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

Authors:
Moussa Sanogo¹, ², ³*, Emmanuel Abatih², Eric Thys², David Fretin⁴, Dirk Berkvens², Claude Saegerman³

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, biovar 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$_2$S 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.
Finally, 13 studies published between 1977 and 2012 with the number of isolates identified ranging between 1 and 181 were considered for review (Tables 1 and 2). Disease-causing agents of bovine brucellosis were investigated and found in The Gambia, Mali, Niger and more frequently in Nigeria, Senegal and Ivory Coast as shown in Fig.1. No record of the results of Brucella typing in cattle was found for Cape Verde, Benin, Burkina Faso, Guinea, Liberia, Mauritania and Sierra Leone.

Brucellae have been reported in cattle from West Africa for many decades (Table 1). These different species and biovars were isolated using various types of samples. By far, hygroma fluid appeared to be the most employed sample for typing (Table 1). Based on this review, only Brucella abortus members were identified in this sub-region, consistently with host preference. Among the classical biovars of B. abortus, only biovars 1, 2, 3, 4, and 6 have been reported in West Africa so far. Moreover, some publications reported intermediate strains, sharing characteristic of both biovars 3 and 6 in Senegal, Guinea Bissau and Niger. These strains were reported as B abortus 3/6. Through the years, isolates with atypical growth characteristics were recorded in many countries (Table 2). Based on the studies included, over six decades of Brucella typing from West Africa, a total of 344 strains were recorded in cattle. All these strains were classified as B. abortus among which about ¾ representing 262 isolates were reported as belonging to biovar 3 or biovar 3/6. These isolates comprised 44 primarily identified as biovar 3/6 and a total of 218 isolates initially described as B. abortus 3 and reclassified as biovar 3/6 (Table 1). Number and proportion per species and/or biovar of Brucella recorded in West Africa and their geographical distribution are respectively presented in Fig. 1 and 2. Considering the studies reporting the different biovars and using an exact logistic regression, biovar 3/6 or 3 appeared to be significantly more likely (Odd Ratio (OR) = 6.9; 95% IC: 1.6,35.0) to be 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 CO₂ requirement on primary isolation, H₂S 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;
Sanogo et al., 2012). They were used as complementary to conventional bacteriological typing methods thus increasing the consistency of the typing results. Thus, the later multilocus variable number tandem repeat analysis (MLVA), a typing method with a good capacity of species identification and also a good discriminatory power (Le Flêche et al., 2006), was recently used in The Gambia and Ivory Coast. Both molecular and bacteriological typing methods are not easy to perform and require facilities and pieces of equipment that are not always available in diagnostic laboratories in Africa, limiting results of investigations on prevailing strains of Brucella (Samartino et al., 2005).

Whatever the typing method, an appropriate sample is essential for identification and typing of Brucella. Depending on the presence of clinical signs, a range of samples is possible including fetal membranes, vaginal secretions, milk, semen, arthritis or hygroma fluids, lymph nodes, spleen, uterus, udder, testes, epididymes, joint exudate, abscesses and other tissues of infected cattle and also the stomach content, spleen and lungs of aborted fetuses (Alton et al., 1988; Corbel, 2006; OIE, 2009; Godfroid et al., 2010). In case of abortion due to brucellosis, concentrations of Brucella in fetal fluids or placenta may reach up to $10^{13}$ colony-forming units (CFUs)/g compared to an estimated minimum infectious doses range of $10^3$ to $10^4$ CFU (Fensterbank, 1986; Olsen and Tatum, 2010; Saegerman et al., 2010). Brucellae may also be shed into milk from the udder and supra mammary lymph nodes of infected cattle at concentrations going from a few hundred up to few million organisms/ml of milk (Corbel, 1988). One clinical sign commonly associated with brucellosis in African cattle herd is the presence of hygroma. In many countries and for years, fluid of hygroma has been used as the sample for biotyping (Thienpont et al., 1961; Akakpo and Bornarel, 1987; Bankole et al, 2010; Sanogo et al., 2012). From this review, except in Nigeria where diverse samples such as semen, testicular exudates, vaginal swabs, 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 biotyping 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
did not seem to be widespread in that sub-region of Africa as well as biovars 2 and 4 similarly reported only in Nigeria in 1980s.

Biovars 5 and 9 have not been reported yet in this sub-region. Conversely with Central Africa, it also appeared that neither *B. melitensis* nor *B. suis* were isolated yet from cattle in West Africa (Domenech et al., 1983; McDermott and Arimi, 2002). This does not necessary mean that they are absent in this sub-region since cattle are sometimes kept and commonly grazed with sheep and goats in West Africa, which can not preclude any cross-infection among hosts (Ocholi et al., 2005).

Whereas *Brucella* are usually oxidase positive except for *B. ovis* and *B. neotomae*, some biovars encountered in different countries of West Africa often appeared to be negative (Verger et al., 1979; Verger et al., 1982; Bankole et al., 2010; Sanogo et al., 2012). Besides this variable oxidase test response reported in Ivory Coast, Guinea Bissau and Senegal so far, atypical characteristics like slow growing characteristics and altered oxidative metabolic profile were also recorded (Verger et al., 1982; Verger and Grayson, 1984). These results highlight the need for more investigations of prevailing strains of *Brucella* from Africa and could justify the use of methods with more discriminative power for typing.

**Public health significance and implications**

Brucellosis is one of the most widespread bacterial zoonosis (Corbel, 2006). Human disease also known as undulant fever or Malta fever may occur through ingestion of contaminated foods, direct contact with an infected animal or material or via aerosol. It principally affects consumers of raw milk and derivatives and field and laboratory animal health professionals (McDermott and Arimi, 2002; Kunda et al., 2007). Rarely fatal, 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
prevailing biovars. Indeed, available data are sometimes two to three decades older for many countries. Moreover, for easy assessment, it might be suggested that studies publishing typing results explicitly report details on typing methods and present sufficiently informative results in compliance with minimal standards for genus, species and biovar definition of *Brucella*.

The presence of *Brucella* strains across West Africa highlighted the reality of a potential public health threat, in such an epidemiological context where close contact may occur between animals and people, where hygienic conditions are usually poor, where customs favour consumption of raw milk and where no control strategies are implemented. More epidemiological investigations are also needed to provide information on possible sources of human infection, on transmission pathways between animals and humans in order to set up an efficient control strategy in a “one health” perspective. Moreover, the reporting of the disease in humans should be drastically improved by considering brucellosis as part of the differential diagnosis for patients with fever of unknown origin. Taking into consideration the presence of *Brucella* in many countries, the existence of movement of cattle between countries and the limited resources allocated for disease control in most of African countries, a collaborative regional control strategy putting strengths together might be a possible approach to contain brucellosis infections and limit its public health impact in West Africa. Such a strategy should adopt a “one health” concept with more cooperation and exchange of information between public health and veterinary authorities. Furthermore, diagnostic and surveillance capacities of veterinary services should be strengthened to provide valuable epidemiological information, notably on prevailing strains of *Brucella*. Hence, initiatives such as the OIE Performance of Veterinary Services are fundamental to improving the efficiency of the control program of brucellosis as well 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


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. 


Organization for Economic Co-operation and Development (OECD), 2008. Livestock and 
regional market in the Sahel and West Africa: potentials and challenges, Paris, 

Osterman, B., Moriyon, I., 2006. International Committee on Systematics of Prokaryotes 
Subcommittee on the taxonomy of Brucella. Minutes of the meeting, September, 17th 


Renoux, G. 1952. La classification des Brucella. Remarques à propos de l'identification de 

Ron-Román, J., Saegerman, C., Minda-Aluisa, E., Benítez-Ortíz, W., Brandt, J., Douce, R., 
2012. First Report of Orchitis in Man Caused by Brucella abortus Biovar 1 in 

Infectious and parasitic diseases of livestock. Lavoisier et Commonwealth 

Samartino L.E., Gil, A., Elzer, P., 2005. Capacity building for surveillance and control of 
bovine and caprine brucellosis. In: Capacity building for surveillance and control of 
zoonotic diseases. FAO Animal Production and Health Proceedings, no. 7;


http://web.oie.int/eng/normes/MMANUAL/2008/pdf/2.04.03_BOVINE_BRUCELL.


http://www.who.int/zoonoses/neglected_zoonotic_diseases/en/

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

Table 2: Summary of growth characteristics reported for isolates of *Brucella* of cattle origin in West Africa, period 1977-2012
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
Table 1: Overview of studies reporting results of identification and typing of *Brucella* from cattle in West Africa, period 1960-2009

<table>
<thead>
<tr>
<th>Authors, year of publication</th>
<th>Country (location)</th>
<th>Data collection period</th>
<th>Samples</th>
<th>Typing references</th>
<th>Typing results</th>
<th>Number of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanogo et al., 2012&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Ivory Coast (Dimbokro)</td>
<td>2009</td>
<td>H</td>
<td>Alton et al., 1988 ; Le Flèche et al., 2006</td>
<td><em>B. abortus</em> 3</td>
<td>1</td>
</tr>
<tr>
<td>Bankole et al., 2010&lt;sup&gt;a&lt;/sup&gt;</td>
<td>The Gambia (Kombo East District)</td>
<td>NA</td>
<td>H</td>
<td>Alton et al., 1988 ; Le Flèche et al., 2006</td>
<td><em>B. abortus</em> 3</td>
<td>3</td>
</tr>
<tr>
<td>Ocholi et al., 2004</td>
<td>Nigeria (Taraba, Plateau, Adamoua, Bauchi, Sokoto)</td>
<td>2004</td>
<td><em>F, H, M, V</em></td>
<td>Alton et al., 1988</td>
<td><em>B. abortus</em> 1</td>
<td>17</td>
</tr>
<tr>
<td>Tounkara et al., 1994</td>
<td>Mali (Region of Koulikoro)</td>
<td>1991</td>
<td>H</td>
<td>Alton et al., 1988</td>
<td><em>B. abortus</em></td>
<td>4</td>
</tr>
<tr>
<td>Rwanda</td>
<td></td>
<td>1982-1983</td>
<td>H</td>
<td></td>
<td><em>B. abortus 3 or 3/6</em></td>
<td>13</td>
</tr>
<tr>
<td>Senegal (Casamance)</td>
<td></td>
<td>1979</td>
<td>H</td>
<td></td>
<td><em>B. abortus 3 or 3/6</em></td>
<td>37</td>
</tr>
<tr>
<td>Togo</td>
<td></td>
<td>1977</td>
<td>H</td>
<td></td>
<td><em>B. abortus 3 or 3/6</em></td>
<td>30</td>
</tr>
<tr>
<td>Akakpo, 1987&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Senegal, Niger, Togo, Rwanda</td>
<td>NA</td>
<td></td>
<td>Corbel, 1984</td>
<td><em>B. abortus 3 or 3/6</em></td>
<td>82</td>
</tr>
<tr>
<td>Study</td>
<td>Country</td>
<td>Years</td>
<td>Haplotype(s)</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>------------------</td>
<td>---------------</td>
<td>------------------------------------</td>
<td>----------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Niger</td>
<td>H</td>
<td>B. abortus 3/6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Senegal</td>
<td>H</td>
<td>B. abortus 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Senegal</td>
<td>H</td>
<td>B. abortus 3/6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Togo</td>
<td>H</td>
<td>B. abortus 3/6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verger et al., 1982</td>
<td>Togo</td>
<td>1977</td>
<td>H</td>
<td>Corbel and Morgan, 1975; Alton et al., 1977</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Sio river’s valley, near Lome)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(northern region, Kano)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pilo-moron et al., 1979</td>
<td>Ivory Coast</td>
<td>1975-1977</td>
<td>H</td>
<td>NS (Results from CNR, Montpellier, France)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Soclo, Jacqueville, Eloka, Toumodi, Karakoro, Raviart, Bouaké, Pokaha)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verger et al., 1979</td>
<td>Senegal</td>
<td>1976-1978</td>
<td>H</td>
<td>Alton et al., 1977</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Koalack, Tambacounda, Ziguinchor, Nioro du Rip, Kédougou, Vélingara, Kolda, Sédhiou, Bignona, Oussouye)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Plateau, Niger, Borno, Kano)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doutre et al., 1977</td>
<td>Senegal</td>
<td>1976</td>
<td>H</td>
<td>NS (Results from INRA, Nouzilly, France)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Kartlack, near Bignona)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chambron, 1965&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Senegal (Kolda, Velingara)</td>
<td>1960</td>
<td>H</td>
<td>Renoux, 1952</td>
<td>B. abortus</td>
<td>5</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-----------------------------</td>
<td>------</td>
<td>---</td>
<td>--------------</td>
<td>------------</td>
<td>---</td>
</tr>
</tbody>
</table>

B: Blood; F: Aborted fetuses; H: Fluid of hygroma; M: milk; S: Semen; T: Testicular exudates; V: Vaginal swabs, NA: not available; NS: not specified.

<sup>a</sup> Except these studies where molecular methods were also used for typing, all the results reported in this review were obtained using bacteriological methods.

<sup>b</sup> These studies were not included in this review.

<sup>c</sup> Heart blood from aborted fetuses was used. Hygroma fluid and milk samples from aborted cows were negative to bacteriological examination.
Table 2: Summary of growth characteristics reported for isolates of *Brucella* of cattle origin in West Africa, period 1977-2012

<table>
<thead>
<tr>
<th>Country</th>
<th>Authors</th>
<th>Year</th>
<th>Species and biotypes</th>
<th>CO₂ dependance</th>
<th>H₂S production</th>
<th>Urease</th>
<th>Oxidase</th>
<th>Anti-serum Agglutination response</th>
<th>Growth in presence of</th>
<th>Lysis by phage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea Bissau</td>
<td>Verger and Grayon,</td>
<td>1984</td>
<td>B. abortus 3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>A(+) ; M(-)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ivory Coast</td>
<td>Sanogo et al., Pilo-moron et al.,</td>
<td>2012</td>
<td>B. abortus 3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>A(+) ; M(-)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1979</td>
<td>B. abortus 1</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>A(+) ; M(-)</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B. abortus 6</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>-</td>
<td>A(+) ; M(-)</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>Mali</td>
<td>Tounkara et al.,</td>
<td>1994</td>
<td>B. abortus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>A(+) ; M(-)</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Niger</td>
<td>Akakpo et al., Verger and Grayon,</td>
<td>1986</td>
<td>B. abortus 3 or 3/6</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>A(+) ; M(-)</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nigeria</td>
<td>Ocholi et al., Bale and Kumi-Diaka,</td>
<td>2004</td>
<td>B. abortus 1</td>
<td>-(15) ; +(2)</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>A(+) ; M(-)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1981</td>
<td>B. abortus 1</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+(3) ; -(2)</td>
<td>A(+) ; M(-)</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B. abortus 3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>A(+) ; M(-)</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B. abortus 4</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>A(+) ; M(-)</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td></td>
<td>B. abortus</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>A(+) ; M(-)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Eze</td>
<td>1978</td>
<td>B. abortus 1</td>
<td>+(15) ;-(4)</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>A(+) ; M(-)</td>
<td>+</td>
<td>+</td>
<td>+(14)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. abortus 2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>A(+) ; M(-)</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Senegal</td>
<td>1987</td>
<td>B. abortus 3 or 3/6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>A(+) ; M(-)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Akakpo and Bornarel</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verger et al,</td>
<td>1979</td>
<td>B. abortus 3 or 3/6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>A(+) ; M(-)</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>The Gambia</td>
<td>2010</td>
<td>B. abortus 3</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>A(+) ; M(-)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bankole et al,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Togo</td>
<td>1982</td>
<td>B. abortus 3 or 3/6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>A(+) ; M(-)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Verger et al,</td>
<td>1984</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verger and Grayon,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

597 Th: Thionin; BF: Basic fushin, Sf: Safranin O
598 (+): positive reaction;
599 (-): Negative reaction;
600 (+/-): variable reaction observed according to the concentration used
601 ?: No precision provided by authors;
602 ND: Not done
603 * Authors erroneously encoded H₂S production of these isolates as negative in the primary publication. All B. abortus 3 are known to produce H₂S.
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