WILDLIFE

Meningitis and orchitis in a hare (*Lepus europaeus*) infected with *Francisella tularensis*

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SUMMARY

In southern Belgium, a brown hare (*Lepus europaeus*) was found moribund and killed for ethical reason. The animal was transmitted for postmortem examination. Major histopathological findings consisted of multifocal subacute necrotising meningoencephalitis and multifocal subacute necrotising orchitis. Infection with *Francisella tularensis* was confirmed by both bacteriological isolation and detection by real-time PCR. Further, subtyping of *F tularensis* colonies stated that it was *F tularensis* subspecies *holarctica* biovar I. It is the first case of tularemia detected in wildlife in Belgium since 2003. The event pushed health professionals to communicate with hunters and other groups with outdoor activities about the *Francisella* risk and the ways to take care of it, such as wearing gloves to handle found-dead or hunted hares and taking protective measures against tick bites.

BACKGROUND

Tularaemia is a zoonotic disease caused by *Francisella tularensis*. Currently, 4 subspecies are described and two of them are known to pose a threat to human health: *F tularensis* subspecies *tularensis* and *F tularensis* subspecies *holarctica*. The first is the most virulent subspecies and occurs in North America while the second is mainly present in Europe and Asia, and to lesser extent, in North America (*Foley and Nieto 2010*). However, some strains of *F tularensis* subspecies *tularensis* were isolated from fleas and mites parasiting on small terrestrial mammals, collected in Slovakia (*Gurycova 1998*). The European brown hare (*Lepus europaeus*) plays an important role in the epidemiology of tularaemia as amplifying host and reservoir in western Europe (*Gyuranecz and others 2010, Decors and others 2011, Rijks and others 2013*) and the species is moderately susceptible to the disease compared with the mountain hare (*Lepus timidus*) (*Morner and others 1988*). The main gross pathological feature of the disease in the brown hare is the presence of numerous necrotic foci in organs. The most often affected tissues in brown hares in Hungary were lungs, pericardia and kidneys (*Gyuranecz and others 2010*). Chronic pattern with poor body condition is also frequently reported in hares (*Morner and others 1988, Gyuranecz and others 2010*). A recent study performed in Switzerland on *F tularensis* subspecies *bolarctica* naturally infected brown hares demonstrated that lesions were different according to the phylogenetic cluster involved; splenitis and hepatitis were associated with one cluster while polymyositis affecting pleura, pericardium and kidney were associated with the other (*Origgi and Pilo 2016*).

Human infection may result from inhalation of infective aerosols, skin and conjunctivae contact with or ingestion of infected hosts or water, and tick bites (*Mailles and Vaillant 2014*).

CASE PRESENTATION

In September 2011, in southern Belgium (province of Liege), a young male brown hare was found moribund by a hunter and killed for ethical reason. The animal was transmitted to the Surveillance Network of Wildlife Diseases for postmortem examination (*Linden and others 2011*). At postmortem examination, numerous whitish foci, less than 1 mm of diameter, were recorded bilaterally on the surface of the testis, the epididymis and the deferent duct (Fig 1). The mesenteric lymph nodes were slightly enlarged. The spleen was doubled in volume, with a firm consistence. No gross lesion was apparent at examination of the brain. The animal showed a poor body condition with muscle atrophy and absence of fat deposits on the organs. Presence of a milk-like fluid was noticed in the stomach. Samples from various organs were examined by histopathology. Lung and kidney showed congestion. A mild subacute multifocal necrotising inflammation was observed in the liver. Major findings were reported in the CNS (cortex, midbrain, cerebellum and medulla oblongata) with multifocal subacute necrotising meningoencephalitis and local congestion (Fig 2), and with several foci of subacute necrotising inflammation at the periphery of the testis, in the tunica albuginea. Mesenteric lymph nodes showed no abnormal microscopic findings.
Infection with *F. tularensis* was confirmed by both conventional bacteriological isolation and detection by real-time PCR (Versage and others 2003) performed in parallel on spleen, liver and tests. All procedures were performed under biosafety level 3 conditions. Tissue samples were cultured on Chocolate agar supplemented with cystein and on Francis agar medium. The putative colonies isolated by culture were identified using phenotypical characters (Gram staining, oxidase and catalase tests), and the species identification was confirmed by specific nested PCR targeting *tul4* gene (Peruchon and others 2006). Further, subtyping of *F. tularensis* colonies was performed by PCR targeting the genomic region RD1 that allows the differentiation between *F. tularensis* subsp *tularensis* and *F. tularensis* subsp *balarctica* (Broekhuijsen and others 2003). Biovar I identification of isolate was carried out by erythromycin sensitivity test (Kudelina and others 1980). Spleen, liver and tests were PCR-positive but brain tissue was not available for genetic analysis. However, meningitis recently described in *F. tularensis* subsp *balarctica* infected hares (Origg and Pilo 2016) suggest that subacute necrotising meningitis observed in the present study could be related to tularemia infection.

**REFERENCES**


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