)
Number of imported cattle (in 2018)	7,139 (FAO, 2024)	4,305 (FAO, 2024)
Number of imported goats (in 2018)	1,000,000 (FAO, 2024)	1,471 (FAO, 2024)
Number of imported sheep (in 2018)	201,926 (FAO, 2024)	353 (FAO, 2024)
Import of livestock	Cattle: imported primarily for domestic consumption, sourced from countries like Australia, India, and Brazil Sheep: imported primarily for domestic consumption, sourced from countries like Australia, New Zealand, and Somalia Goats: minimal import for domestic consumption	Cattle, sheep and goat: minimal import for local consumption and nomadic herding
Native wild ruminant species	Sand gazelle, mountain gazelle, Arabian tahr, Arabian oryx	In the OROAFR: dorcas gazelle, addax, scimitar-horned oryx (reintrodcued), dama gazelle
Wild animal density	Lower due to arid environment and urbanization	Moderate to high in some regions
Zoonotic diseases	Presence of zoonotic diseases such as Middle East respiratory syndrome (MERS), Q fever, Crimean-Congo hemorrhagic fever (CCHF), Leptospirosis	Presence of zoonotic diseases such as anthrax, brucellosis, Rift Valley fever, Crimean-Congo hemorrhagic fever
Vector Species	Presence of ticks (*Hyalomma dromedarii, Rhipicephalus annulatus*), mosquitoes (*Culex* spp., *Aedes* spp.)…	presence of ticks (*Rhipicephalus spp., Hyalomma spp*.), flies (*Stomoxys calcitrans*)…
Animal Vaccination against	pleuropneumonia, foot-and-mouth disease, haemorrhagic septicaemia, lumpy skin disease, paratuberculosis, Peste des petits Ruminants, Sheep pox and goat pox (WAHIS, 2024)	Anthrax, haemorrhagic septicaemia, contagious bovine pleuropneumonia, Peste des Petits Ruminants (WAHIS, 2024)

Annex 2: Number of results in two scientific literature search engines? The queries entered comprehended the keywords indicated in the first column, and either “Chad”, “UAE”, or “Belgium”. (performed March 15, 2024)

	PubMed			Google Scholar		
Keywords	**Chad**	**UAE**	**Belgium**	**Chad**	**UAE**	**Belgium**
zoonotic disease	50	58	960	8,860	9,790	55,700
brucellosis	12	7	164	2,820	2,510	14,500
foot-and-mouth disease	5	6	104	2,350	2,240	13,600
contagious caprine pleuropneumonia	2	0	4	789	321	1,390
bovine tuberculosis	14	2	58	4,870	3,570	26,700
Crimean-Congo hemorrhagic fever	3	11	10	738	1,240	2,220
vector borne diseases	156	63	2,814	10,700	7,600	36,600
lumpy skin disease	1	2	33	665	434	2,800
toxoplasmosis	1	13	145	2,130	2,250	16,200
Q fever	6	4	46	11,600	14,400	61,900
tuberculosis	59	81	2,185	24,300	18,300	152,000
Mers CoV	0	44	40	15,800	17,600	26,700
bluetongue	0	2	129	600	518	4,830
total	309	293	6,692	86,222	80,773	415,140
average	24	23	515	6,632	6,213	31,934

Chapter 5 - References

[A]

Abdel-Aziz, A. I., Romey, A., Relmy, A., Gorna, K., Laloy, E., Métras, R., Muñoz, F., Blaise-Boisseau, S., Zientara, S., Lancelot, R., & Bakkali Kassimi, L. (2019). Seroprevalence and molecular characterization of foot-and-mouth disease virus in Chad. *Veterinary Medicine and Science*, *6*(1), 114–121. https://doi.org/10.1002/vms3.206

Abdelwhab, E.-S. M., Veits, J., & Mettenleiter, T. C. (2013). Genetic changes that accompanied shifts of low pathogenic avian influenza viruses toward higher pathogenicity in poultry. *Virulence*, *4*(6), 441–452. https://doi.org/10.4161/viru.25710

Ackelsberg, J., Liddicoat, A., Burke, T., Szymczak, W. A., Levi, M. H., Ostrowsky, B., Hamula, C., Patel, G., Kopetz, V., Saverimuttu, J., Sordillo, E. M., D'Souza, D., Mitchell, E. A., Lowe, W., Khare, R., Tang, Y.-W., Bianchi, A. L., Egan, C., Perry, M. J., … Layton, M. (2020). *Brucella* exposure risk events in 10 clinical laboratories, New York City, USA, 2015 to 2017. *Journal of Clinical Microbiology*, *58*(2), e01096-19. https://doi.org/10.1128/JCM.01096-19

ADFCA. (2010). *Statistic Book 2010* (p. 158). ADFCA. Retrieved March 17, 2024 https://www.adafsa.gov.ae/English/MediaCenter/Publications/Reports/Statistics%20Book.pdf

ADFCA. (2011). *Statistic Book 2011* (p. 215). ADFCA. Retrieved March 17, 2024 https://www.adafsa.gov.ae/English/MediaCenter/Publications/Reports/S%20BOOK%20Nov%202011%20Final%20Dec%2010.pdf

African Development Bank Group. (2021). *Chad—Project to support the productivity and competitiveness of the meat and dairy value chains in Chad (PAPCV-VL)* (p. 43) [Project appraisal report]. African Development Bank Group. https://www.afdb.org/en/documents/tchad-project-support-productivity-and-competitiveness-meat-and-dairy-value-chains-chad-papcv-vl-project-appraisal-report

Ahmad, S., Mokaddas, E., & Al-Mutairi, N. M. (2018). Prevalence of tuberculosis and multidrug resistant tuberculosis in the Middle East Region. *Expert Review of Anti-Infective Therapy*, *16*(9), 709–721. https://doi.org/10.1080/14787210.2018.1519393

Al Dabal, L. M., Rahimi Shahmirzadi, M. R., Baderldin, S., Abro, A., Zaki, A., Dessi, Z., Al Eassa, E., Khan, G., Shuri, H., & Alwan, A. M. (2016). Crimean-Congo Hemorrhagic Fever in Dubai, United Arab Emirates, 2010: Case Report. *Iranian Red Crescent Medical Journal*, *18*(8), e38374. https://doi.org/10.5812/ircmj.38374

Al Shehhi, N., Aziz, F., Al Hosani, F., Aden, B., & Blair, I. (2016). Human brucellosis in the Emirate of Abu Dhabi, United Arab Emirates, 2010–2015. *BMC Infectious Diseases*, *16*. https://doi.org/10.1186/s12879-016-1900-9

Alexandersen, S., Zhang, Z., Donaldson, A. I., & Garland, A. J. M. (2003). The pathogenesis and diagnosis of foot-and-mouth disease. *Journal of Comparative Pathology*, *129*(1), 1–36. https://doi.org/10.1016/s0021-9975(03)00041-0

Antunes, S., Rosa, C., Couto, J., Ferrolho, J., & Domingos, A. (2017). Deciphering *Babesia*-vector interactions. *Frontiers in Cellular and Infection Microbiology*, *7*. https://doi.org/10.3389/fcimb.2017.00429

ArcGIS StoryMaps. (2022). *Bringing the scimitar-horned oryx back to life*. https://storymaps.arcgis.com/stories/d20032af2d694b13bfdb22004120aeb7

Arif, A., Schulz, J., Thiaucourt, F., Taha, A., & Hammer, S. (2007). Contagious caprine pleuropneumonia outbreak in captive wild ungulates at Al Wabra Wildlife Preservation, State of Qatar. *Journal of Zoo and Wildlife Medicine*, *38*(1), 93–96. https://doi.org/10.1638/05-097.1

Aubry, P., & Geale, D. W. (2011). A review of bovine anaplasmosis. Transboundary and Emerging Diseases, 58(1), 1–30. https://doi.org/10.1111/j.1865-1682.2010.01173.x

[B]

Babiuk, S., Bowden, T. R., Boyle, D. B., Wallace, D. B., & Kitching, R. P. (2008). Capripoxviruses: An emerging worldwide threat to sheep, goats and cattle. *Transboundary and Emerging Diseases*, *55*(7), 263–272. https://doi.org/10.1111/j.1865-1682.2008.01043.x

Badiaga, S., Imbert, G., La Scola, B., Jean, P., Delmont, J., & Brouqui, P. (2005). Imported brucellosis associated with *Plasmodium falciparum* malaria in a traveler returning from the Tropics. *Journal of Travel Medicine*, *12*(5), 282–284. https://doi.org/10.2310/7060.2005.12508

Balkhy, H. H., & Memish, Z. A. (2003). Rift Valley fever: An uninvited zoonosis in the Arabian peninsula. *International Journal of Antimicrobial Agents*, *21*(2), 153–157. https://doi.org/10.1016/S0924-8579(02)00295-9

Belsham, G. J., Kristensen, T., & Jackson, T. (2020). Foot-and-mouth disease virus: Prospects for using knowledge of virus biology to improve control of this continuing global threat. *Virus Research*, *281*, 197909. https://doi.org/10.1016/j.virusres.2020.197909

Bemadjim, N. E., Newby, J., Desbiez, A., Lees, C., & Miller, P. (2012). *L'atelier technique sur la réintroduction de l'oryx algazelle dans la réserve de faune de Ouadi Rimé-Ouadi Achim, Tchad* (p. 44). IUCN/SSC Conservation Breeding Specialist Group,. http://www.cbsg.org/sites/cbsg.org/files/documents/SHO_Chad_WorkshopReport_French_FINAL.pdf

Beyouli, H. chedli T., & Neffati, M. (2016). Vegetative habitat selection of scimitar-horned oryx (*Oryx dammah*) in Bouhedma National Park, Southern Tunisia. Journal of King Saud University - Science, 3(28), 261–267. https://doi.org/10.1016/j.jksus.2016.04.003

Black, J. M. (1991). Reintroduction and restocking: Guidelines for bird recovery programmes. *Bird Conservation International*, *1*(4), 329–334. https://doi.org/10.1017/S095927090000068X

Brito, B. P., Rodriguez, L. L., Hammond, J. M., Pinto, J., & Perez, A. M. (2017). Review of the global distribution of foot-and-mouth disease virus from 2007 to 2014. *Transboundary and Emerging Diseases*, *64*(2), 316–332. https://doi.org/10.1111/tbed.12373

[C]

Caley, M. J., O'Leary, R. A., Fisher, R., Low-Choy, S., Johnson, S., & Mengersen, K. (2014). What is an expert? A systems perspective on expertise. *Ecology and Evolution*, *4*(3), 231–242. https://doi.org/10.1002/ece3.926

Caporale, M., Di Gialleonorado, L., Janowicz, A., Wilkie, G., Shaw, A., Savini, G., Van Rijn, P. A., Mertens, P., Di Ventura, M., & Palmarini, M. (2014). Virus and host factors affecting the clinical outcome of bluetongue virus infection. *Journal of Virology*, *88*(18), 10399–10411. https://doi.org/10.1128/jvi.01641-14

Cardinale, B. J., Duffy, J. E., Gonzalez, A., Hooper, D. U., Perrings, C., Venail, P., Narwani, A., Mace, G. M., Tilman, D., Wardle, D. A., Kinzig, A. P., Daily, G. C., Loreau, M., Grace, J. B., Larigauderie, A., Srivastava, D. S., & Naeem, S. (2012). Biodiversity loss and its impact on humanity. *Nature*, *486*(7401), 59–67. https://doi.org/10.1038/nature11148

Casaubon, J., Chaignat, V., Vogt, H.-R., Michel, A. O., Thür, B., & Ryser-Degiorgis, M.-P. (2013). Survey of bluetongue virus infection in free-ranging wild ruminants in Switzerland. *BMC Veterinary Research*, *9*(1), 166. https://doi.org/10.1186/1746-6148-9-166

Ceballos, G., & Ehrlich, P. R. (2002). Mammal population losses and the extinction crisis. *Science*, *296*(5569), 904–907. https://doi.org/10.1126/science.1069349

Chaber, A., & Saegerman, C. (2017). Biosecurity measures applied in the United Arab Emirates–a comparative study between livestock and wildlife sectors. Transboundary and Emerging Diseases, 64(4), 1184–1190. https://doi.org/10.1111/tbed.12488

Contreras, M., Kasaija, P. D., Merino, O., de la Cruz-Hernandez, N. I., Gortazar, C., & de la Fuente, J. (2019). Oral vaccination with a formulation combining *Rhipicephalus microplus* subolesin with heat inactivated *Mycobacterium bovis* reduces tick infestations in cattle. *Frontiers in Cellular and Infection Microbiology*, *9*. https://www.frontiersin.org/articles/10.3389/fcimb.2019.00045

Cossu, C. A., Collins, N. E., Oosthuizen, M. C., Menandro, M. L., Bhoora, R. V., Vorster, I., Cassini, R., Stoltsz, H., Quan, M., & van Heerden, H. (2023). Distribution and prevalence of *Anaplasmataceae, Rickettsiaceae and Coxiellaceae* in African ticks: A systematic review and meta-analysis. *Microorganisms*, *11*(3), 3. https://doi.org/10.3390/microorganisms11030714

[D]

Deem, S. L., Cruz, M. B., Higashiguchi, J. M., & Parker, P. G. (2012). Diseases of poultry and endemic birds in Galapagos: Implications for the reintroduction of native species. *Animal Conservation*, *15*(1), 73–82. https://doi.org/10.1111/j.1469-1795.2011.00489.x

Delafosse, A., & Doutoum, A. A. (2004). Prevalence of *Trypanosoma evansi* infection and associated risk factors in camels in eastern Chad. *Veterinary Parasitology*, *119*(2), 155–164. https://doi.org/10.1016/j.vetpar.2003.11.010

Delafosse, A., Goutard, F., & Thebaud, E. (2002). Epidémiologie de la tuberculose et de la brucellose des bovins en zone périurbaine d'Abéché, Tchad. *Revue d'Elevage et de Médecine Vétérinaire Des Pays Tropicaux*, *55*(1). https://doi.org/10.19182/remvt.9846

Delafosse, A., Thébaud, E., Desquesnes, M., & Michaux, Y. (2006). Epidemiology of *Trypanosoma vivax* infection in cattle in the tse-tse free area of Lake Chad. *Preventive Veterinary Medicine*, *74*(2), 108–119. https://doi.org/10.1016/j.prevetmed.2005.10.006

Delgado, M., Ferrari, N., Fanelli, A., Muset, S., Thompson, L., Sleeman, J. M., White, C. L., Walsh, D., Wannous, C., & Tizzani, P. (2023). Wildlife health surveillance: Gaps, needs and opportunities. *Revue Scientifique et Technique (International Office of Epizootics)*, *42*, 161–172. https://doi.org/10.20506/rst.42.3359

Devillers, P., & Devillers-Terschuren, J. (2005). *Oryx dammah*. In *Status and Perspectives. Report on the conservation status of the six Sahelo-Saharan Antelopes* (p. 26). UNEP/CMS Secretariat. https://www.cms.int/sites/default/files/publication/antilopes-ss-tech11-fr-part2.pdf

Di Teodoro, G., Marruchella, G., Di Provvido, A., D'Angelo, A. R., Orsini, G., Di Giuseppe, P., Sacchini, F., & Scacchia, M. (2020). Contagious Bovine Pleuropneumonia: A comprehensive overview. *Veterinary Pathology*, *57*(4), 476–489. https://doi.org/10.1177/0300985820921818

Diguimbaye-Djaibé, C., Hilty, M., Ngandolo, R., Mahamat, H. H., Pfyffer, G. E., Baggi, F., Hewinson, G., Tanner, M., Zinsstag, J., & Schelling, E. (2006). *Mycobacterium bovis* isolates from tuberculous lesions in Chadian zebu carcasses. *Emerging Infectious Diseases*, *12*(5), 769–771. https://doi.org/10.3201/eid1205.050691

Dipeolu, O. O., & Amoo, A. (1984). The presence of kinetes of a *Babesia* species in the haemolymph smears of engorged *Hyalomma* ticks in Nigeria. *Veterinary Parasitology*, *17*(1), 41–46. https://doi.org/10.1016/0304-4017(84)90063-3

Dissanayake, S., Usmani, A., Sheikh, F. A., Sheek-Hussein, M., Ameen, A. M., & Lukic, M. L. (2001). *Mycobacterium tuberculosis* in the United Arab Emirates: Evidence of local transmission with unique strains. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *95*(6), 591–594. https://doi.org/10.1016/S0035-9203(01)90088-4

Dufour, B., Plée, L., Moutou, F., Boisseleau, D., Chartier, C., Durand, B., Ganière, J. P., Guillotin, J., Lancelot, R., Saegerman, C., Thébault, A., Hattenberger, A. M., & Toma, B. (2011). A qualitative risk assessment methodology for scientific expert panels. *Revue Scientifique Et Technique (International Office of Epizootics)*, *30*(3), 673–681. https://doi.org/10.20506/rst.30.3.2063

[E]

East, R. (1999). *African antelope database 1998* (Occasional Paper of the IUCN Species Survival Commission, p. x+434) [21]. IUCN/SSC Antelope Specialist Group.IUCN, Gland, Switzerland and Cambridge, UK. https://portals.iucn.org/library/efiles/documents/ssc-op-021.pdf

EFSA Scientific Committee. (2012). Scientific opinion on risk assessment terminology. European Food Safety Authority Journal, 10(5), 2664. Retrieved March 17, 2024 from https://doi.org/10.2903/j.efsa.2012.2664

El Tigani-Asil, E. T. A., Blanda, V., Abdelwahab, G. E., Hammadi, Z. M. A., Habeeba, S., Khalafalla, A. I., Alhosani, M. A., La Russa, F., Migliore, S., Torina, A., Loria, G. R., & Al Muhairi, S. S. (2021). Molecular investigation on tick-borne hemoparasites and *Coxiella burnetii* in dromedary camels (*Camelus dromedarius*) in Al Dhafra Region of Abu Dhabi, UAE. *Animals*, *11*(3), 666. https://doi.org/10.3390/ani11030666

Eltahir, Y. M., Ishag, H. Z. A., Wadsworth, J., Hicks, H. M., Knowles, N. J., Mioulet, V., King, D. P., Mohamed, M. S., Bensalah, O. K., Yusof, M. F., Gasim, E. F. M., Hammadi, Z. M. A., Shah, A. A. M., Abdelmagid, Y. A., Gahlan, M. A. meguid E., Kassim, M. F., Kayaf, K., Zahran, A., & Nuaimat, M. M. A. (2024). Molecular epidemiology of foot-and-mouth disease viruses in the emirate of Abu Dhabi, United Arab Emirates. *Veterinary Sciences*, *11*(1), 1. https://doi.org/10.3390/vetsci11010032

European Commission. (2021). *Programme Indicatif Multiannuel 2021-2027 en faveur de la république du Tchad*. European Commission. Retrieved March 17, 2024 from https://international-partnerships.ec.europa.eu/system/files/2022-01/mip-2021-c2021-9104-chad-annex_fr.pdf

European Commission. (2023). *European Commission—International partnerships—Chad*. Retrieved March 17, 2024 from https://international-partnerships.ec.europa.eu/countries/chad_en

European Commission, Joint Research Centre. (2023). *The Digital Observatory for Protected Areas (DOPA) Explorer—Ouadi Rimé-Ouadi Achim*. European Commission. Retrieved March 17, 2024 from https://dopa-explorer.jrc.ec.europa.eu/wdpa/1250

[F]

FAO. (2024). *FAOSTAT* [Database]. Crops and Livestock Products - Food and Agriculture Organization of the United Nations. Retrieved March 20, 2024 from https://www.fao.org/faostat/en/#data/QCL

Fitzgerald, S. D., & Kaneene, J. B. (2013). Wildlife reservoirs of bovine tuberculosis worldwide: Hosts, pathology, surveillance, and control. *Veterinary Pathology*, *50*(3), 488–499. https://doi.org/10.1177/0300985812467472

Foster, J. T., Walker, F. M., Rannals, B. D., Hussain, M. H., Drees, K. P., Tiller, R. V., Hoffmaster, A. R., Al-Rawahi, A., Keim, P., & Saqib, M. (2018). African Lineage *Brucella melitensis* Isolates from Omani livestock. *Frontiers in Microbiology*, *8*. https://doi.org/10.3389/fmicb.2017.02702

Frölich, K., Hamblin, C., Jung, S., Ostrowski, S., Mwanzia, J., Streich, W. J., Anderson, J., Armstrong, R. M., & Anajariyah, S. (2005). Serologic surveillance for selected viral agents in captive and free-ranging populations of Arabian oryx (*Oryx leucoryx*) from Saudi Arabia and the United Arab Emirates. *Journal of Wildlife Diseases*, *41*(1), 67–79. https://doi.org/10.7589/0090-3558-41.1.67

[G]

Gascon, C., Brooks, T. M., Contreras-MacBeath, T., Heard, N., Konstant, W., Lamoreux, J., Launay, F., Maunder, M., Mittermeier, R. A., Molur, S., Al Mubarak, R. K., Parr, M. J., Rhodin, A. G. J., Rylands, A. B., Soorae, P., Sanderson, J. G., & Vié, J.-C. (2015). The Importance and Benefits of Species. *Current Biology*, *25*(10), R431–R438. https://doi.org/10.1016/j.cub.2015.03.041

Gates Foundation. (2019). *Routine immunization strengthening in Chad*. Bill & Melinda Gates Foundation. Retrieved March 17, 2024 from https://www.gatesfoundation.org/about/committed-grants/2019/07/inv002136

Gilbert, T., & Woodfine, T. (2004). *The biology, husbandry and conservation of scimitar-horned oryx (Oryx dammah)*. Marwell Preservation Trust.

Gortazar, C., Diez-Delgado, I., Barasona, J. A., Vicente, J., De La Fuente, J., & Boadella, M. (2015). The wild side of disease control at the wildlife-livestock-human interface: A review. *Frontiers in Veterinary Science*, *1*, 27. https://doi.org/10.3389/fvets.2014.00027

Greth, A., Gourreau, J. M., Vassart, M., Wyers, M., & Lefevre, P. C. (1992). Capripoxvirus disease in an Arabian oryx (*Oryx leucoryx*) from Saudi Arabia. *Journal of Wildlife Diseases*, *28*(2), 295–300.

Griffith, B., Scott, J. M., Carpenter, J. W., & Reed, C. (1993). Animal translocations and potential disease transmission. *Journal of Zoo and Wildlife Medicine*, *24*(3), 231–236.

Guinat, C., Gogin, A., Blome, S., Keil, G., Pollin, R., Pfeiffer, D. U., & Dixon, L. (2016). Transmission routes of African swine fever virus to domestic pigs: Current knowledge and future research directions. *Veterinary Record*, *178*(11), 262–267. https://doi.org/10.1136/vr.103593

[H]

Hamdi, J., Munyanduki, H., Omari Tadlaoui, K., El Harrak, M., & Fassi Fihri, O. (2021). Capripoxvirus infections in ruminants: A review. *Microorganisms*, *9*(5), 5. https://doi.org/10.3390/microorganisms9050902

Hartley, M., & Sainsbury, A. (2017). Methods of disease risk analysis in wildlife translocations for conservation purposes. *EcoHealth*, *14*(S1), 16–29. https://doi.org/10.1007/s10393-016-1134-8

Hartemink, N., Vanwambeke, S. O., Purse, B. V., Gilbert, M., & Van Dyck, H. (2015). Towards a resource-based habitat approach for spatial modelling of vector-borne disease risks. Biological Reviews, 90(4), 1151–1162. https://doi.org/10.1111/brv.12149

Haulisah, N. A., Hassan, L., Bejo, S. K., Jajere, S. M., & Ahmad, N. I. (2021). High levels of antibiotic resistance in isolates from diseased livestock. *Frontiers in Veterinary Science*, *8*. https://doi.org/10.3389/fvets.2021.652351

Haydon, D. T., Cleaveland, S., Taylor, L. H., & Laurenson, M. K. (2002). Identifying reservoirs of infection: A conceptual and practical challenge. *Emerging Infectious Diseases*, *8*(12), 1468–1473. https://doi.org/10.3201/eid0812.010317

Hedger, R. S. (1972). Foot-and-mouth disease and the African buffalo (*Syncerus caffer*). *Journal of Comparative Pathology*, *82*(1), 19–28. https://doi.org/10.1016/0021-9975(72)90022-9

Horigan, V., Simons, R., Kavanagh, K., & Kelly, L. (2023). A review of qualitative risk assessment in animal health: Suggestions for best practice. Frontiers in Veterinary Science, 10. https://doi.org/10.3389/fvets.2023.1102131

Humble, E., Dobrynin, P., Senn, H., Chuven, J., Scott, A. F., Mohr, D. W., Dudchenko, O., Omer, A. D., Colaric, Z., Lieberman Aiden, E., Al Dhaheri, S. S., Wildt, D., Oliaji, S., Tamazian, G., Pukazhenthi, B., Ogden, R., & Koepfli, K.-P. (2020). Chromosomal-level genome assembly of the scimitar-horned

oryx: Insights into diversity and demography of a species extinct in the wild. *Molecular Ecology Resources*, *20*(6), 1668–1681. https://doi.org/10.1111/1755-0998.13181

Humble, E., Stoffel, M. A., Dicks, K., Ball, A. D., Gooley, R. M., Chuven, J., Pusey, R., Remeithi, M. A., Koepfli, K.-P., Pukazhenthi, B., Senn, H., & Ogden, R. (2023). Conservation management strategy impacts inbreeding and mutation load in scimitar-horned oryx. *Proceedings of the National Academy of Sciences*, *120*(18), e2210756120. https://doi.org/10.1073/pnas.2210756120

[I]

ICTV. (2024). *Current International Committee on Taxonomy of Viruses Taxonomy Release*. Retrieved March 17, 2024 from https://ictv.global/taxonomy

IMF. (2023). *United Arab Emirates and the International Monetary Fund*. Retrieved March 17, 2024 from https://www.imf.org/en/Countries/ARE

IMF. (2023). *Chad and the International Monetary Fund*. Retrieved March 17, 2024 from https://www.imf.org/en/Countries/TCD.

IPBES. (2019). Global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES). *E. S. Brondizio, J. Settele, S. Díaz, and H. T. Ngo, version 1*, 1148. https://doi.org/10.5281/zenodo.3831673

IUCN, & SSC. (2013). *Guidelines for reintroductions and other conservation translocations. Version 1.0*. IUCN Species Survival Commission. Retrieved March 17, 2024 from https://portals.iucn.org/library/node/10386

IUCN PAPACO. (2009). *Management Effectiveness Tracking Tool (METT)—Tchad—Réserve de faune Ouadi Rimé—Ouadi Achim*. IUCN Programme on African Protected Areas & Conservation. Retrieved March 17, 2024 from https://papaco.org/fr/wp-content/uploads/2015/07/Tchad-METT-oudi-Rime.pdf

IUCN SSC Antelope Specialist. (2023). *IUCN Red List of Threatened Species: Oryx dammah*. IUCN Red List of Threatened Species. Retrieved March 17, 2024 from https://www.iucnredlist.org/species/15568/197393805

Iyengar, A., Gilbert, T., Woodfine, T., Knowles, J. M., Diniz, F. M., Brenneman, R. A., Louis, E. E., & Maclean, N. (2007). Remnants of ancient genetic diversity preserved within captive groups of scimitar-horned oryx (*Oryx dammah*). *Molecular Ecology*, *16*(12), 2436–2449. https://doi.org/10.1111/j.1365-294X.2007.03291.x

[J]

Jakob-Hoff, R. M., MacDiarmid, S. C., Lees, C., Miller, P. S., Travis, D., & Kock, R. (2014). *Manual of procedures for wildlife disease risk analysis*. WOAH Published in association with the IUCN and the SSC.

Jamal, S. M., & Belsham, G. J. (2018). Molecular epidemiology, evolution and phylogeny of foot-and-mouth disease virus. Infection, Genetics and Evolution, 59, 84–98. https://doi.org/10.1016/j.meegid.2018.01.020

Jebara, K. B., Cáceres, P., Berlingieri, F., & Weber-Vintzel, L. (2012). Ten years' work on the World Organisation for Animal Health (OIE) Worldwide Animal Disease Notification System. *Preventive Veterinary Medicine*, *107*(3), 149–159. https://doi.org/10.1016/j.prevetmed.2012.08.008

Jia, B., Colling, A., Stallknecht, D. E., Blehert, D., Bingham, J., Crossley, B., Eagles, D., & Gardner, I. A. (2020). Validation of laboratory tests for infectious diseases in wild mammals: Review and recommendations. *Journal of Veterinary Diagnostic Investigation*, *32*(6), 776–792. https://doi.org/10.1177/1040638720920346

[K]

Kasem, S., Saleh, M., Qasim, I., Hashim, O., Alkarar, A., Abu-Obeida, A., Gaafer, A., Hussien, R., AL-Sahaf, A., Al-Doweriej, A., Bayoumi, F., Hodhood, A., & Abdelatif, M. (2018). Outbreak investigation and molecular diagnosis of Lumpy Skin Disease among livestock in Saudi Arabia 2016. *Transboundary and Emerging Diseases*, *65*(2), e494–e500. https://doi.org/10.1111/tbed.12769

Khalafalla, A. I., Gaffar, E. M., & Abbas, Z. (1993). Lumpy skin disease: Observations on the recent outbreaks of the disease in the Sudan. *Revue d'elevage et de Medecine Veterinaire Des Pays Tropicaux*, *46*(4), 548–550.

Khan, A. S., Maupin, G. O., Rollin, P. E., Noor, A. M., Shurie, H. H., Shalabi, A. G., Wasef, S., Haddad, Y. M., Sadek, R., & Ijaz, K. (1997). An outbreak of Crimean-Congo hemorrhagic fever in the United Arab Emirates, 1994-1995. *The American Journal of Tropical Medicine and Hygiene*, *57*(5), 519–525. https://doi.org/10.4269/ajtmh.1997.57.519

Kilgallon, C., Bailey, T., O'Donovan, D., Wernery, U., & Alexandersen, S. (2008). Temporal assessment of seroconversion in response to inactivated foot-and-mouth disease vaccine in Arabian oryx (*Oryx leucoryx*). *Veterinary Record*, *163*(24), 717–720. https://doi.org/10.1136/vr.163.24.717

Kitching, R. P., McGrane, J. J., & Taylor, W. P. (1986). Capripox in the Yemen Arab Republic and the Sultanate of Oman. *Tropical Animal Health and Production*, *18*(2), 115–122. https://doi.org/10.1007/BF02359725

Knowles, N. J., Samuel, A. R., Davies, P. R., Midgley, R. J., & Valarcher, J.-F. (2005). Pandemic strain of foot-and-mouth disease virus serotype O. *Emerging Infectious Diseases*, *11*(12), 1887–1893. https://doi.org/10.3201/eid1112.050908

Kock, R. A., Woodford, M. H., & Rossiter, P. B. (2010). Disease risks associated with the translocation of wildlife. *Revue Scientifique et Technique (International Office of Epizootics)*, *29*(2), 329–350. https://doi.org/10.20506/rst.29.2.1980

[L]

Lachish, S., & Murray, K. A. (2018). The certainty of uncertainty: Potential sources of bias and imprecision in disease ecology studies. *Frontiers in Veterinary Science*, *5*. https://doi.org/10.3389/fvets.2018.00090

Leach, C. B., Webb, C. T., & Cross, P. C. (2016). When environmentally persistent pathogens transform good habitat into ecological traps. Royal Society Open Science, 3(3), 160051. https://doi.org/10.1098/rsos.160051

Lebov, J., Grieger, K., Womack, D., Zaccaro, D., Whitehead, N., Kowalcyk, B., & MacDonald, P. D. M. (2017). A framework for One Health research. *One Health*, *3*, 44–50. https://doi.org/10.1016/j.onehlt.2017.03.004

Leighton, F. A. (2002). Health risk assessment of the translocation of wild animals. *Revue Scientifique Et Technique (International Office of Epizootics)*, *21*(1), 187–195.

Lefèvre, P.-C. (1991). *Atlas des maladies infectieuses des ruminants* (CIRAD-IEMVT). CIRAD-IEMVT.

Li, N., Yu, F., Peng, F., Zhang, X., & Jia, B. (2020). Probable sexual transmission of brucellosis. *IDCases*, *21*, e00871. https://doi.org/10.1016/j.idcr.2020.e00871

Lignereux, L., Chaber, A.-L., Saegerman, C., Heath, L., Knowles, N. J., Wadsworth, J., Mioulet, V., & King, D. P. (2020). Foot-and-mouth disease outbreaks in captive scimitar-horned oryx (*Oryx dammah*). *Transboundary and Emerging Diseases*. https://doi.org/10.1111/tbed.13502

Limon, G., Gamawa, A. A., Ahmed, A. I., Lyons, N. A., & Beard, P. M. (2020). Epidemiological characteristics and economic impact of Lumpy Skin Disease, Sheeppox and Goatpox among subsistence farmers in northeast Nigeria. *Frontiers in Veterinary Science*, *7*. https://www.frontiersin.org/articles/10.3389/fvets.2020.00008

Loire, E., Ibrahim, A. I., Manso-Silván, L., Lignereux, L., & Thiaucourt, F. (2020). A whole-genome worldwide molecular epidemiology approach for contagious caprine pleuropneumonia. *Heliyon*, *6*(10), e05146. https://doi.org/10.1016/j.heliyon.2020.e05146

Lu, L., Zhang, F., Munnink, B. B. O., Munger, E., Sikkema, R. S., Pappa, S., Tsioka, K., Sinigaglia, A., Molin, E. D., Shih, B. B., Günther, A., Pohlmann, A., Ziegler, U., Beer, M., Taylor, R. A., Bartumeus, F., Woolhouse, M., Aarestrup, F. M., Barzon, L., … Koopmans, M. P. G. (2024). West Nile virus spread in Europe: Phylogeographic pattern analysis and key drivers. *PLOS Pathogens*, 20(1), e1011880. https://doi.org/10.1371/journal.ppat.1011880

Luizza, M. (2017). *Transhumant pastoralism in Central Africa: Emerging impacts on conservation and security* [Unpublished report.]. US Fish & Wildlife Service. https://doi.org/10.13140/RG.2.2.15410.91843/1

[M]

Machtinger, E. T., Poh, K. C., Pesapane, R., & Tufts, D. M. (2024). An integrative framework for tick management: The need to connect wildlife science, One Health, and interdisciplinary perspectives. *Current Opinion in Insect Science*, *61*, 101131. https://doi.org/10.1016/j.cois.2023.101131

Mamman, A. H., Lorusso, V., Adam, B. M., Dogo, G. A., Bown, K. J., & Birtles, R. J. (2021). First report of *Theileria annulata* in Nigeria: Findings from cattle ticks in Zamfara and Sokoto States. *Parasites & Vectors*, *14*(1), 242. https://doi.org/10.1186/s13071-021-04731-4

Manso-Silván, L., & Thiaucourt, F. (2019). Contagious caprine pleuropneumonia. In M. Kardjadj, A. Diallo, & R. Lancelot (Eds.), *Transboundary animal diseases in Sahelian Africa and connected regions* (pp. 439–458). Springer International Publishing. https://doi.org/10.1007/978-3-030-25385-1_21

Martinelle, L., Haegeman, A., Lignereux, L., Chaber, A.-L., Dal Pozzo, F., De Leeuw, I., De Clercq, K., & Saegerman, C. (2022). Orbivirus screening from imported captive oryx in the United Arab Emirates stresses the importance of pre-import and transit measures. *Pathogens*, *11*(6), 6. https://doi.org/10.3390/pathogens11060697

Masiga, W. N., Rossiter, P., & Bessin, B. (1998). *The present situation of CBPP in Africa and epidemiological trends*. 116. https://www.fao.org/3/ae716e/ae716e.pdf

Messina, J. P., Pigott, D. M., Golding, N., Duda, K. A., Brownstein, J. S., Weiss, D. J., Gibson, H., Robinson, T. P., Gilbert, M., William Wint, G. R., Nuttall, P. A., Gething, P. W., Myers, M. F., George, D. B., & Hay, S. I. (2015). The global distribution of Crimean-Congo hemorrhagic fever. *Transactions of The Royal Society of Tropical Medicine and Hygiene*, *109*(8), 503–513. https://doi.org/10.1093/trstmh/trv050

Michel, A. L., Van Heerden, H., Prasse, D., Rutten, V. P. M. G., Al Dahouk, S., & Crossley, B. M. (2021). *Pathogen detection and disease diagnosis in wildlife: Challenges and opportunities*. https://doi.org/10.20506/rst.40.1.3211

Mick, V., Carrou, G. L., Corde, Y., Game, Y., Jay, M., & Garin-Bastuji, B. (2014). *Brucella melitensis* in France: Persistence in wildlife and probable spillover from Alpine ibex to domestic animals. *PLOS ONE*, *9*(4), e94168. https://doi.org/10.1371/journal.pone.0094168

Miquelle, D. G., Poole, C. M., Mahood, S. P., Travers, H., Linkie, M., Goodrich, J., Walston, J., Rotha, K., & Rabinowtiz, A. (2018). Comments on "a framework for assessing readiness for tiger reintroductions." *Biodiversity and Conservation*, *27*(12), 3287–3293. https://doi.org/10.1007/s10531-018-1591-1

Ministère des Armées. (2022, February 14). *Opération BARKHANE*. Retrieved March 17, 2024 from https://www.defense.gouv.fr/operations/bande-sahelo-saharienne/operation-barkhane

Morrison, I. W. (2022). Theileriosis in Animals. In *MSD Veterinary Manual*. Merck & Co., Inc., Rahway, NJ, USA. Retrieved March 17, 2024 from https://www.msdvetmanual.com/circulatory-system/blood-parasites/theileriosis-in-animals?query=theileriosis

Mucheka, V. T., Pillay, A., & Mukaratirwa, S. (2023). Prevalence of tick-borne pathogens in *Rhipicephalus* species infesting domestic animals in Africa: A systematic review and meta-analysis. *Acta Tropica*, *246*, 106994–106994. https://doi.org/10.1016/j.actatropica.2023.106994

[N]

Newby, J. (1980). Can Addax and Oryx be saved in the Sahel? *Oryx*, *15*(3), 262–266. https://doi.org/10.1017/S0030605300024662

Newby, J. (1988). Aridland wildlife in decline: The case of the scimitar-horned oryx. In *Conservation and biology of desert antelopes* (Vol. 146). Christopher Helm, London.

[O]

Oberem, P. T., & Bezuidenhout, J. D. (1987). Heartwater in hosts other than domestic ruminants. *The Onderstepoort Journal of Veterinary Research*, *54*(3), 271–275.

Ogden, R., Chuven, J., Gilbert, T., Hosking, C., Gharbi, K., Craig, M., Al Dhaheri, S. S., & Senn, H. (2020). Benefits and pitfalls of captive conservation genetic management: Evaluating diversity in scimitar-horned oryx to support reintroduction planning. *Biological Conservation*, *241*, 108244. https://doi.org/10.1016/j.biocon.2019.108244

Orsel, K., Dekker, A., Bouma, A., Stegeman, J. A., & de Jong, M. C. M. (2007). Quantification of foot-and-mouth disease virus excretion and transmission within groups of lambs with and without vaccination. *Vaccine*, *25*(14), 2673–2679. https://doi.org/10.1016/j.vaccine.2006.11.048

Ouagal, M., Brocchi, E., Grazioli, S., Adel, B. Y., Keith, S., Kiram, D., Oussiguere, A., Hendrikx, P., Berkvens, D., & Saegerman, C. (2018). Study on seroprevalence and serotyping of foot-and-mouth disease in Chad. *Revue Scientifique et Technique (International Office of Epizootics)*, *37*(3), 937–947. https://doi.org/10.20506/37.3.2897

Özcelik, R., Abakar, M. F., Counotte, M. J., Zakaria, F. A., Kimala, P., Issa, R., & Dürr, S. (2023). Seroprevalence and associated risk factors of brucellosis, Rift Valley fever and Q fever among settled and mobile agro-pastoralist communities and their livestock in Chad. *PLoS Neglected Tropical Diseases*, *17*(6), e0011395–e0011395. https://doi.org/10.1371/journal.pntd.0011395

[P]

Penczykowski, R. M., Hall, S. R., Civitello, D. J., & Duffy, M. A. (2014). Habitat structure and ecological drivers of disease. Limnology and Oceanography, 59(2), 340–348.

Penzhorn, B. L. (2006). Babesiosis of wild carnivores and ungulates. *Veterinary Parasitology*, *138*(1–2), 11–21. https://doi.org/10.1016/j.vetpar.2006.01.036

Perveen, N. (2021). *Livestock ticks in the UAE: prevalence, distribution, population dynamics* [United Arab Emirates University]. Retrieved March 17, 2024 from https://scholarworks.uaeu.ac.ae/cgi/viewcontent.cgi?article=1134&context=all_dissertations

Perveen, N., & Khan, G. (2022). Crimean–Congo hemorrhagic fever in the Arab world: A systematic review. *Frontiers in Veterinary Science*, *9*. https://doi.org/10.3389/fvets.2022.938601

Perveen, N., Muzaffar, S. B., & Al-Deeb, M. A. (2021). Prevalence, distribution, and molecular record of four hard ticks from livestock in the United Arab Emirates. *Insects*, *12*(11), 1016. https://doi.org/10.3390/insects12111016

Peter, T. F., Burridge, M. J., & Mahan, S. M. (2002). *Ehrlichia ruminantium* infection (heartwater) in wild animals. *Trends in Parasitology*, *18*(5), 214–218. https://doi.org/10.1016/S1471-4922(02)02251-1

[R]

Rhyan, J. C., & Spraker, T. R. (2010). Emergence of diseases from wildlife reservoirs. *Veterinary Pathology*, *47*(1), 34–39. https://doi.org/10.1177/0300985809354466

Rinchen, S., Tenzin, T., Hall, D., & Cork, S. (2020). A qualitative risk assessment of rabies reintroduction into the rabies low-risk zone of Bhutan. *Frontiers in Veterinary Science*, *7*. https://doi.org/10.3389/fvets.2020.00366

Ringot, D., Durand, J.-P., Tolou, H., Boutin, J.-P., & Davoust, B. (2004). Rift Valley Fever in Chad. *Emerging Infectious Diseases*, *10*(5), 945–947. https://doi.org/10.3201/eid1005.030621

Riojas, M. A., McGough, K. J., Rider-Riojas, C. J., Rastogi, N., & Hazbón, M. H. (2018). Phylogenomic analysis of the species of the *Mycobacterium tuberculosis* complex demonstrates that *Mycobacterium africanum*, *Mycobacterium bovis*, *Mycobacterium caprae*, *Mycobacterium microti* and *Mycobacterium pinnipedii* are later heterotypic synonyms of *Mycobacterium tuberculosis*. *International Journal of Systematic and Evolutionary Microbiology*, *68*(1), 324–332. https://doi.org/10.1099/ijsem.0.002507

Ristic, M. (1981). Babesiosis. In M. Ristic & I. McIntyre (Eds.), *Diseases of cattle in the tropics: Economic and zoonotic relevance* (pp. 443–468). Springer Netherlands. https://doi.org/10.1007/978-94-015-6895-1_35

Roche, S. E., Costard, S., Meers, J., Field, H. E., & Breed, A. C. (2015). Assessing the risk of Nipah virus establishment in Australian flying-foxes. *Epidemiology and Infection*, *143*(10), 2213–2226. https://doi.org/10.1017/S0950268813003336

Rodríguez, O., de la Fuente, G., Fernández de Mera, I. G., Vaz-Rodrigues, R., Gortázar, C., & de la Fuente, J. (2022). The Saharan antelope addax (*Addax nasomaculatus*) as a host for *Hyalomma marginatum*, tick vector of Crimean-Congo hemorrhagic fever virus. *Ticks and Tick-Borne Diseases*, *13*(6), 102034. https://doi.org/10.1016/j.ttbdis.2022.102034

Roper, T. J. (2003). Badger cull culled. *Nature*, *426*(6968), 6968. https://doi.org/10.1038/nature02219

Rostal, M. K., Liang, J. E., Zimmermann, D., Bengis, R., Paweska, J., & Karesh, W. B. (2017). Rift Valley Fever: Does Wildlife Play a Role? *ILAR Journal*, *58*(3), 359–370. https://doi.org/10.1093/ilar/ilx023

[S]

Sainsbury, A. W., & Vaughan-Higgins, R. J. (2012). Analyzing disease risks associated with translocations. *Conservation Biology*, *26*(3), 442–452. https://doi.org/10.1111/j.1523-1739.2012.01839.x

Schelling, E., Diguimbaye, C., Daoud, S., Nicolet, J., Boerlin, P., Tanner, M., & Zinsstag, J. (2003). Brucellosis and Q-fever seroprevalences of nomadic pastoralists and their livestock in Chad. *Preventive Veterinary Medicine*, *61*(4), 279–293. https://doi.org/10.1016/j.prevetmed.2003.08.004

Scientific Committee on Animal Health and Animal Welfare. (2001). *Brucellosis in sheep and goats* (p. 89). European Commission. Retrieved on March 23, 2024 from https://food.ec.europa.eu/system/files/2020-12/sci-com_scah_out59_en.pdf

Shimshony, A., Orgad, U., Baharav, D., Prudovsky, S., Yakobson, B., Moshe, B. B., & Dagan, D. (1986). Malignant foot-and-mouth disease in mountain gazelles. *Veterinary Record*, *119*(8), 175–176. https://doi.org/10.1136/vr.119.8.175

Soorae, P. S. (2018). *Global reintroduction perspectives: 2018. Case studies from around the globe* (6th ed.). IUCN/SSC Reintroduction Specialist Group and Environment Agency, Abu Dhabi. https://doi.org/10.2305/IUCN.CH.2018.08.en

Springer, A., Shuaib, Y. A., Isaa, M. H., Ezz-Eldin, M. I.-E., Osman, A. Y., Yagoub, I. A., Abdalla, M. A., Bakiet, A. O., Mohmed-Noor, S. E.-T., Schaper, S., Rieß, R., Dobler, G., Strube, C., Bakkes, D. K., & Chitimia-Dobler, L. (2020). Tick fauna and associated *Rickettsia*, *Theileria*, and *Babesia* spp. In domestic animals in Sudan (North Kordofan and Kassala States). *Microorganisms*, *8*(12), 12. https://doi.org/10.3390/microorganisms8121969

Suárez-Esquivel, M., Ruiz-Villalobos, N., Jiménez-Rojas, C., Barquero-Calvo, E., Chacón-Díaz, C., Víquez-Ruiz, E., Rojas-Campos, N., Baker, K. S., Oviedo-Sánchez, G., Amuy, E., Chaves-Olarte, E., Thomson, N. R., Moreno, E., & Guzmán-Verri, C. (2017). *Brucella neotomae* infection in humans, Costa Rica. *Emerging Infectious Diseases*, *23*(6), 997–1000. https://doi.org/10.3201/eid2306.162018

SDC. (2016). Support Project for the Health Districts in Chad (PADS)—Yao and Danamadji. Swiss Agency for Development and Cooperation. Retrieved March 17, 2024 from https://www.swisstph.ch/en/projects/project-detail/project/support-project-for-the-health-districts-in-chad-yao-and-danamadji

[T]

Tabor, A. E. (2022). Anaplasmosis in ruminants. In *MSD Veterinary Manual*. Merck & Co., Inc., Rahway, NJ, USA. Retrieved March 17, 2024 from https://www.msdvetmanual.com/circulatory-system/blood-parasites/anaplasmosis-in-ruminants

Temur, A. I., Kuhn, J. H., Pecor, D. B., Apanaskevich, D. A., & Keshtkar-Jahromi, M. (2021). Epidemiology of Crimean-Congo Hemorrhagic Fever (CCHF) in Africa—Underestimated for decades. *The American Journal of Tropical Medicine and Hygiene*, *104*(6), 1978–1990. https://doi.org/10.4269/ajtmh.20-1413

The World Bank. (2024). *World Development Indicators | DataBank* [Database]. Retrieved March 20, 2024 from https://databank.worldbank.org/source/world-development-indicators

Thomas, P. (1873). Rapport médical sur le Bou Frida. In *Publication du gouvernement général civil de l'Algérie* (p. 35). Jourdan A., Alger.

Thomson, M. C., & Connor, S. J. (2000). Environmental information systems for the control of arthropod vectors of disease. Medical and Veterinary Entomology, 14(3), 227–244. https://doi.org/10.1046/j.1365-2915.2000.00250.x

[U]

Uilenberg, G. (1970). Existence of *Haematoxenus veliferus* (Sporozoa, Theileriidae) in Central African Republic [and Chad]. Presence of *Haematoxenus* sp. In African buffalo. *Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux*, *23*, 455–456.

Ulziibat, G., Raizman, E., Lkhagvasuren, A., Bartels, C. J. M., Oyun-Erdene, O., Khishgee, B., Browning, C., King, D. P., Ludi, A. B., & Lyons, N. A. (2023). Comparison of vaccination schedules for foot-and-

mouth disease among cattle and sheep in Mongolia. *Frontiers in Veterinary Science*, *10*, 990043. https://doi.org/10.3389/fvets.2023.990043

UNEP-WCMC, & IUCN. (2024). Protected Planet: The World Database on Protected Areas (WDPA) and World Database on Other Effective Area-based Conservation Measures (WD-OECM). Retrieved March 17, 2024 from https://www.protectedplanet.net

[V]

Vallat, B., Thiermann, A., Ben Jebara, K., & Dehove, A. (2013). Notification of animal and human diseases: The global legal basis. *Revue Scientifique Et Technique (International Office of Epizootics)*, *32*(2), 331–335.

Viggers, K. L., Lindenmayer, D. B., & Spratt, D. M. (1993). The importance of disease in reintroduction programmes. *Wildlife Research*, *20*(5), 687–698. https://doi.org/10.1071/wr9930687

Vourchakbé, J., Tiofack, A. A. Z., Kante, S. T., Barka, P. A., & Simo, G. (2022). Prevalence of pathogenic trypanosome species in naturally infected cattle of three sleeping sickness foci of the south of Chad. *PLOS ONE*, *17*(12), e0279730. https://doi.org/10.1371/journal.pone.0279730

[W]

WAHIS. (2019). *Event ID 2907—Chad—Rift Valley fever virus (Inf. With)* (Immediate Notification IN_15240). Retrieved March 17, 2024 from https://wahis.woah.org/#/in-review/2907?reportId=15240&fromPage=event-dashboard-url

WAHIS. (2024). [Database]. World Animal Health Information System - Control Measures Dashboard. Retrieved March 20, 2024 from https://wahis.woah.org/#/dashboards/control-measure-dashboard

Walker, A. R., Bouattour, A., Camicas, J.-L., Estrada-Peña, A., Horak, I. G., Latif, A. A., Pegram, R. G., & Preston, P. M. (2003). *Ticks of domestic animals in Africa: A guide to identification of species*. Bioscience Reports, Edinburgh Scotland,U.K. https://www.alanrwalker.com/assets/PDF/tickguide-africa.pdf

WFP. (2019). *Chad country strategic plan (2019—2024) | World Food Programme*. Retrieved March 17, 2024 from https://www.wfp.org/operations/td01-chad-country-strategic-plan-2019-2024

Wildt, D., Miller, P., Koepfli, K.-P., Pukazhenthi, B., Palfrey, K., Livingston, G., Beetem, D., Shurter, S., Gregory, J., Takács, M., & Snodgrass, K. (2019). Breeding centers, private ranches, and genomics for creating sustainable wildlife populations. *BioScience*, *69*(11), 928–943. https://doi.org/10.1093/biosci/biz091

Wilkowsky, S. E. (2022). Trypanosomiasis in ruminants. In *MSD Veterinary Manual*. Merck & Co., Inc., Rahway, NJ, USA. Retrieved March 17, 2024 from https://www.msdvetmanual.com/circulatory-system/blood-parasites/trypanosomiasis-in-animals

WOAH. (2023). *Manual of diagnostic tests and vaccines for terrestrial animals (Terrestrial Manual)* (12th ed.). World Organisation for Animal Health. Retrieved March 17, 2024 from https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/A_summry.htm

WOAH, & IUCN. (2014). *Guidelines for wildlife disease risk analysis*. WOAH & IUCN. Retrieved March 17, 2024 from https://portals.iucn.org/library/sites/library/files/documents/2014-006.pdf

Woodfine, T., & Gilbert, T. (2016). The fall and rise of the scimitar-horned oryx: A case study of ex-situ conservation and reintroduction in practice. In *Antelope conservation: From diagnosis to action* (First, pp. 280–296). John Wiley & Sons, Ltd.

World Bank. (2023). *Climate Change Knowledge Portal for Development Practitioners and Policy Makers—Chad*. Climate Change Knowledge Portal. Retrieved March 17, 2024 from https://climateknowledgeportal.worldbank.org/country/chad/climate-data-historical

[Z]

Zhou, K., Wu, B., Pan, H., Paudyal, N., Jiang, J., Zhang, L., Li, Y., & Yue, M. (2020). ONE Health approach to address zoonotic brucellosis: A spatiotemporal associations study between animals and humans. *Frontiers in Veterinary Science*, *7*. https://doi.org/10.3389/fvets.2020.00521

[Y]

Yu, Z., Wang, T., Sun, H., Xia, Z., Zhang, K., Chu, D., Xu, Y., Xin, Y., Xu, W., Cheng, K., Zheng, X., Huang, G., Zhao, Y., Yang, S., Gao, Y., & Xia, X. (2013). *Contagious caprine pleuropneumonia in endangered tibetan antelope, China, 2012*. *19*(12), 2051–2053.

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