Title:

Importance of identification and typing of *Brucellae* from West African cattle: a review.

Authors:

Moussa Sanogo[1, 2, 3*], Emmanuel Abatih[2], Eric Thys[2], David Fretin[4], Dirk Berkvens[2], Claude Saegerman[3]

1) LANADA/Central Veterinary Laboratory of Bingerville, P.O. Box 206 Bingerville, Ivory Coast
2) Unit of Epidemiology and Biostatistics, Department of Biomedical Sciences, Institute of Tropical Medicine (ITM), Nationalestraat, 155, B-2000, Antwerp, Belgium
3) Research Unit of Epidemiology and Risk Analysis applied to Veterinary Sciences (UREAR-ULg), Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, University of Liege (ULg), Boulevard de Colonster 20, B42, B-4000 Liege, Belgium
4) Department of Bacteriology and Immunology, Veterinary and Agro-chemical Research Centre (VAR), Uccle, Groeselenberg 99, 1180 Brussels, Belgium

* Corresponding author at: LANADA/Central Veterinary Laboratory of Bingerville, P.O. Box 206 Bingerville, Ivory Coast, Tel: +22507523356. *E-mail address*: ssanogomoussas@gmail.com

Abstract

Bovine brucellosis is an endemic infectious disease which can impact cattle productivity and welfare negatively, as well as human health. Sufficient knowledge on its epidemiology, particularly on species and biotypes of *Brucella* at national and/or regional scale are important to set up and implement efficient control measures against brucellosis in a "One health" perspective. The main objective of this review was to investigate available literature on strains of *Brucella* in order to provide a state of art-knowledge on species and biovars reported in cattle from West Africa. A literature search was conducted to identify relevant data on species and biovars of *Brucella* in cattle from Western African countries. This search included studies presenting bacteriological and/or molecular results of identification and typing, relied on international classification methods with no time limit and no language restrictions. Studies reporting results of identification at genus level only were not considered for this review. This review revealed that *Brucella abortus* was the most prevalent species in cattle from West Africa, in line with host preference for *Brucellae*. So far, biovars 1, 2, 3, 4, 6 and intermediate biovar 3/6 of *B. abortus* were reported in cattle in the region. Among these strains, biovars 3, recently identified in The Gambia and Ivory Coast, was the most commonly isolated. *Brucella melitensis* and/or *B. suis* have not been mentioned yet in cattle in this part of Africa. The public health significance of prevailing strains is discussed and a regional collaborative control program of brucellosis is suggested.

Keywords: Cattle; Brucellosis; Identification; Typing; *Brucella*; biovar; West Africa.

Introduction

In Africa, livestock development is continuously being challenged by several constraints among which are many parasitic, viral and bacterial infectious diseases. Brucellosis is one of the major bacterial infectious diseases, affecting domestic animals in many developing countries (Akakpo and Bornarel, 1987; Corbel, 1997; Wastling et al. 1999; McDermott and Arimi., 2002). In sub-Saharan Africa, bovine brucellosis remains the most widespread form of the disease in livestock (Akakpo and Bornarel, 1987; Corbel; 1997; McDermott and Arimi, 2002; Bronvoort et al., 2009). It is responsible for considerable economic losses through its negative impacts on livestock production including late term abortion, birth of weak calf, retention of placenta, metritis, infertility, orchitis or epididymitis with or without sterility and hygroma. Brucellosis is caused by slow-growing, small, Gram negative, cocco-bacilli bacteria composing the genus *Brucella*. These bacteria are facultative intracellular pathogen which can be transmitted to a susceptible host mostly by direct contact, ingestion or via aerosol. When transmission occurs, lymphatic tissues, blood and other tissues and organs of the host are invaded, with a particular tropism for the reproductive tract (Olsen and Tatum, 2010). On the basis of pathogenicity, host preference and phenotypic characteristics, six species of *Brucella* are commonly listed: *Brucella (B.) neotomae (desert rat), B. canis (dogs), B. suis (pigs), B.ovis (rams), B. melitensis (sheep, goats)* and *B. abortus (cattle)* (Osterman and Moriyon, 2006). Besides these six common species of *Brucella*, some strains were newly reported like *B. ceti* and *B. pinnipediae* identified in marine mammals, *B. microti* in the common vole (*Microtus arvalis*), in wild red fox *(Vulpes vulpes)* and in soil and *B. inopinata* in human (Ewalt et al., 1994; Foster et al., 1996; Clavareau et al., 1998; Scholz et al., 2008; scholz et al., 2010; Tiller et al., 2010; Banai and Corbel, 2010; Nymo et al., 2011). Based on their cultural morphology, serotyping and biochemical characteristics, these species may be sub-divided into sub-types (also known as biovars, or biotypes) (Alton et al., 1988).

In cattle, the disease is mainly caused by one of the seven biovars of *B. abortus (1, 2, 3, 4, 5, 6, and 9)* but also occasionally by biovars of *B. melitensis* and *B. suis* (Corbel, 2006; OIE, 2009; Fretin et al., 2012). Among species encountered in cattle, *B. suis* (biovars 1 and 3) *and B. melitensis* can cause disease in human, with more severe cases related to *B. melitensis* (Acha and Szyfres, 2005; Corbel, 2006). In addition to these common zoonotic species, newly reported strains of *Brucella* in marine mammals were also alleged to have a zoonotic potential but further investigations are still needed (Godfroid et al., 2005).

For a better understanding of its epidemiology in cattle, prevalence of brucellosis has been investigated throughout the years in Africa (Akakpo and Bornarel, 1987; Mangen et al., 2002). Besides these investigations, species and biotypes infecting cattle have also been investigated. By providing information on the actual evidence of the presence of the disease-causing agent, identification and typing of strains are relevant in the "one health" perspective. They are also useful for a better knowledge of the disease epidemiology, for managing outbreaks, for identification of appropriate antigens for testing and for setting up efficient preventive and control measures (Crawford et al., 1979; Ica et al., 1998; Saegerman et al., 2010; Godfroid et al., 2010). Since infected animals and particularly infected cattle may be sources of human brucellosis, knowledge on prevailing strains in these hosts may supply information that can be used to assess potential threats for public health at national and/or at regional levels.

The aim of this review was to determine strains of *Brucella* reported in cattle from West Africa in order to provide a summary of species and biovars reported in that sub-region of Africa, determine geographical distribution of strains, identify samples used for typing and discuss potential implications on public health.

Methodology

Study area

The area of concern in this review included West African countries. West Africa is one of the four major regions of sub-Saharan Africa. It covers almost one fifth of the geographical area of the whole continent with 5 112 903 km² and comprised of members of the Economic Community Of West African States (ECOWAS) (Fig. 1). Four main climatic zones are encountered from south to north in this area namely, humid, sub-humid, semi-arid and arid zones (McDermott and Arimi, 2002). West Africa is an important area of livestock production with the largest population of ruminants after East Africa and ahead of the southern regions (OECD, 2008). About 60 million heads of cattle, representing approximately 21 % of the cattle population of the continent, are found in this sub-region of Africa (FAO, 2010). These cattle belong to two subspecies of *Bos Taurus*: the West African humpless breeds (*Bos taurus* type) and the humped zebus of *Bos indicus* type. Compared to the rest of the continent, significant populations of both subspecies of cattle are found in this part of Africa, with different crossbreds. Besides a sedentary production system, cattle are also reared under a nomadic production system, known as transhumance system. Thus, herds move across areas and borders to find better pasture and secure places (Bassett and Turner, 2007; OECD, 2008).

Literature search

Using a systematic approach, a literature search was undertaken to identify available information on species and biovars of *Brucella* reported in cattle from Western African countries. This search was conducted through online general search engine and particularly in Google Scholar and in PubMed using combination of keywords such as "*Brucella*" and "cattle" or "bovine brucellosis" and "identification" or "typing" associated with each country name. Studies reporting bacteriological and/or molecular results of identification

and typing of *Brucella* from cattle at species and/or biotypes level and complying with international standards on classification of *Brucella* were summarised with no time limit and no language restriction. Reference lists of retrieved literature were also scanned. Both primary research and review articles were included. Studies reporting results of identification at genus level only were not considered for this review. Relevant studies were then submitted to the data extraction and analysis process.

Data extraction and analysis

Relevant articles were screened for data on country and year of identification, identified strains, identification and typing characteristics and samples used for typing. Data were extracted, compiled and submitted to a descriptive analysis to provide a state of the art-knowledge on prevailing strains of *Brucella* in West Africa. Distribution and number of species and biovar(s) per country were provided. Public health significance and threats related to reported strains were discussed.

Results

A total of 15 studies reporting results of identification and/or typing of *Brucella* in cattle were gathered by the literature search (Table 1). Results published by Chambron (1965), were not expressed according to recommendations of the Subcommittee on Taxonomy of *Brucella* of the International Committee on Systematic Bacteriology on classification of *Brucella* and were not included in this review. Bale and Kumi-Diaka (1981) erroneously encoded H_2S production for two Nigerian isolates among the eleven reported in that their study. Results published by Akakpo (1987) and Akakpo and Bornarel (1987) reported similar information on strains from Senegal, Niger and Togo. Verger and Grayon (1984) also mentioned the same results but provided supplementary results from Guinea Bissau.

147 Finally, 13 studies published between 1977 and 2012 with the number of isolates identified
148 ranging between 1 and 181 were considered for review (Tables 1 and 2). Disease-causing
149 agents of bovine brucellosis were investigated and found in The Gambia, Mali, Niger and
150 more frequently in Nigeria, Senegal and Ivory Coast as shown in Fig.1. No record of the
151 results of *Brucella* typing in cattle was found for Cape Verde, Benin, Burkina Faso,
152 Guinea, Liberia, Mauritania and Sierra Leone.
153 *Brucellae* have been reported in cattle from West Africa for many decades (Table 1).
154 These different species and biovars were isolated using various types of samples. By far,
155 hygroma fluid appeared to be the most employed sample for typing (Table 1). Based on
156 this review, only *Brucella abortus* members were identified in this sub-region, consistently
157 with host preference. Among the classical biovars of *B. abortus*, only biovars 1, 2, 3, 4,
158 and 6 have been reported in West Africa so far. Moreover, some publications reported
159 intermediate strains, sharing characteristic of both biovars 3 and 6 in Senegal, Guinea
160 Bissau and Niger. These strains were reported as *B abortus* 3/6. Through the years, isolates
161 with atypical growth characteristics were recorded in many countries (Table 2). Based on
162 the studies included, over six decades of *Brucella* typing from West Africa, a total of 344
163 strains were recorded in cattle. All these strains were classified as *B. abortus* among which
164 about ¾ representing 262 isolates were reported as belonging to biovar 3 or biovar 3/6.
165 These isolates comprised 44 primarily identified as biovar 3/6 and a total of 218 isolates
166 initially described as *B. abortus 3* and reclassified as biovar 3/6 (Table 1). Number and
167 proportion per species and/or biovar of *Brucella* recorded in West Africa and their
168 geographical distribution are respectively presented in Fig. 1 and 2. Considering the studies
169 reporting the different biovars and using an exact logistic regression, biovar 3/6 or 3
170 appeared to be significantly more likely (Odd Ratio (OR) = 6.9; 95% IC: 1.6,35.0) to be
171 encountered in this sub-region in comparison with biovar 1 as a reference (P = 0.006).

Discussion

Samples and typing methods of Brucella in West Africa

The primary objective of this review was to provide an overview on strains of *Brucella* reported in cattle in West African epidemiological context through a literature search aiming to be as exhaustive as possible. Data collected through this review were based on both conventional phenotypic and/or genotypic characteristics. Phenotypic identification of *Brucella* at biovar level using bacteriological methods commonly consisted in a combination of morphological, cultural and biochemical characteristics. Classification of strains into species is based on natural host preference, sensitivity to *Brucella* phages (Tbilisi (Tb), Weybridge (Wb), BK2, R/C) and oxidative metabolic profiles. Subtypes or biovar rely on CO_2 requirement on primary isolation, H_2S production, sensitivity to inhibition by thionin, basic fuchsin and safranin O dyes, and agglutination response to monospecific antisera for the A antigen of *B. abortus* and for the M antigen of *B. melitensis* M (Corbel and Morgan, 1975; Alton et al., 1988; Godfroid et al., 2010). For all but two of the studies included in this review, results of identification were only culture-based typing. These results complied with available recommendations for typing at the time of publication (Alton and Jones, 1964; Brinley-Morgan and McCullough, 1974; Alton et al., 1975; Corbel and Morgan, 1975; Alton et al., 1977; Corbel, 1984; Alton et al., 1988). These methods have been used for typing for years and enable differentiation among species and biotypes of *Brucella.* However, differences between some isolates might be unclear as for biotypes 3 and 6 which can be distinguished only on dye sensitivity (Banai and Corbel, 2010).

Molecular typing methods based on the detection of *Brucella* DNA (Yu and Nielsen, 2010) and comparatively less fastidious, were also applied in a few cases (Bankole et al., 2010;

197 Sanogo et al., 2012). They were used as complementary to conventional bacteriological
198 typing methods thus increasing the consistency of the typing results. Thus, the later
199 multilocus variable number tandem repeat analysis (MLVA), a typing method with a good
200 capacity of species identification and also a good discriminatory power (Le Flêche et al.,
201 2006), was recently used in The Gambia and Ivory Coast. Both molecular and
202 bacteriological typing methods are not easy to perform and require facilities and pieces of
203 equipment that are not always available in diagnostic laboratories in Africa, limiting results
204 of investigations on prevailing strains of *Brucella* (Samartino et al., 2005).
205 Whatever the typing method, an appropriate sample is essential for identification and
206 typing of *Brucella*. Depending on the presence of clinical signs, a range of samples is
207 possible including fetal membranes, vaginal secretions, milk, semen, arthritis or hygroma
208 fluids, lymph nodes, spleen, uterus, udder, testes, epididymes, joint exudate, abscesses and
209 other tissues of infected cattle and also the stomach content, spleen and lungs of aborted
210 fetuses (Alton et al., 1988; Corbel, 2006; OIE, 2009; Godfroid et al., 2010). In case of
211 abortion due to brucellosis, concentrations of *Brucella* in fetal fluids or placenta may reach
212 up to 10^{13} colony-forming units (CFUs)/g compared to an estimated minimum infectious
213 doses range of 10^3 to 10^4 CFU (Fensterbank, 1986; Olsen and Tatum, 2010; Saegerman et
214 al., 2010). *Brucellae* may also be shed into milk from the udder and supra mammary lymph
215 nodes of infected cattle at concentrations going from a few hundred up to few million
216 organisms/ml of milk (Corbel, 1988). One clinical sign commonly associated with
217 brucellosis in African cattle herd is the presence of hygroma. In many countries and for
218 years, fluid of hygroma has been used as the sample for biotyping (Thienpont et al., 1961;
219 Akakpo and Bornarel, 1987; Bankole et al, 2010; Sanogo et al., 2012). From this review,
220 except in Nigeria where diverse samples such as semen, testicular exudates, vaginal swabs,
221 aborted foetuses, and blood were used, fluid of hygroma appeared to be the preferred

sample for *Brucella* typing in West Africa (Table 1). A possible explanation is that hygroma fluid stays comparatively easier to collect compared to samples related to abortions which are poorly recorded and rarely submitted to laboratory investigations in African epidemiological context. Some strains were also isolated at high rates from milk samples like in Nigeria where 48% of the 25 strains isolated by Ocholi et al. (2004) came from milk samples. This implies an existing risk for public health particularly for people coming from ethnic groups of the region where customs encourage the consumption of unpasteurized raw milk (Schelling et al., 2003; Ocholi et al., 2004).

Decades of identification and typing of Brucella from cattle in West Africa

Throughout the years, serological evidence of brucellosis in cattle population was found in many sub-Saharan African countries where investigations were undertaken including Benin, Burkina Faso, The Gambia, Ghana, Guinea, Ivory Coast, Mali, Niger, Nigeria, Senegal, Sierra Leone and Togo (Mangen et al., 2002; Unger et al., 2003). Seroprevalence by Rose Bengal test was estimated to range between 10.2 and 25.7% in cattle population of sub-Saharan Africa. Even if detection of antibodies produced against *Brucella* is indicative of the presence of brucellosis, identification of the disease-causing agent stays the ultimate evidence of the actual presence of the disease (Nielsen, 2002). As shown in Table 1, so far this evidence was regularly provided in many West African countries where investigations were made including The Gambia, Guinea Bissau, Ivory Coast, Mali, Niger, Nigeria, Senegal and Togo and confirming the endemicity of brucellosis in that region. Furthermore, this endemicity seems to be consistent with the absence of a sustainable efficient control program in that area.

Based on data retrieved from the published literature, *Brucella abortus* appeared to be the main species infecting cattle in West Africa, confirming the host preference of this species.

Biovar 3 seemed to be the most common strain in West Africa (Table 1). Except in Mali, this biovar was identified in 7 out of the 8 countries of that sub-region where biotying studies were undertaken. Even if not established in this review, the presence of that biovar was argued to be associated with the presence of hygroma in nomadic or semi-nomadic cattle herds in Africa (Corbel, 2006). It has been described through the years in Senegal (Verger et al., 1979), in Togo (Verger et al., 1982), in Niger (Akakpo et al., 1986), and most recently in The Gambia (Bankole et al, 2010) and in Ivory Coast (Sanogo et al., 2012). A similar trend was noticed in Central Africa by Domenech et al. (1983) where most isolates were also *B. abortus biovar 3*. Furthermore, isolates from Senegal, Togo and Niger initially described as *B. abortus 3* with some particular phenotypic characteristics were reclassified as *B. abortus 3/6* in compliance with recommendations of the Subcommittee on Taxonomy of *Brucella* of the International Committee on Systematic Bacteriology on classification on *Brucella* (Corbel, 1984). This proposition to merge the two biovars in a single biovar 3/6 was made since differences were not always neat between biovar 3 and biovar 6 regarding growth characteristics on thionin and basic fuchsin (Verger and Grayon, 1984). These differences were not sufficiently taken into account when originally defining these biovars, due to a limited number of strains from Africa (Corbel, 1984).

Throughout the world, *B. abortus* biovar 1 is the most widely isolated in cattle (Acha and Szyfres, 2003). It was also naturally reported in West Africa in Ivory Coast, Senegal and particularly in Nigeria where most of the isolates were identified as belonging to this biovar (Table 1). It was assumed to be the prevailing strain associated with brucellosis infection in livestock in Nigeria (Ocholi et al., 2004). *Brucella abortus* biovar 6 is another strain reported in West Africa. So far, this strain was mentioned only in Ivory Coast and

271 did not seem to be widespread in that sub-region of Africa as well as biovars 2 and 4
272 similarly reported only in Nigeria in 1980s.
273 Biovars 5 and 9 have not been reported yet in this sub-region. Conversely with Central
274 Africa, it also appeared that neither *B. melitensis* nor *B. suis* were isolated yet from cattle
275 in West Africa (Domenech et al., 1983; McDermott and Arimi, 2002). This does not
276 necessary mean that they are absent in this sub-region since cattle are sometimes kept and
277 commonly grazed with sheep and goats in West Africa, which can not preclude any cross-
278 infection among hosts (Ocholi et al., 2005).
279 Whereas *Brucella* are usually oxidase positive except for *B. ovis* and B. *neotomae*, some
280 biovars encountered in different countries of West Africa often appeared to be negative
281 (Verger et al., 1979; Verger et al., 1982; Bankole et al., 2010; Sanogo et al., 2012). Besides
282 this variable oxidase test response reported in Ivory Coast, Guinea Bissau and Senegal so
283 far, atypical characteristics like slow growing characteristics and altered oxidative
284 metabolic profile were also recorded (Verger et al., 1982; Verger and Grayon, 1984).
285 These results highlight the need for more investigations of prevailing strains of *Brucella*
286 from Africa and could justify the use of methods with more discriminative power for
287 typing.
288
289 ***Public health significance and implications***
290 Brucellosis is one of the most widespread bacterial zoonosis (Corbel, 2006). Human
291 disease also known as undulant fever or Malta fever may occur through ingestion of
292 contaminated foods, direct contact with an infected animal or material or via aerosol. It
293 principally affects consumers of raw milk and derivatives and field and laboratory animal
294 health professionals (McDermott and Arimi, 2002; Kunda et al., 2007). Rarely fatal,
295 infection of human can be severely debilitating and disabling through diverse non-specific

clinical signs including an undulant fever, fatigue, depression, loss of appetite, headache, sweating, joint pain, muscular pain, lumber pain, weight loss, hepatomegaly, splenomegaly and arthritis (Corbel, 2006). About 500,000 cases of human brucellosis are reported annually worldwide (Corbel, 1997; Pappas et al., 2006; Franco et al., 2007). Despite its incidence, the disease is one of the neglected endemic zoonotic diseases in the world (WHO, 2012). Within West Africa, knowledge on the actual impact of the disease in humans stays limited and human cases stay under-reported. Nevertheless, serological evidences of the presence of *Brucella* in humans were already recorded in some Western African countries like in Benin, Burkina Faso, Ivory Coast, Guinea, Guinea Bissau, Mali, Togo and Nigeria (Pappas et al., 2006; Akakpo et al., 2010). In Burkina Faso and in Nigeria, seroprevalence estimates were reported to be respectively 10% and 26% (McDermott and Arimi, 2002). In West Africa isolation and identification of *Brucellae* in human are rarely performed (McDermott and Arimi, 2002; Corbel, 2006). In addition to little interest in human brucellosis, this situation could also be due to poor diagnostic capacities (McDermott and Arimi, 2002). Particularly in this part of the continent, acute brucellosis might be misdiagnosed and missed out in cases of febrile illness similarly encountered in others endemic human diseases like malaria or typhoid (McDermott and Arimi, 2002; Pappas et al., 2006; Akakpo et al, 2010). The introduction of less fastidious molecular methods in that part of Africa might be an alternative to improve reporting of human cases and assessment of human exposition to *Brucellae*.

Based on this review, only the presence of *B. abortus* was reported in cattle in the West African epidemiological context among species of public health interest so far. This species remains the most widespread among the ones associated with infection in man, as recently demonstrated in Ecuador (Ron-Roman et al., 2012) even if that species is fortunately less associated with severe human infections (Corbel, 2006).

Species and biovars of *B. abortus* were isolated more or less persistently since 1960s in many countries of the sub-region, what is consistent with an endemicity of bovine brucellosis and with a persistent risk of infection of cattle in that area. The presence of *Brucella* among cattle should also be considered as an indicator of the existence of a possible risk of exposure for human, even if factors such as methods of food preparation, heat treatment of dairy products, and the amount of effective direct contact with infected cattle might interfere with risk of transmission to human population (McDermott et al., 2002, Samartino et al., 2005).

Indeed, besides their epidemiological importance, knowledge on prevailing strains of *Brucella* are of key importance for developing adapted control programs. They could be helpful to appreciate the appropriateness of antigens used for testing and to identify appropriate vaccination strains. From a public health perspective, data on prevailing strains could give an indication of the sources of infection and also to identify the level of exposure and the potential risks of human infections.

Conclusion and perspectives

Data on species and biovars of *Brucella* in cattle remain crucial for a better understanding of the epidemiology of bovine brucellosis in the West African sub-region. This review summarized available published data of decades of typing in cattle since 1960s but cannot be assumed to be exhaustive of strains actually present in 2012 in that region. At least, the proposed summary provided indication of the presence of *Brucella* sp. and gave a global and updated map of disease-causing agents of bovine brucellosis reported in West Africa so far. Considering the geographical and the time scale covered by this review, the limited number of strains retrieved suggests the need to continue efforts on identification and typing of *Brucella* strains in order to provide more extended and updated information on

346 prevailing biovars. Indeed, available data are sometimes two to three decades older for
347 many countries. Moreover, for easy assessment, it might be suggested that studies
348 publishing typing results explicitly report details on typing methods and present
349 sufficiently informative results in compliance with minimal standards for genus, species
350 and biovar definition of *Brucella*.
351 The presence of *Brucella* strains across West Africa highlighted the reality of a potential
352 public health threat, in such an epidemiological context where close contact may occur
353 between animals and people, where hygienic conditions are usually poor, where customs
354 favour consumption of raw milk and where no control strategies are implemented. More
355 epidemiological investigations are also needed to provide information on possible sources
356 of human infection, on transmission pathways between animals and humans in order to set
357 up an efficient control strategy in a "one health" perspective. Moreover, the reporting of
358 the disease in humans should be drastically improved by considering brucellosis as part of
359 the differential diagnosis for patients with fever of unknown origin. Taking into
360 consideration the presence of *Brucella* in many countries, the existence of movement of
361 cattle between countries and the limited resources allocated for disease control in most of
362 African countries, a collaborative regional control strategy putting strengths together might
363 be a possible approach to contain brucellosis infections and limit its public health impact in
364 West Africa. Such a strategy should adopt a "one health" concept with more cooperation
365 and exchange of information between public health and veterinary authorities.
366 Furthermore, diagnostic and surveillance capacities of veterinary services should be
367 strengthened to provide valuable epidemiological information, notably on prevailing
368 strains of *Brucella*. Hence, initiatives such as the OIE Performance of Veterinary Services
369 are fundamental to improving the efficiency of the control program of brucellosis as well
370 as other zoonoses.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Acknowledgements

The authors would like to thank the Institute of Tropical Medicine of Antwerp, the University of Liege (UREAR-ULg) for academic support. We would also like to thank Dr David Shamaki and his colleagues, from National Veterinary Research Institute, Vom, Nigeria and Ms Sylvie Courtault and Marie-Estelle Esnault from Institut de Recherche Agronomique (INRA), Tours, Nouzilly, France for helping us during the literature search.

References

Acha, P.N., Szyfres, B., 2003. Brucellosis. In Zoonoses and Communicable Diseases Common to Man and Animals: Vol 1: Bacterioses and Mycoses. 3rd Ed. Washington, Pan American Health Organization, pp. 40-67.

Akakpo, A.J. 1987. Brucelloses animales en Afrique tropicale. Particularités épidémiologique, clinique et bactériologique. Rev. Elev. Méd. vét. Pays trop. 40, 307-320.

Akakpo, A.J., Bornarel, P., 1987. Epidémiologie des brucelloses animales en Afrique tropicale : Enquêtes clinique, sérologique et bactériologique. Rev. sci. tech. Off. int. Epiz. 6, 981-1027.

Akakpo, A.J., Saley, M., Bornarel, P., Sarradin, P., 1986. Epidémiologie de la brucellose bovine en Afrique tropicale : Enquête sérologique au Niger, identification des deux premières souches de *B. abortus* biotype 3. Rev. Elev. Méd. vét. Pays trop. 39, 175-179.

396 Akakpo, A.J., Teko-Agbo, A., Kone, Ph., 2010. L'impact de la brucellose sur l'économie et
397 la santé publique en Afrique. 18th Conference of the OIE Regional Commission for
398 Africa, N'Djamena, Chad, 23-27 February 2009, Recueil des thèmes présentés à
399 l'assemblée mondiale des délégués et aux commissions, pp. 71-98.
400 http://www.oie.int/doc/document.php?numrec=3978303.
401 Alton, G.G., Jones, L.M., 1964. Laboratory techniques in brucellosis, FAO, Animal Health
402 Branch Monograph no 7, Rome. Italie, 53 p.
403 Alton, G.G., Jones, L.M., Angus, R.D., Verger, J.M., 1988. Techniques for the brucellosis
404 laboratory. INRA, Paris, France, 192 p.
405 Alton, G.G., Jones, L.M., Pietz, D.E., 1975. Laboratory techniques in Brucellosis. 2nd ed .,
406 World Health Organization, Geneva, Switzerland, 163 p.
407 Alton, G.G., Jones, L.M., Pietz, D.E., 1977. La brucellose : technique de laboratoires. 2nd
408 éd. FAO-OMS, Genève, Suisse, 177 p.
409 Bale, O.O.J., Kumi-Diaka, J., 1981. Serological and bacteriological study of bovine
410 *Brucellae* from livestock investigation and breeding centers in Nigeria. Br. vet. J. 137,
411 256-261.
412 Banai, M., Corbel, M., 2010. Taxonomy of *Brucella*. Open Vet Sci J. 4, 85-101.
413 Bankole, A.A., Saegerman, C., Berkvens, D., Fretin, D., Geerts, S., Ieven, G., Walravens,
414 K., 2010. Phenotypic and genotypic characterisation of *Brucella* strains isolated from
415 cattle in the The Gambia. Vet. Rec. 166, 753-756.
416 Bassett, T.J., Turner, M.D., 2007. "Sudden Shift of Migratory Drift? FulBe Herd
417 Movements to the Sudano-Guinean Region of West Africa." Hum. Ecol. 35, 33-49.
418 https://netfiles.uiuc.edu/bassett/shared/Bassett%20pubs/Bassett%20and%20Turner%2
419 02007.pdf.

Brinley-Morgan, W.J., McCullough, N.B., 1974. Genus *Brucella* Meyer and Shaw 1920, In R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th Ed. The Williams & Wilkins Co., Baltimore, pp. 278-282.

Bronsvoort, B.M.d., Koterwas, B., Land, F., Handel, I.G., Tucker, J., Morgan, K.L., Tanya, V.N., Abdoel, T.H., Smits, H.L., 2009. Comparison of a Flow Assay for Brucellosis Antibodies with the Reference cELISA Test in West African *Bos indicus*. PLoS ONE 4(4): e5221. doi:10.1371/journal.pone.0005221.

Clavareau, C., Wellemans, V., Walravens, K., Tryland, M., Verger, J.M., Grayon, M., Cloeckaert, A., Letesson, J.J., Godfroid, J., 1998. Phenotypic and molecular characterization of a *Brucella* strain isolated from a minke whale (*Balaenoptera acutorostrata*), Vet. Microbiol. 144, 3267–3273.

Corbel, M.J., 1984. International Committee on Systematic Bacteriology. Subcommittee on Taxonomy of *Brucella*. Minutes of the Meeting, 10 August 1982, Boston, Massachusetts. Int. J. syst. Bact. 34, 366-367.

Corbel, M.J., 1988. Brucellosis, In Laing, J.A. (ed.) Fertility and Infertility in Veterinary Practice. 4th ed. ELBS, Bailliere Tindall, pp. 189-221.

Corbel, M.J., 1997. Brucellosis: an overview (1st International conference on emerging zoonosis). Emerg. Infect. Dis. 3, 213-221. http://www.cdc.gov/ncidod/EID/vol3no2/corbel.htm.

Corbel, M.J., 2006. Brucellosis in Humans and Animals. WHO press (ed), World Health Organization, Geneva, Switzerland, pp.1-21.

Corbel, M.J., Morgan, W.J., 1975. Proposal for minimal standards for descriptions of new species and biotypes of the genus *Brucella*. Int. J. syst. Bact. 25, 83–89.

443 Crawford, R.P., Williams, J.D., Huber, J.D., Childers, A.B., 1979. Biovars of *Brucella*
444 *abortus* and their value in epidemiological studies of infected cattle herds. J. Am. Vet.
445 Med. Assoc. 175, 1274-1277.
446 Domenech, J., Corbel M.J., Thomas, E.L., Lucet, Ph., 1983. La brucellose bovine en
447 Afrique centrale : VI. Identification et typage des souches isolées au Tchad et au
448 Cameroun. Rev. Elev. Méd. vét. Pays trop. 36, 19–25.
449 Doutre, M.P., Fensterbank, R., Sagna, F., 1977. Etude de la brucellose bovine dans un
450 village de Basse-Casamance (Sénégal). I.- Diagnostic sérologique et bactériologique.
451 Rev. Elev. Méd. vét. Pays trop. 30, 345-351.
452 Ewalt, D.R., Payeur, J.B., Martin, B.M., Cummings, D.R., Miller, G., 1994. Characteristics
453 of a *Brucella* species from a bottlenose dolphin (*Tursiops truncatus*), J. Vet. Diagn.
454 Invest. 6, 448–452.
455 Eze, E.N., 1978. Isolation of *Brucellae* from the Nigerian livestock and the typing of such
456 isolates. Bull. Anim. Hlth. Prod. Afr., 26, 29-36.
457 Fensterbank, R., 1986. Brucellose des bovines et des petits ruminants : diagnostic,
458 prophylaxie et vaccination. Rev. Sci. tech. Off. int. Epiz. 5, 587-603.
459 Food and Agricultural Organization (FAO), 2010. FAO Statistic Division, FAOSTAT.
460 http://faostat.fao.org/site/573/DesktopDefault.aspx?PageID=573#ancor; updated:
461 7/08/2012.
462 Foster, G., Jahans, K.L., Reid, R.J., Ross, H.M., 1996. Isolation of *Brucella* species from
463 cetaceans, seals and an otter. Vet. Rec. 138, 583-585.
464 Franco, M.P., Mulder, M., Gilman, R.H., Smits, H.L., 2007. Human brucellosis. Lancet
465 Infect Dis. 7, 775-786.
466 Fretin, D., Czaplicki, G., Quinet, C., 2012. Brucellosis, porcine – Belgium (02): serovar 2,
467 wild boar, bovine. http://www.promedmail.org/direct.php?id=20120710.1196658.

Godfroid, J., Cloeckaert, A., Liautard, J.-P., Kohler, S., Fretin, D., Walravens, K., Garin-Bastuji, B., Letesson, J.-J., 2005. From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis. Vet. Res. 36, 13-25.

Godfroid, J., Nielsen, K., Saegerman, C., 2010.Diagnosis of Brucellosis in livestock and wildlife. Croat. Med. J. 51, 296-305.

Ica, T., Aydin, F., Erdenlig, S., Guler, L., Büyükcangaz, E., 2008. Characterisation of *Brucella abortus* biovar 3 isolates from Turkey as biovar 3b. Vet. Rec. 63, 659-661.

Kunda, J., Fitzpatrick, J., Kazwala, R., French, N.P., Shirima, G., MacMillan, A., Kambarage, D., Bronsvoort, M., Cleaveland, S., *2007.* Health-Seeking Behavior of Human *brucellosis* cases in Rural Tanzania, BMC. Public Health 7, 315.

Le Flêche, P., Jacques, I., Grayon, M., Al Dahouk, S., Bouchon, N.P., Denoeud, F., Nockler, 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, 9.

Mangen, M.J., Otte, J., Pfeiffer, D., Chilonda, P., 2002. Bovine Brucellosis in sub-Saharan Africa: Estimation of Sero-Prevalence and Impact on Meat and Milk Offtake Potential. FAO Livestock Information and Policy Branch, AGAL, Livestock Policy Paper n°8. Rome, Italie, pp. 39-41.

McDermott, J.J., Arimi, S.M., 2002. Brucellosis in sub-Saharan Africa: epidemiology, control and impact. Vet. Microbiol. 90, 111-134.

Nielsen, K., 2002. Diagnosis of brucellosis by serology. Vet. Microbiol. 90, 447-459.

Nymo, I.H., Tryland, M., Godfroid, J., 2011. A review of *Brucella* infection in marine mammals, with special emphasis on *Brucella pinnipedialis* in the hooded seal (*Cystophora cristata*). Vet. Res. 42, 93.

493 Ocholi, R.A., Kwaga, J.K.P., Ajogi, I., Bale, J.O.O, 2004. Phenotypic characterization of
494 *Brucella* strains isolated from livestock in Nigeria. Vet. Microbiol. 103, 47-53.
495 Ocholi, R.A., Kwaga, J.K.P., Ajogi, I., Bale, J.O.O., 2005. Abortion due to *Brucella*
496 *abortus* in sheep in Nigeria. Rev. Sci. tech. Off. int. Epiz. 24, 973-979.
497 Organization for Economic Co-operation and Development (OECD), 2008. Livestock and
498 regional market in the Sahel and West Africa: potentials and challenges, Paris,
499 France, 170 p. http://www.oecd.org/swac/publications/41848366.pdf
500 Osterman, B., Moriyon, I., 2006. International Committee on Systematics of Prokaryotes
501 Subcommittee on the taxonomy of *Brucella*. Minutes of the meeting, September, 17th
502 2003, Pamplona, Spain. Int. J. Syst. Evol. Microbiol. 56, 1173–1175.
503 Pappas, G., Papadimitriou, P., Akritidis, N., Christou, L., Tsianos, E. V., 2006. The new
504 global map of human brucellosis. Lancet Infect. Dis. 6, 91-99.
505 Pilo-Moron, E., Pierre, F., Kouame, J.B., 1979. Bovine brucellosis in Ivory Coast.
506 Epidemiology. Rev. Elev. Méd. vét. Pays trop. 32, 325-333.
507 Renoux, G. 1952. La classification des *Brucella*. Remarques à propos de l'identification de
508 2,598 souches. Ann. Inst. Pasteur 82, 289–298.
509 Ron-Román, J., Saegerman, C., Minda-Aluisa, E., Benítez-Ortíz, W., Brandt, J., Douce, R.,
510 2012. First Report of Orchitis in Man Caused by *Brucella abortus* Biovar 1 in
511 Ecuador. Am. J. Trop. Med. Hyg. 87, 524-528.
512 Saegerman, C., Berkvens, D., Godfroid, J., Walravens, K., 2010. Bovine brcuellosis. In
513 Infectious and parasitic diseases of livestock. Lavoisier et Commonwealth
514 Agricultural Bureau – International (ed.), France, pp. 971-1001.
515 Samartino L.E., Gil, A., Elzer, P., 2005. Capacity building for surveillance and control of
516 bovine and caprine brucellosis. In: Capacity building for surveillance and control of
517 zoonotic diseases. FAO Animal Production and Health Proceedings, no. 7;

518 FAO/WHO/OIE Expert and Technical Consultation on Capacity Building for
519 Surveillance and Control of Zoonotic Diseases, Rome, Italy. pp. 55-66.
520 Sanogo, M., Thys, E.,Achi, Y.L., Fretin, D., Michel, P., Abatih, E., Berkvens, D.,
521 Saegerman, C., 2013. Bayesian estimation of true prevalence, sensitivity and
522 specificity of Rose Bengal test and indirect ELISA for the diagnosis of bovine
523 brucellosis. Vet. J. 195, 114-120.
524 Schelling, E., Diguimbaye, C., Daoud, S., Nicolet, J., Boerlin, P., Tanner, M., Zinsstag, J.,
525 2003. Brucellosis and Q-fever seroprevalences of nomadic pastoralists and their
526 livestock in Chad. Prev.vet. Med. 61, 279-293.
527 Scholz, H.C., Hubalek, Z., Sedlácek, I., Vergnaud, G., Tomaso, H., Al Dahouk, S., Melzer,
528 F., Kämpfer, P., Neubauer, H., Cloeckaert, A., Maquart, M., Zygmunt, M.S.,
529 Whatmore, A.M., Falsen, E., Bahn, P., Göllner, C., Pfeffer, M., Huber, B., Busse,
530 H.J., Nöckler, K., 2008. *Brucella microti* sp. nov., isolated from the common vole
531 Microtus arvalis. Int. J. Syst. Evol. Microbiol. 58, 375-382.
532 Scholz, H.C., Nockler, K., Gollner, C., Bahn, P., Vergnaud, G., Tomaso, H., Al Dahouk,
533 S., Kampfer, P., Cloeckaert, A., Maquart, M., Zygmunt, M.S., Whatmore, A.M.,
534 Pfeffer, M., Huber, B., Busse, H.J., De, B.K., 2010. *Brucella inopinata* sp. nov.,
535 isolated from a breast implant infection. Int. J. Syst. Evol. Microbiol. 60, 801-808.
536 Thienpont, D., Vandervelden, M., Fagard, P., Mortelmans J., 1961. L'hygroma brucellique:
537 l'aspect clinique caractéristique de la brucellose bovine au Rwanda-Burundi. Rev.
538 Elev. Méd. vét. Pays Trop. 14, 257-266.
539 Tiller, R.V., Gee, J.E., Lonsway, D.R., Gribble, S., Bell, S.C., Jennison, A.V., Bates, J.,
540 Coulter, C., Hoffmaster, A.R., De, B.K. 2010. Identification of an unusual *Brucella*
541 strain (BO2) from a lung biopsy in a 52 year-old patient with chronic destructive
542 pneumonia. BMC Microbiol. 10, 23.

543 Tounkara, K., Maiga, S., Traore, A., Seck, B.M., Akakpo, A.J., 1994. Epidemiology of
544 Bovine Brucellosis in Mali - Serological Survey and Initial Isolation of *Brucella*
545 *abortus* Strains. Rev. Sci. tech. Off. int. Epiz. 13, 777-786.
546 Unger, F., Münstermann, S., Gou Mou, A., Apia, C. N., Konte, M., Hempen, M. A., 2003.
547 Risk associated with bovine brucellosis in selected study herds and market places in
548 four countries of West Africa. Animal Health Working Paper 2. ITC. pp 16-54.
549 Grayon, M., 1984. Characteristics of 273 strains of *Brucella abortus* of African origin.
550 Dev. Biol. Stand. 56, 63-71.
551 Verger, J.M., Grayon, M., Chantal, J., Akakpo, J.A., 1982. Characteristics of Togo strains
552 of *Brucella abortus* from cattle. Ann. Rech. Vét., 13, 177-184.
553 Verger, J.M., Grayon, M., Doutre, M.P., Sagna, F., 1979. *Brucella abortus* d'origine
554 bovine au Sénégal : Identification et typage. Rev. Elev. Med. Vet. Pays trop. 32, 25-
555 32.
556 Wastling, J.M, Akanmori B.D.; Williams, D.J.L. 1999. Zoonoses in West Africa: impact
557 and control. Parasitol. Today 15, 309-310.
558 World Animal Health Organization (OIE), 2009. Chapter 2.4.3. Bovine Brucellosis. OIE
559 Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, pp 1-35.
560 http://web.oie.int/eng/normes/MMANUAL/2008/pdf/2.04.03_BOVINE_BRUCELL.
561 pdf; updated: May 2009.
562 World Health Organization (WHO), 2012. Zoonoses and veterinary public health. Seven
563 neglected endemic zoonoses. Basic facts.
564 http://www.who.int/zoonoses/neglected_zoonotic_diseases/en/
565 Yu, W.L., Nielsen, K., 2010. Review of detection of *Brucella* spp. by Polymerase Chain
566 Reaction. Croat. Med. J. 51, 306-313.
567

568 **Tables**

569

570 **Table 1:**

571 Overview of studies reporting results of identification and typing of *Brucella* from cattle in

572 West Africa, period 1960-2009

573

574 **Table 2:**

575 Summary of growth characteristics reported for isolates of *Brucella* of cattle origin in West

576 Africa, period 1977-2012

577

Figure legends

Fig. 1:

A map showing Western African countries and the geographical distribution with cumulative total number of species and biovars of *Brucella* reported through years in cattle, period 1960-2009

Fig. 2:

Number and proportion of isolates of *Brucella* of cattle origin per species and/or biovar in West Africa, period 1960-2009

588 **Table 1**: Overview of studies reporting results of identification and typing of *Brucella* from cattle in West Africa, period 1960-2009

Authors, year of publication	Country (location)	Data collection period	Samples	Typing references	Typing results	Number of isolates
Sanogo et al., 2012 [a]	Ivory Coast (Dimbokro)	2009	*H*	Alton et al., 1988 ; Le Flèche et al., 2006	*B. abortus 3*	1
Bankole et al., 2010 [a]	The Gambia (Kombo East District)	NA	*H*	Alton et al., 1988 ; Le Flèche et al., 2006	*B. abortus 3*	3
Ocholi et al., 2004	Nigeria (Taraba, Plateau, Adamoua, Bauchi, Sokoto)	2004	*F, H, M , V*	Alton et al., 1988	*B. abortus 1*	17
Tounkara et al., 1994	Mali (Region of Koulikoro)	1991	*H*	Alton et al., 1988	*B. abortus*	4
Akakpo and Bornarel, 1987	Niger	1980-1981	*H*	Alton et al., 1977	*B. abortus 3 or 3/6*	2
	Rwanda	1982-1983	*H*		*B. abortus 3 or 3/6*	13
	Senegal (Casamance)	1979	*H*		*B. abortus 3 or 3/6*	37
	Togo	1977	*H*		*B. abortus 3 or 3/6*	30
Akakpo, 1987 [b]	Senegal, Niger, Togo, Rwanda	NA		Corbel, 1984	*B. abortus 3 or 3/6*	82
Akakpo et al., 1986	Niger (Niamey, Zinder)	1980-1981	*H*	Alton et al., 1977	*B. abortus 3*	2

Verger and Grayon, 1984	Guinea Bissau	1976-1982	*H*	Brinley-Morgan and McCullough, 1974	*B. abortus 3/6*	7
	Niger		*H*		*B. abortus 3/6*	1
	Senegal		*H*		*B. abortus 1*	1
	Senegal		*H*		*B. abortus 3/6*	212
	Togo		*H*		*B. abortus 3/6*	30
Verger et al., 1982	Togo (Sio river's valley, near Lome)	1977	*H*	Corbel and Morgan, 1975; Alton et al., 1977	*B. abortus 3*	30
Bale and Kumi-Diaka, 1981	Nigeria (northern region, Kano)	NA	*B, F, H, M, S, T* [c]	Alton et al., 1975	*B. abortus 1*	5
					B. abortus 3	2
					B. abortus 4	1
					B. abortus	3
Pilo-moron et al., 1979	Ivory Coast (Soclo, Jacqueville, Eloka, Toumodi, Karakoro, Raviart, Bouaké, Pokaha)	1975-1977	*H*	NS (Results from CNR, Montpellier, France)	*B. abortus 1*	9
					B. abortus 6	8
Verger et al., 1979	Senegal (Koalack, Tambacounda, Ziguinchor, Nioro du Rip, Kédougou, Vélingara, Kolda, Sédhiou, Bignona, Oussouye)	1976-1978	*H*	Alton et al., 1977	*B. abortus 1*	1
					B. abortus 3/6	180
Eze, 1978	Nigeria (Plateau, Niger, Borno, Kano)	1974-1976	*F, H, M , V*	Alton and Jones, 1964	*B. abortus 1*	19
					B. abortus 2	1
Doutre et al., 1977	Senegal (Kartiack, near Bignona)	1976	*H*	NS (Results from INRA, Nouzilly, France)	*B. abortus*	14

Chambron, 1965 [b]	Senegal (Kolda, Velingara)	1960	*H*	Renoux, 1952	*B. abortus*	5

589 B: Blood; F: Aborted fetuses; H: Fluid of hygroma; M: milk; S: Semen; T: Testicular exudates; V: Vaginal swabs, NA: not available; NS: not
590 specified.
591 [a] Except these studies where molecular methods were also used for typing, all the results reported in this review were obtained using
592 bacteriological methods.
593 [b] These studies were not included in this review.
594 [c] Heart blood from aborted fetuses was used. Hygroma fluid and milk samples from aborted cows were negative to bacteriological examination.
595

596 **Table 2:** Summary of growth characteristics reported for isolates of *Brucella* of cattle origin in West Africa, period 1977-2012

Country	*Authors*	Year	Species and biotypes	Growthcharacteristics										
				CO_2 dependance	H_2S production	Urease	Oxidase	Anti-serum Agglutination response	Growth in presence of			Lysis by phage			
									Th	BF	Sf	Tb	Wb	Bk_2	R/C
Guinea Bissau	Verger and Grayon,	1984	*B. abortus 3*	+	+	+	-	*A(+) ; M(-)*	+	+	+	+	+	+	-
Ivory Coast	Sanogo et al.,	2012	*B.abortus 3*	+	+	+	-	*A(+) ; M(-)*	+	+	+	*ND*	*ND*	*ND*	*ND*
	Pilo-moron et al.,	1979	*B.abortus 1*	-	+	+	-	*A(+) ; M(-)*	+/-	+	*ND*	+	*ND*	*ND*	*ND*
			B.abortus 6	-	*?*	+	-	*A(+) ; M(-)*	+/-	+	+	+	*ND*	*ND*	*ND*
Mali	Tounkara et al.,	1994	*B.abortus*	+	+	+	+	*A(+) ; M(-)*	+	+	+	*ND*	*ND*	*ND*	*ND*
Niger	Akakpo et al,	1986	*B.abortus 3 or 3/6*	-	+	+	+	*A(+) ; M(-)*	+	+	+	+	+	+	-
	Verger and Grayon,	1984													
Nigeria	Ocholi et al.,	2004	*B.abortus 1*	*-(15) ; +(2)*	+	+	*ND*	*A(+) ; M(-)*	+	+	*ND*	+	+	+	*ND*
	Bale and Kumi-Diaka,	1981	*B.abortus 1*	-	+	*+(3) ;-(2)*	+	*A(+) ; M(-)*	-	+	*ND*	+	*ND*	*ND*	*ND*
			B.abortus 3	+	+*	*-(1) ; trace(1)*	*ND*	*A(+) ; M(-)*	+	+	*ND*	+	*ND*	*ND*	*ND*
			B.abortus 4	+	+	-	+	*A(+) ; M(-)*	-	+	*ND*	+	*ND*	*ND*	*ND*

			B. abortus	+	*ND*	*ND*	+	*A(+) ; M(-)*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
	Eze	1978	*B.abortus 1*	*+(15) ;-(4)*	+	+	*ND*	*A(+) ; M(-)*	+	+	*+(14) ;-(5)*	+	*ND*	*ND*	*ND*
			B.abortus 2	+	+	+	*ND*	*A(+) ; M(-)*	+	-	-	+	*ND*	*ND*	*ND*
Senegal	Akakpo and Bornarel	1987	*B.abortus 3 or 3/6*	+	+	+	-	*A(+) ; M(-)*	+	+	+	+	+	+	-
	Verger et al,	1979	*B.abortus 3 or 3/6*	+	+	+	*+(1) ; -(179)*	*A(+) ; M(-)*	+	+	+	+	*ND*	*ND*	*ND*
			B.abortus 1	+	+	+	+	*A(+) ; M(-)*	-	+	+	+	*ND*	*ND*	*ND*
	Doutre et al,	1977	*B.abortus*	*?*	*?*	*?*	*?*	*?*	*?*	*?*	*?*	*?*	*?*	*?*	*?*
The Gambia	Bankole et al.,	2010	*B.abortus 3*	+	+	-	+	*A(+) ; M(-)*	+	+	+	*ND*	*ND*	*ND*	*ND*
Togo	Verger et al.,	1982	*B. abortus 3 or 3/6*	+	+	+	+	*A(+) ; M(-)*	+	+	+	+	+	+	-
	Verger and Grayon,	1984													

Th: Thionin; BF: Basic fushin, Sf: Safranin O

(+): positive reaction;

(-): Negative reaction;

(+/-)**:** variable reaction observed according to the concentration used

? : No precision provided by authors;

ND: Not done

* Authors erroneously encoded H_2S production of these isolates as negative in the primary publication. All *B. abortus 3* are known to produce H_2S.

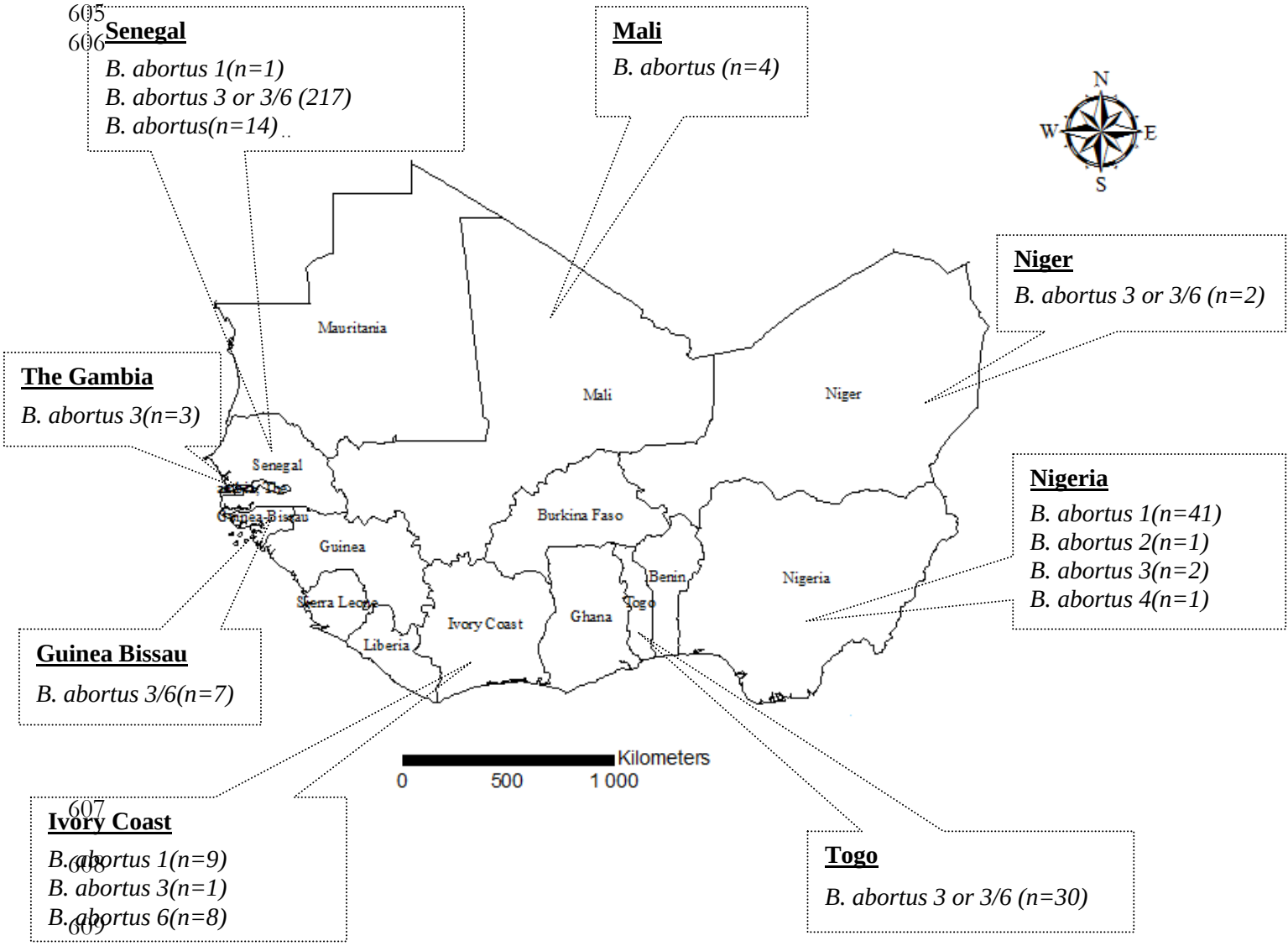

Fig. 1: A map showing Western African countries and the geographical distribution with cumulative total number of species and biovars of *Brucella* reported through years in cattle, period 1960-2009

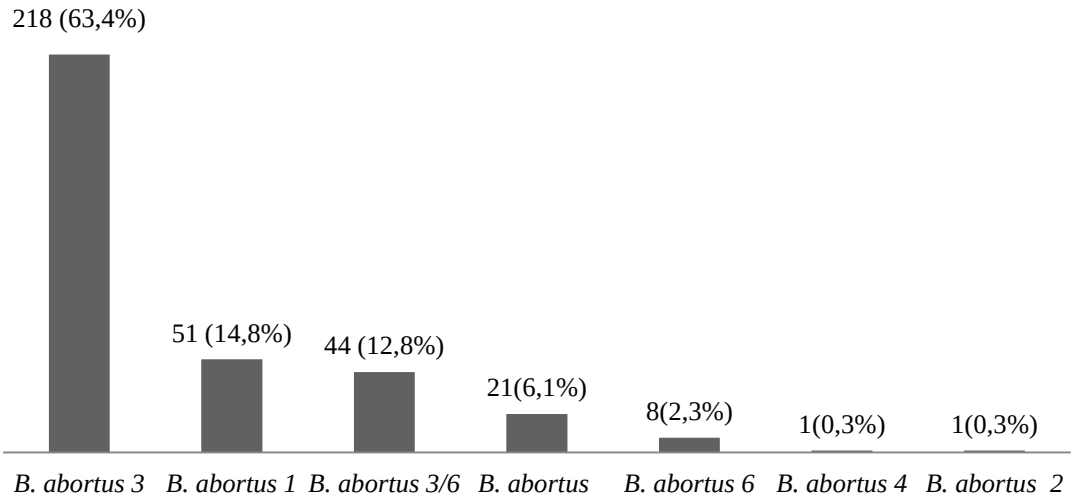

Fig. 2: Number and proportion of isolates of *Brucella* of cattle origin per species and/or biovar in West Africa, period 1960-2009