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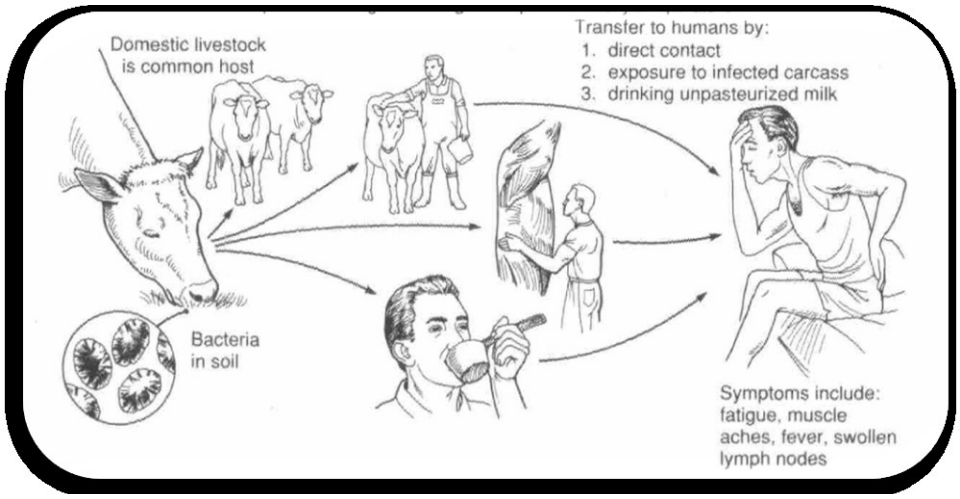
Contribution à l'épidémiologie de la brucellose bovine en Côte d'Ivoire



Contribution to the epidemiology of bovine brucellosis in Ivory Coast

Moussa SANOGO

THESE PRESENTEE EN VUE DE L'OBTENTION DU GRADE DE
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The illustration of the title page is a schematic representation of the main routes of brucellosis from livestock to human proposed by Sir David BRUCE (1855-1931) available at the following URL :
http://m2002.tripod.com/brucellosis.jpg

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In the memory of my father

To my mother

To my lovely wife

And to my son and my daughter

ABSTRACT

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Bovine brucellosis is an endemic infectious disease which can negatively impact on cattle productivity and welfare as well as on human health. In many developing countries such as Ivory Coast, there is a need for knowledge on the distribution and the frequency of the disease (or evidence of its presence) within the animal population and the possible factors associated with the disease. Information is also needed on species and biovars of *Brucella* at national and regional scales, on the performance of commonly used diagnostic tests for accurate estimation of the true disease prevalence, and on determination of risk factors associated with the disease. These informations are of key importance to set up and implement appropriate and efficient prevention and control measures against brucellosis. For these reasons, the research presented in this thesis aimed to contribute to a better understanding of the epidemiology of bovine brucellosis in Ivory Coast.

The thesis is structured into three main parts. The introduction part includes three chapters. The first chapter presents an overview of the literature on the pathogen causing brucellosis, their characteristics and distribution. The impact and the existing strategies for preventing and controlling brucellosis are discussed with a particular reference to the situation of bovine brucellosis in Ivory Coast. The presence and the importance of the disease were confirmed in the country but the disease is still uncontrolled. In the second chapter (Chapter 2), an insight on statistical, epidemiological principles and concepts applied to achieve the different objectives (Chapter 3) is given, including a discussion on available approaches to estimate diagnostic test characteristics and the true prevalence of a disease.

The second part of the thesis includes research on different aspects of the epidemiology of bovine brucellosis in Ivory Coast and West Africa (Chapter 4, 5, 6 and 7). Chapter 4 specifically provides a state-of–the-art knowledge on species and biovars of *Brucella* reported in cattle from Ivory Coast and all other countries of West Africa, through a review of available literature. From the synthesized literature, *Brucella abortus* was demonstrated to be the most prevalent species in cattle in West Africa, in line with the known host preference for *Brucellae*. So far, biovars 3 appeared to be commonly the most isolated in West Africa and was also recently identified in Ivory Coast. However, the presence of *B. melitensis* and/or *B. suis* was not reported yet in cattle in this part of Africa.

Results on prevailing strains of *Brucella* in cattle were related with commonly used serological diagnostic tools. Thus, chapter 5 was dedicated to verify their appropriateness and to assess the performance of two serological tests, Rose Bengal Test (RBT) and indirect Enzyme-linked Immunosorbent assay (iELISA). Using a Bayesian approach, the correlation-adjusted sensitivity of iELISA was estimated at 96.1 % (Credibility Interval (CrI): 92.7-99.8) whereas that of RBT was 54.9 % (CrI: 23.5-95.1). High correlation-adjusted specificities were found for both tests, 95.0 % (CrI: 91.1-99.6) for iELISA and 97.7 % (CrI: 95.3-99.4) for RBT, respectively. The true prevalence of brucellosis was also estimated using the 1228 tested serum samples to be 4.6 with a 95% credibility interval ranging from 0.6 to 9.5% (Chapter 5 and 6). These results also revealed a good performance for the iELISA, which might consequently be a valuable screening assay under the epidemiological conditions prevailing in Ivory Coast.

In Chapter 7, risk factors associated with bovine brucellosis seropositivity were investigated using serological results obtained from 907 serum samples collected from unvaccinated cattle of at least 6 months of age in the savannah-forest region of Ivory Coast. Serum samples were tested using the Rose Bengal test (RBT) and indirect enzyme linked immunosorbent assay (iELISA). The logistic regression analysis indicated that brucellosis seropositivity was associated with age and herd size. Cattle above 5 years of age were found to be more likely seropositive (Odd Ratio (OR) =2.8; 95% Confidence Interval (CI): 1.3, 6.4) compared to cattle under 3 years of age. Similarly, the odds of brucellosis seropositivity for herds with more than 100 cattle was 3.3 (95% CI: 1.2, 8.9) times higher compared to those with less than 50 cattle.

The third part presents a general discussion on the overall contribution of the current research (Chapter 8), by highlighting the main results and pointing out their significance. The need for more investigations on the epidemiology of brucellosis, in Ivory Coast and at West African scale, is highlighted. It is neccessary to provide additional knowledge on prevailing field strains of *Brucella*, on the distribution of the disease and on associated risk factors to implement preventive and control measures. Finally, for more cost-effectiveness and efficiency, the need to strengthen the capabilities of the veterinary services and national laboratories and to consider the control of brucellosis and other zoonotic diseases through a regional, integrated and collaborative perspective is also highlighted.

RESUME

La brucellose bovine est une maladie infectieuse endémique qui peut impacter négativement la productivité et le bien-être des bovins ainsi que sur la santé humaine. Dans de nombreux pays en voie de développement tels que la Côte d'Ivoire, les connaissances sur la distribution et la fréquence de la maladie (ou les preuves de sa présence) dans la population animale ainsi que sur les facteurs de risque associés à la maladie restent limitées. La disponibilité d'informations sur les espèces et biovars de *Brucella* à l'échelle nationale et/ou régionale, sur la performance des tests de diagnostic communément utilisés pour déterminer la prévalence réelle de la maladie, et sur les facteurs de risque est également essentielle. Toutes ces informations sont d'une importance clé pour la définition et la mise en œuvre de mesures de prévention et de contrôle appropriées et efficaces contre la brucellose.

Cette thèse vise donc à contribuer à une meilleure connaissance et compréhension de l'épidémiologie de la brucellose bovine en Côte d'Ivoire. Elle est organisée en trois parties principales. La partie introductive comprend trois chapitres. Le premier chapitre présente une revue de la littérature sur les agents pathogènes responsables de la brucellose, sur leurs caractéristiques et sur leur distribution. L'impact ainsi que les stratégies existantes de prévention et de contrôle de la brucellose sont également discutés avec des références à la situation particulière de la brucellose bovine en Côte d'Ivoire. La présence et l'importance de la maladie ont été confirmées dans ce pays, mais elle y reste toujours incontrôlée. Le deuxième chapitre (Chapitre 2) comprend un aperçu des principes et concepts épidémiologiques et des statistiques appliqués dans le cadre de cette thèse pour atteindre les différents objectifs présentés au chapitre trois (chapitre 3). Les différentes approches méthodologiques disponibles pour l'estimation des caractéristiques des tests de diagnostic ainsi que de la prévalence réelle d'une maladie sont aussi discutées.

La deuxième partie de cette thèse présente, dans un enchainement logique, les recherches effectuées sur différents aspects de l'épidémiologie de la brucellose bovine en Côte d'Ivoire, avec des références à la situation en Afrique de l'Ouest (Chapitre 4, 5, 6 et 7). Le chapitre 4 présente, à travers une revue de la littérature disponible, un état des lieux des connaissances sur les espèces et biovars de *Brucella* signalés chez les bovins de Côte d'Ivoire mais aussi chez ceux de tous les autres pays de l'Afrique de l'Ouest. Il en ressort que *Brucella abortus* a été l'espèce la plus répandue chez les bovins en Afrique de l'Ouest, en conformité avec la préférence d'hôte connue pour les *Brucella*. A ce jour, le biovar 3 semble être le plus généralement isolé dans les pays d'Afrique de l'Ouest y compris en Côte d'Ivoire où il a été

récemment identifié pour la première fois. Cependant, la présence de *B. melitensis* et/ou de *B. suis* n'a pas encore été signalée chez les bovins dans cette partie de l'Afrique.

Les résultats sur les souches dominantes de *Brucella* chez les bovins ont été mis en relation avec des outils de diagnostic sérologique couramment utilisés. Ainsi, le chapitre 5 a été consacré à vérifier leur pertinence et à évaluer la performance de deux tests sérologiques, le test de Rose Bengale (TRB) et l'ELISA indirect (iELISA). En utilisant une approche bayésienne, la sensibilité de iELISA ajusté en prenant en compte la corrélation entre les deux tests, a été estimée à 96,1% (intervalle de crédibilité (ICr): 92,7-99,8), tandis que celle de TRB était de 54,9% (ICr: 23,5-95,1). De hautes valeurs de spécificités ont été trouvées pour les deux tests, respectivement 95,0% (ICr: 91,1-99,6) pour l'iELISA et 97,7% (ICr: 95,3-99,4) pour le TRB. La prévalence réelle de la brucellose a également été estimée à 4,6% avec un intervalle de crédibilté à 95% entre 0,6 et 9,5% sur la base de 1228 sérums analysés (Chapitres 5 et 6). Ces résultats ont mis en évidence une bonne performance pour l'iELISA, qui pourrait être par conséquent un test de dépistage précieux dans les conditions épidémiologiques de la Côte d'Ivoire.

Dans le chapitre 7, les facteurs de risque associés à la séropositivité de brucellose bovine ont été étudiés sur base des résultats sérologiques obtenus de 907 échantillons de sérum prélevés chez des bovins non vaccinés d'au moins 6 mois dans la région intermédiaire entre la savane et la forêt, au centre de la Côte d'Ivoire. Les sérums ont été testés en utilisant le TRB et l'iELISA. L'analyse de régression logistique a indiqué que la séropositivité de la brucellose était associée à l'âge des animaux et à la taille du troupeau d'origine. Les bovins de plus de 5 ans présentaient une plus grande probabilité d'être séropositif (Odd Ratio (OR) = 2,8 (Intervalle de confiance (IC) 95%: 1,3-6,4)) par rapport ceux de moins de 3 ans. De même, la côte de séropositivité à la brucellose pour les troupeaux de plus de 100 bovins était 3,3 (IC 95%: 1,2-8,9) fois plus élevée par rapport à ceux de moins de 50 bovins.

La troisième partie de cette thèse présente une discussion générale sur la contribution globale de cette recherche (Chapitre 8). La nécessité d'entreprendre plus d'études sur l'épidémiologie de la brucellose, en Côte d'Ivoire et en Afrique de l'Ouest, a été soulignée. Il est nécessaire de fournir des connaissances supplémentaires sur les souches circulantes de *Brucella*, sur la distribution de la maladie et sur les facteurs de risque associés pour la prise de mesures de prévention et de contrôle appropriés. Enfin, pour un meilleur rapport coût-efficacité, il est également nécessaire de renforcer les capacités des services vétérinaires et des laboratoires nationaux et d'appréhender la lutte contre la brucellose et les autres maladies zoonotiques dans une perspective régionale, intégrée et concertée.

RESUMEN

La brucelosis bovina es una enfermedad infecciosa endémica que puede tener un impacto negativo en la productividad y en el bien estar de los bovinos, así como en la salud humana. En varios países en vías de desarrollo, como Costa de Marfil, son limitados el conocimiento sobre la distribución y la frecuencia de la enfermedad, pruebas de su presencia, en la población animal así como los factores de riesgo asociados. Son igualmente necesarios los conocimientos en las especies y biotipos de *Brucella* en el país y en la región, la validación de las pruebas de diagnóstico comúnmente utilizadas, y los factores de riesgo. Estas informaciones son de gran relevancia para la puesta en marcha de medidas de prevención o y para el control adecuado y eficaz contra la brucelosis.

Esta tesis contribuye a mejorar el conocimiento y comprensión de la epidemiología de la brucelosis bovina en Costa de Marfil. Está estructurada en tres partes principales. La parte introductoria comprende tres capítulos. El primer capítulo presenta un acercamiento a la literatura sobre los agentes patógenos responsables de la brucelosis, sus características y su distribución. Se discute el impacto y las estrategias existentes para prevenir y combatir la brucelosis con referencias a la situación particular de la brucelosis bovina en Costa de Marfil. La presencia y la importancia de la enfermedad han sido confirmadas en dicho país, sin embargo, la enfermedad es aún incontrolada. En el segundo capítulo, capítulo dos, se muestra los principios y conceptos epidemiológicos y estadísticos aplicados en el marco de la presente tesis para lograr los diferentes objetivos, visto en el capítulo tres. Son también discutidos las diferentes aproximaciones metodológicas disponibles para la estimación de las características de las pruebas de diagnóstico, así como, de la prevalencia real de una enfermedad.

Basados en investigaciones realizadas, la segunda parte de esta tesis relaciona diferentes aspectos epidemiológicos de la brucelosis bovina en Costa de Marfil y en África del Oeste, presente en los capítulos cuatro, cinco, seis y siete. El capítulo cuatro muestra el estado serológico de los biotipos de *Brucella* en los bovinos de Costa de Marfil, y de varios países de África del Oeste a través de la literatura disponible. Así, la *Brucella abortus* demostró ser la especie más extendida en los bovinos de África del Oeste, de acuerdo a la preferencia de hospedador conocido por la *Brucella*. Actualmente, el biotipo 3 es el más aislado en los países de África del Oeste, incluyendo a Costa de Marfil, país confirmado por primera vez. Sin embargo, la presencia de *Brucella melitensis* o/y *Brucella suis* no ha sido aún encontrada en los bovinos de esta parte de África.

Los resultados de las cepas de *Brucella* de los bovinos se relacionaron con las herramientas de diagnóstico serológico comúnmente usadas. De esa manera, el capítulo cinco verifica su pertinencia y evalúa su desempeño en dos pruebas serológicas, la prueba de Rosa de Bengala (RBT) y la de ELISA indirecto (iELISA). Haciendo uso de un enfoque bayesiano, la sensibilidad de iELISA ajustada a la correlación entre dos pruebas, fue de 96,1% (Interval de credibilidad (ICr): 92,7-99,8), mientras que aquella para RBT fue 54,9% (ICr: 23,5-95,1). Así mismo, fueron encontrados fuertes valores de especificidad para las dos pruebas, siendo para iELISA de 95,0% (ICr: 91,1-99,6) y para RBT de 97,7% (ICr; 95,3-99,4). A partir de los 1228 sueros ensayados, la prevalencia real de la brucelosis fue estimada a 4,6% con un interval de credibilidad de 95% entre el 0,6 y el 9,5% (Capítulo cinco y seis). Estos resusltados muestran el buen desempeño de la prueba iELISA para ser usado en las pruebas de despistaje epidemiológico en Costa de Marfil.

En el capítulo 7, son evaluados los factores de riesgo asociados con la seropositividad de brucelosis bovina mediante resultados serológicos obtenidos a partir de 907 muestras de suero tomadas de bovinos no vacunados de por lo menos 6 meses de edad en la región límite entre la sabana y la selva, en el centro de Costa de Marfil. Las muestras de suero se analizaron mediante la prueba RBT e iELISA. El análisis de regresión logística indicó que la seropositividad de la brucelosis se asoció con la edad del animal y el tamaño del rebaño. Los bovinos de más de cinco años presentan 2,8 veces más de riesgo (Interval de confidencia (IC) 95%: 1,3 - 6,4), que aquellos de más de tres años de edad. Por otro lado, el riesgo para la brucelosis en hatos de más de cien cabezas de ganado es de 3,3 veces mayor (IC 95%: 1,2 - 8,9) que aquellos con menos de 50 vacas.

La tercera parte, vista en el capítulo ocho de la presente tesis, muestra una discusión general sobre la contribución global de la investigación actual poniendo en evidencia los principales resultados y señalando su importancia. En tal sentido, se hacen necesarios mayores trabajos epidemiológicos sobre la brucelosis tanto en Costa de Marfil como en África del Oeste, a fin de proveer conocimientos adicionales y suficientes relativos al origien de la *Brucella* circulante, la distribución de la enfermedad y los factores de riesgo asociados orientados a las medidas de prevención o y de control. Para una mejor relación entre el costo y la eficacia, se hace necesaria fortalecer las capacidades de los servicios veterinarios y de los laboratorios nacionales, así como, de concebir la lucha contra la brucelosis y las otras zoonosis sobre una perspectiva regional, integrada y colaborativa.

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"[...] Great things can happen to ordinary people if we work hard and never give up."

Barak Obama

"You have the responsibility to persist until you succeed"

T. Newberry

"[...] Making the most of what we have"

T. Newberry

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LIST OF ABBREVIATIONS

°C	Degree Celsius
AMOS	Abortus-Melitensis-Ovis-Suis
ANADER	Agence Nationale d'Appui au Développement Rural (National Agency for rural development)
AP	Apparent Prevalence
AUC	Area Under the Receiver-Operating-Characteristic (ROC) Curve
Bayesp	Bayesian P-value
BK	Berkeley
BSL3	Biosafety Level 3
CERVA	Centre d'Etude et de Recherches Vétérinaires et Agro-chimiques
CFT	Complement Fixation Test
CFU	Colony Forming Unit
CI	Confidence Interval
CO2	Carbon dioxide
CrI	Credibility Interval
DIC	Deviation Information Criterion
DNA	Deoxyribonucleic Acid
DOR	Diagnostic Odd Ratio
EDTA	Ethylene Diamine Tetraacetic Acid
ECOWAS	Ecomonic Community of West African States
FAO	Food and Agriculture Organisation
FAOSTAT	Food and Agriculture Organization Statistics Division
FCFA	CFA Franc (West African currency: 1 EURO= 655.957 FCFA)
FN	False negative
FP	False Positive
FPA	Fluorescence Polarization Assay
FPSR	False Positive Serological Reaction
GDP	Gross Domestic Product
H2S	Dihydrogen sulfide
H38	Vaccine strain H38(killed *B. melitenis* strain 53H38)
HSe	Herd Sensitivity
HSp	Herd specificity
iELISA	Indirect Enzyme Linked Immunosorbent Assay
IFAD	International Fund for Agricultural Development
Ig	Immunoglobulin
ILRI	International Livestock Research Institute
IMT	Intsitute of Tropical Medicine
J	Youden index
K	Kappa coefficient
LANADA	Laboratoire National d'Appui au Développement Agricole
LFA	Lateral Flow Assays
LID	Livestock in Development

LPS	Lipopolysaccharide
LR	Likelihood Ratio
MCMC	Markov Chain Monte Carlo
MIPARH	Ministry of animal and fishery production and resources
MLSA	Multilocus Sequence Analysis
MLST	Multilocus Sequence Typing
MLVA	Multiple Loci Variable number tandem repeat Analysis
MRT	Milk Ring Test
Neg	Negative test result
NPV	Negative Predictive Value
OIE	World Animal Health Organization
OPS	O Polysaccarhide
OR	Odd Ratio
P	True prevalence
Pc	Proportion of observed agreement
PLS	Projet laitier Sud
PNDL	Programme national de Développement Laitier
Pneg	Index of negative agreement
Po	Proportion of agreement due to chance
Pos	Positive test result
Ppos	Index of positive agreement
PPV	Positive Predictive Value
PSE	Programme Sectoriel de l'Elevage,
PVS	Performance of Veterinary Services
R	Rough
R/C	A brucellaphage active on rough *Brucella*
RB51	Vaccine RB51
RBT	Rose Bengal Test
RLPS	Rough Lipopolysccharide
RTD	Routine Test Dilution
S	Smooth
S19	Vaccine strain 19
SAW	Slow Agglutination of Wright
Se	Sensitivity
SLPS	Smooth Lipopolysaccharide
SNP	Single Nucleotide Polymorphism
SODEPRA	Société pour le Développement de la Production Animale
Sp	Specificity
Tb	Tbilisi
TN	True Negative
TP	True positive
ULg	University of Liège
USD	United States Dollar (1 USD= 0.788454 EUROS)
USDA	United States Department of Agriculture
VNTR	Variable Number Tandem Repeat

Wb	Weybridge
WGS	Whole Genome Sequencing
WHO	World Health Organization
γ-IFN	Gamma interferon

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GENERAL INTRODUCTION

The majority of the world's poor population (about 75%) works and lives in rural areas. About 600 million of the 1.3 billion of the poor worldwide keep livestock as means to produce food and generate cash income (IFAD, 2001; Thornton *et al.*, 2002; ILRI, 2012). Hence, livestock is of key importance in people's everyday lives in most of the sub-Saharan African countries where a quarter of the world's poor come from (**Figure 1**). Livestock contributes to their financial security, their food security and to the development of their agriculture through animal traction and manure (Starkey, 2010; FAO, 2011). The development of livestock production and its productivity are therefore part of the solution for food security and poverty alleviation, especially in low-income areas. Consequently, there is a need to tackle the different constraints to this development, especially the pathological ones.

With its negative impact on animal health and productivity, and its threat to human health, brucellosis is one of the pathological constrains to be considered. On a global basis, this disease is among the thirteen animal diseases and syndromes identified as having a significant impact on poor people worldwide and in West Africa (Perry, 2002). Brucellosis is a bacterial infectious disease affecting domestic, wild animals and humans (Maurin *et al.*, 2005). It is one of the most widespread bacterial zoonotic diseases (Corbel, 2006). According to the World Health Organization (WHO), about 500,000 new cases of human brucellosis are reported annually worldwide (Corbel, 1997, Pappas *et al.*, 2006).

In animals, brucellosis is responsible for many economic losses because of abortions, decrease in production (particularly reduced milk production), newborn mortality, reproductive disorders, and costs of intervention. With its impact on productivity, it contributes to worsen the deficit of animal protein especially for populations in developing countries, where the needs are continuously increasing. In areas where people's livelihood heavily depends on livestock, the impact of brucellosis might also exacerbate poverty (Cáceres, 2010). The most common and widespread form of the disease in animals is bovine brucellosis (Akakpo and Bornarel, 1987; Corbel, 1997; McDermott and Arimi, 2002; Bronvoort *et al.*, 2009). Therefore, it is the main concern in sub-Saharan African countries (McDermott and Arimi, 2002) where average prevalence rates ranging between 10.2 and 25.7% were reported (Mangen *et al.*, 2002). In West Africa, the disease (or evidence of its presence) was reported in 12 out of the 14 countries so far (Mangen *et al*., 2002; Boukary,

2013) with higher seroprevalences estimated in Senegal, Togo, Mali, Niger, Burkina Faso and Ivory Coast (Mangen *et al.*, 2002).

In Ivory Coast[1], the disease is considered as one of the dominant pathologies affecting livestock productivity, with negative impact on livestock breeders' financial security and annual income (Angba *et al.*, 1987; Mangen *et al.*, 2002). Located between 3° to 9° Longitude West and 5° to 11° Latitude North, Ivory Coast is a West African country of 322 462 kilometers of square (Km²) (**Annex 1**). It is surrounded by Mali and Burkina Faso in the North, Ghana in the East and Guinea and Liberia in the West (**Figure 2**). Its population is about 21 millions of inhabitants, of which almost a half live in rural areas (FAO, 2014a). Three main agro-ecological areas are encountered (**Figure 3**): The Guinean zone or forest area, in the south, is the most humid and covers almost the whole forest region with annual rainfall generally above 1,500 mm. The Soudano-Guinean zone (or savannah-forest area) is an area of transition between the forest zone and the north. In this area, annual rainfall varies between 1,200 and 1,500 mm. The Soudanean zone in the northern part of the country is the savannah region with rainfall ranging between 900 and 1,200 mm per year. According to the FAO, there are about 1.6 millions cattle, 3.3 millions small ruminants, and almost 353,000 pigs (FAO, 2014b). Most cattle herds are concentrated in the northern and central part of the country, which is more favorable for livestock breeding with around 85% of the country's cattle population. These cattle are of four different breeds: The N'Dama, the Baoulé, and the Lagunaire which belongs to the humpless *Bos taurus* type and the Zebus of the humped *Bos indicus* type. There are also various crossbred animals (*Bos taurus* X *Bos indicus*). Conversely to *B. Taurus* breeds which are mostly raised in the sedentary system, Zebus and their crossbred are mostly associated with the transhumance[2] or semi-transhumance system, with movement of cattle toward the central part or within the northern and central part of the countries. The extensive system is the dominant type of farming in the country.

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[1] *The official name of the country is "**Côte d'Ivoire**", but it is popularly named "**Ivory Coast**" in english. "Ivory Coast" will be used throughout this manuscript.*

[2] *Transhumance is defined as an oscillating, seasonal movement of livestock under the care of herders, following precise routes in order to exploit pastoral resources. It is distinguished from nomadism, which is characterised by more random movements and is followed by the herder's whole family (OECD, 2008). There are agreements for transhumance between member countries of the Economic Community of West African States (ECOWAS) since 1998 , allowing inter-states animal movements (Anomynous, 1998; Anonymous, 2003b)*

In Ivory Coast, livestock breeding is still a secondary activity compared to agriculture that is practiced by 2/3 of the whole population. According to available figures, livestock breeding accounts for only 4.5 % of the agricultural Gross Domestic Product (GDP) and 2% of the total GDP of the country (Anonymous, 2003a). As a result, the national coverage of needs in meat products (59%) and dairy products (18%) is still insufficient (Anonymous, 2003a). Therefore, many efforts are required to cover these needs. Meanwhile, the country is dependent on imports from neighbouring countries such as Mali and Burkina Faso. To reduce the magnitude of this dependence, many initiatives have been undertaken. Institutions, projects and programs were promoted for the development of livestock production and increased productivity of local breeds through genetic improvement (e.g., Société pour le Développement de la Production Animale (SODEPRA), Agence Nationale pour le Développement Rural (ANADER), Programme National de Développement Laitier (PNDL) , Programme Sectoriel de l'Elevage (PSE), Projet Laitier Sud (PLS) (Anonymous, 1997)). The sustainability of these initiatives also implies tackling the numerous animal diseases of food producing animals, including the endemic and zoonotic ones such as brucellosis.

Controlling brucellosis efficiently requires good diagnostic tools and sufficient and reliable information on the epidemiology of the disease. Until now, different aspects of the disease have been investigated throughout years in Ivory Coast before this research (Gidel *et al.*, 1974; Pilo-Moron *et al*., 1979; Camus, 1980a; Angba *et al.*, 1987; Thys *et al.*, 2005) since first evidences of brucellosis were reported in the 1970s (Böhnel, 1971; Pilo-Moron *et al.*, 1979). However, available information is still scarce or outdated. Therefore, there is a need to update information on the epidemiology of brucellosis, especially on its distribution, the causes (which *Brucella* spp. are involved), and the factors favoring the spread of the disease. All these preliminary pieces of knowledge are necessary to understand the epidemiology of the disease and to elaborate future preventive and control programs for countries facing brucellosis in West Africa, including Ivory Coast. It is particularily important to consider the regional perspective, knowing the existence of frequent cattle movements between West African countries through transhumance or commercial exchanges (**Figure 4**).

This thesis aims to improve the current knowledge on the status of brucellosis in Ivory Coast and it is structured in three main parts. The introduction part includes three chapters. In Chapter 1, an overview is presented on the disease-causing agents of brucellosis, their characteristics, and their distribution. In addition, the impact, the prevention and the control measures of brucellosis are presented with references to the situation of bovine brucellosis in

Ivory Coast. The chapter 2 includes a review of statistical methodological approaches for accurate estimation of diagnostic test characteristics and true prevalence of disease. It also brings an insight on statistical, epidemiological principles and concepts applied to achieve the different objectives of the thesis, presented in Chapter 3. The second part of the thesis includes the research contribution to the different aspects of the epidemiology of bovine brucellosis in Ivory Coast and West Africa (Chapter 4, 5, 6 and 7). Finally, the last part presents a general discussion on the overall contribution of the thesis (Chapter 8).

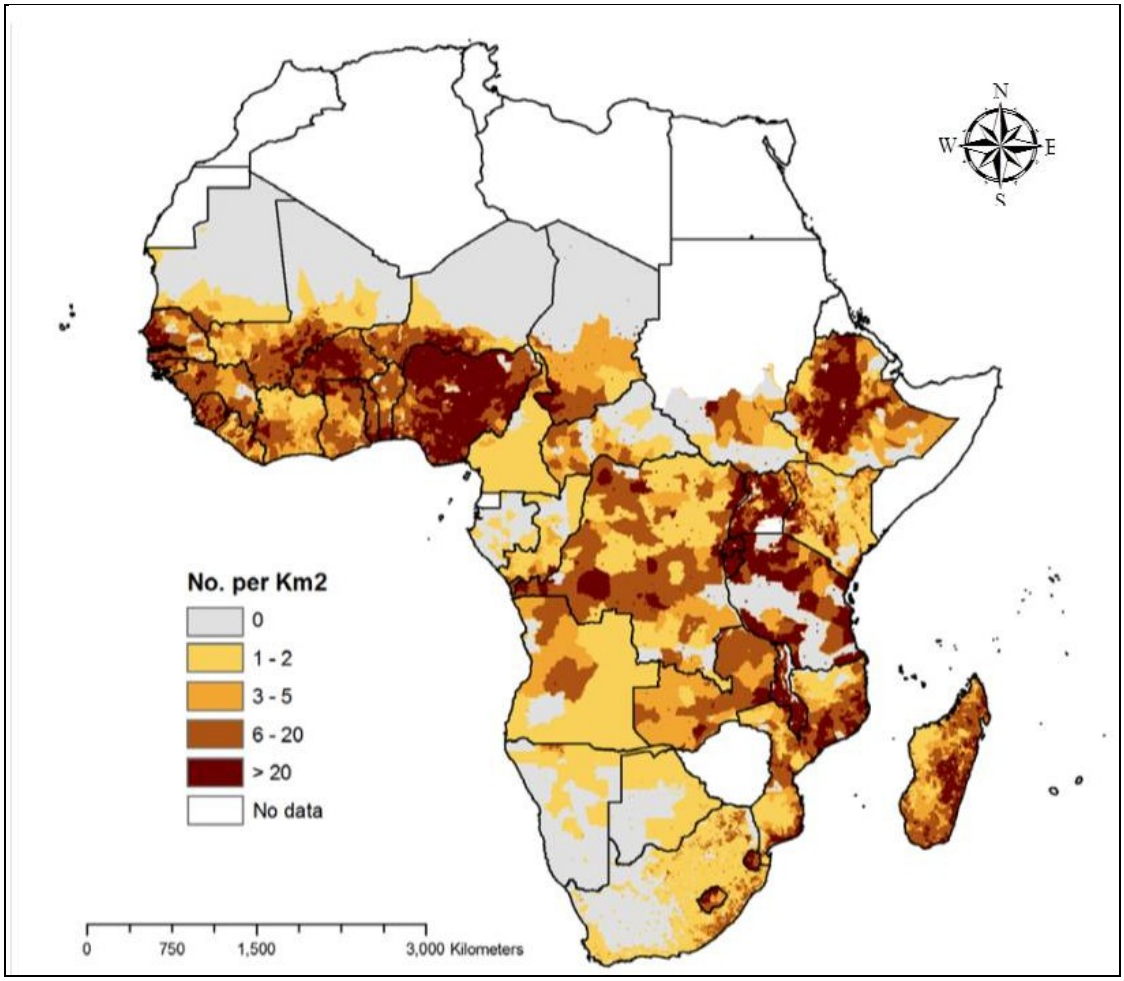

Figure 1: Map showing the density of poor people keeping livestock in Africa, 2005 (ILRI, 2012)

Figure 2: Administrative map of Ivory Coast (Source: http://www.nationsonline.org/oneworld/map/cote-dIvoire-administrative-map.htm)

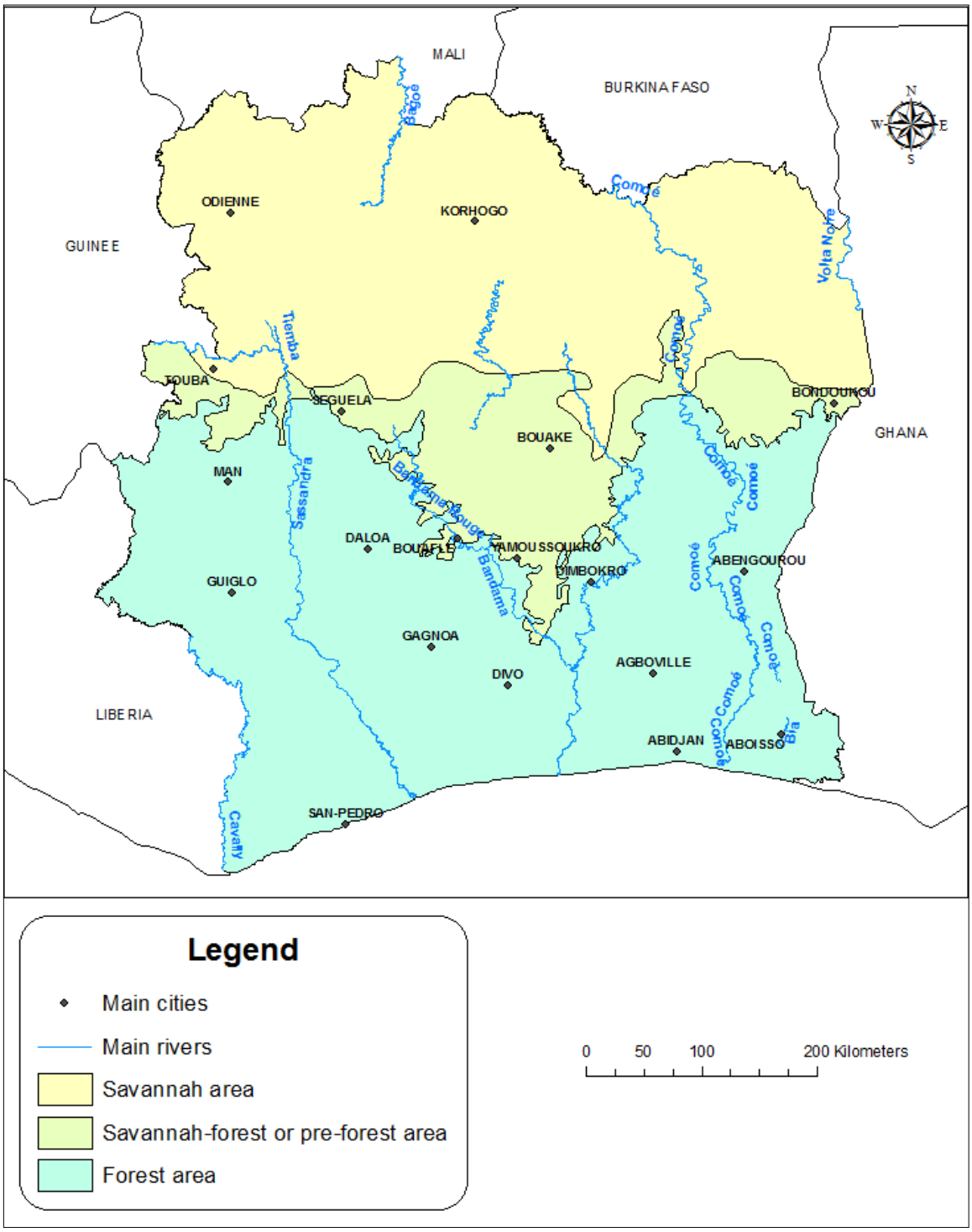

Figure 3: Map showing the agro-ecological areas of Ivory Coast

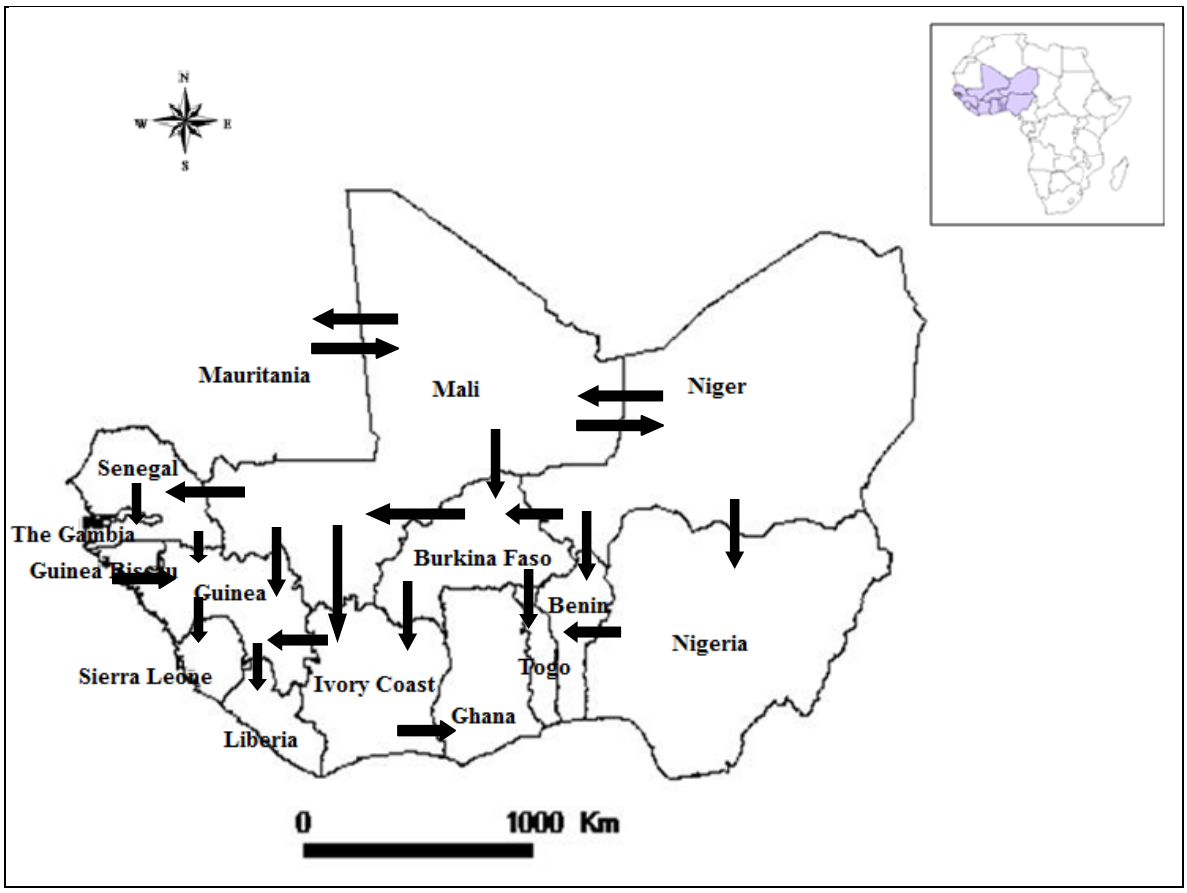

Figure 4: Cross-border transhumance routes in West Africa and Central Africa (adapted from OECD, 2008)

PART ONE:
LITERATURE REVIEW

CHAPTER 1:

BRUCELLOSIS: ETIOLOGY, IMPORTANCE, PREVENTION AND CONTROL WITH SPECIAL REFERENCES TO THE SITUATION OF THE DISEASE IN CATTLE IN IVORY COAST

CHAPTER 1: BRUCELLOSIS: ETIOLOGY, IMPORTANCE, PREVENTION AND CONTROL WITH SPECIAL REFERENCES TO THE SITUATION OF THE DISEASE IN CATTLE IN IVORY COAST

1.1. Etiological agents of brucellosis

1.1.1. Brief history on *Brucella*

Brucellosis is an ancient disease. References to what is now known as brucellosis are argued to exist in the history up to about five century before Jesus Christ, with a description of a resembling condition by Hippocrates (Fernando *et al.*, 2003; Cutler *et al.*, 2005). During the 17th and 18th centuries, cases of a mysterious undulant fever were recorded in many areas all over the Mediterranean region, with different local names (e.g., Mediterranean fever, Rock fever, Gibraltar fever, Cyprus fever, Danube fever, Neapolitan fever, Crimean fever, Cartagena fever, Barcelonan fever, Corps disease, undulant fever). In 1859, Dr Jeffrey Alan Marston, an assistant surgeon of the British royal artillery on duty in the island of Malta, contracted a similar illness, also characterized by an undulant fever (Wyatt, 2013). By describing his own case, Dr Marston produced the first detailed clinical description of "Malta fever". His illness was later associated with brucellosis, after another army surgeon, Captain David Bruce, identified the causal agent of this disease, a small bacterium (designated *Micrococcus melitensis* and later named *Brucella melitenis*), isolated from the liver of a British soldier who died from a similar disease (Bruce, 1887).

In 1897, about 10 years after the works of Captain Bruce, Prof. Almroth Wright described the first serological diagnostic test for the disease, the sero-agglutination tube test. Meanwhile, a new bacterium designated *Bacillus abortus* was isolated from repetitive abortive cows by Bernhard Bang, a Danish veterinarian. The first relation between the disease in human and an animal source was made about fifty years after the first clinical description, in 1905, by Dr Themistocles Zammit who associated the disease in humans with unpasteurized goat milk (Zammit, 1905). In 1917, Alice Evans, an American bacteriologist related *Bacillus abortus* and *Micrococcus melitensis* and the two bacteria were grouped into a single genus designated *Brucella*, as a tribute to Captain David Bruce (Meyer and Show, 1920).

In sub-saharan Africa, the first references to Malta fever were made in the early 1900s in Senegal and Mauritania (Bourret, 1910). However, evidence of the disease in animals started to be reported in the 1930s especially in West African countries (Akakpo and

Bornarel, 1987). In Ivory Coast, Böhnel (1971) provided the first evidence of the disease in cattle during a serological survey in the northern part of the country. Some years later, serological evidences in humans were also provided (Gidel *et al.*, 1974).

1.1.2. Taxonomy, description and characteristics of *Brucella*

The etiological agents of brucellosis are bacteria members of the genus *Brucella*. The genus *Brucella* belongs to the family *Brucellaceae* within the order *Rhizobiales* of the class *Alphaproteobacteria* (Meyer and Shaw, 1920; Godfroid *et al.*, 2011).

Brucellae are facultative intracellular bacteria that grow slowly in aerobic conditions at 37°C but some strains may require 5 to 10% carbon dioxide for growth. Phenotypically, *Brucellae* appear as short rods (0.5-0.7 µm×0.6-1.5µm), non-motile, non-capsulate, small Gram-negative coccobacilli. After three to seven days of incubation on culture plates (Quinn *et al.*, 1999; Alton *et al.*, 1988), *Brucellae* colonies appear with round (2-4 mm in diameter), pin-point shape, smooth, rough or mucoid (intermediate) aspect (Corbel and Brinley-Morgan, 1984). Contrarly to rough strains, smooth strains contain an O antigen on the lipopolysaccharide (LPS), a structural component of the outer membrane of the bacteria and appear translucent with a honey-color (**Figure 5).** Among the known species, so far, only *B. canis* and *B. ovis* have a rough shape (Alton *et al.*, 1988).

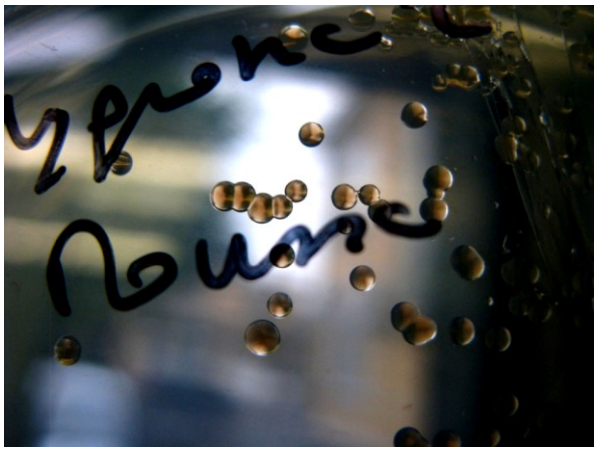

Figure 5 : *Brucella* colonies on a solid culture media, showing translucent honey-colored appearance (credit picture: M. Sanogo)

Using bacteriological examination, complete identification of *Brucella* until biovar level is made with a combination of morphological, cultural and biochemical characteristics (**Table I**). Classification of *Brucella* into species is dependent on criteria as natural host

preference, sensitivity to *Brucella* phages (Tbilisi (Tb), Weybridge (Wb), BK2, R/C) and oxidative metabolic profiles. Requirement of CO_2 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 are used to determine subtypes or biovars (Corbel and Morgan, 1975; Alton *et al.*, 1988; Saegerman *et al.*, 2010; Godfroid *et al.*, 2010). Nevertheless, the classification into subtypes or biovars may be sometimes problematic due to variability of some of the characteristics used for typing, such as sensitivity to dyes (thionine, fuschine, and safranine O), H_2S production and CO_2 requirement for growth (Acha and Zysfres, 2003). When they are available, DNA-based methods are also useful tools to characterize the different species and biovars of *Brucella.* They are particularly useful when a high discrimative power is needed and can be used in combination with other identification and typing methods (Adone *et al.*, 2001; Bricker, 2002; Bricker *et al.*, 2003). Various methods have been developed over the time including the Multilocus sequence analysis (MLSA), the whole genome sequencing and the global genome-wide Single nucleotide polymorphism (SNP) analysis (Le Flèche *et al.*, 2006; Yu and Nielsen, 2010; Bankole *et al.*, 2010; Sanogo *et al.*, 2013a, Jiang *et al.*, 2013; Scholz and Vergnaud, 2013). In addition to their high resolution, molecular based methods limit the manipulation of living agent (Le Flèche *et al.*, 2006).

Traditionally, species of *Brucellae* are determined according to their host preference and pathogenicity. Thus, the different species of *Brucella* and their associated hosts are as follows: *B. abortus* (cattle), *B. melitensis* (goats), *B. suis* (swine), *B. canis* (dogs), *B. ovis* (sheep), and *B. neotomae* (rodent) (Godfroid *et al.*, 2005; Corbel, 2006; Saegerman *et al.*, 2010). In addition to these common species, new strains of *Brucella* were later described. *B. microti* were isolated from common vole (*Microtus arvalis*) and wild red fox (*Vulpes vulpes*), *B. pinnipediae* and *B. ceti in* marine mammals (Ewalt *et al.*, 1994; Foster *et al.*, 1996; Clavareau *et al.*, 1998; Godfroid *et al*., 2005; Cutler *et al*., 2005; Scholz *et al.*, 2008; Scholz *et al.*, 2010; Banai and Corbel, 2010; Nymo *et al.*, 2011). Up to date, at least 10 species have been reported as members of the genus *Brucella* (Godfroid *et al.*, 2011; Scholz and Vergaund, 2013). A summary of known species until now and a description of the different biovars are presented in **Table I and Figure 6**. Moreover, because of a high phylogenetic homogeny, it was suggested to consider all *Brucella* as belonging to the same single species namely *B. melitensis* and the other species of *Brucella* becoming the biotypes or biovars (Verger *et al.* 1985, 1987; De Ley *et al.*, 1987; Cutler *et al.*, 2005; Scholz *et al.*, 2008).

Despite the scientific accuracy of this homogeny, the suggested classification is still not widely adopted due to lack of practicability (Pappas *et al.*, 2005) and because the different species are also considered as different ecotypes (Cohan, 2002). Regarding the host preference-based classification, even if it appears more convenient, it is now apparent that a given host can be infected by different species of *Brucella* (Cutler *et al.*, 2005). Thus, *B. melitensis* and *B. suis* were also reported in cattle in some epidemiological contexts, where cattle have contact with pigs and where cattle and small ruminants are grazing on common pastures as it is the case in Latina America, southern European countries and in the middle East (e.g. Godfroid and Kasbohrer, 2002; Godfroid *et al.*, 2005, Szulowski *et al.*, 2013, Fretin *et al.*, 2013). The presence of *B. melitensis* in cattle was also documented in North Africa (e.g. Samaha *et al.*, 2008) and in Eastern Africa (e.g. Muendo *et al.*, 2012). In Western Africa, the lack of this kind of report did not preclude the possible presence of other species of *Brucella* in cattle population.

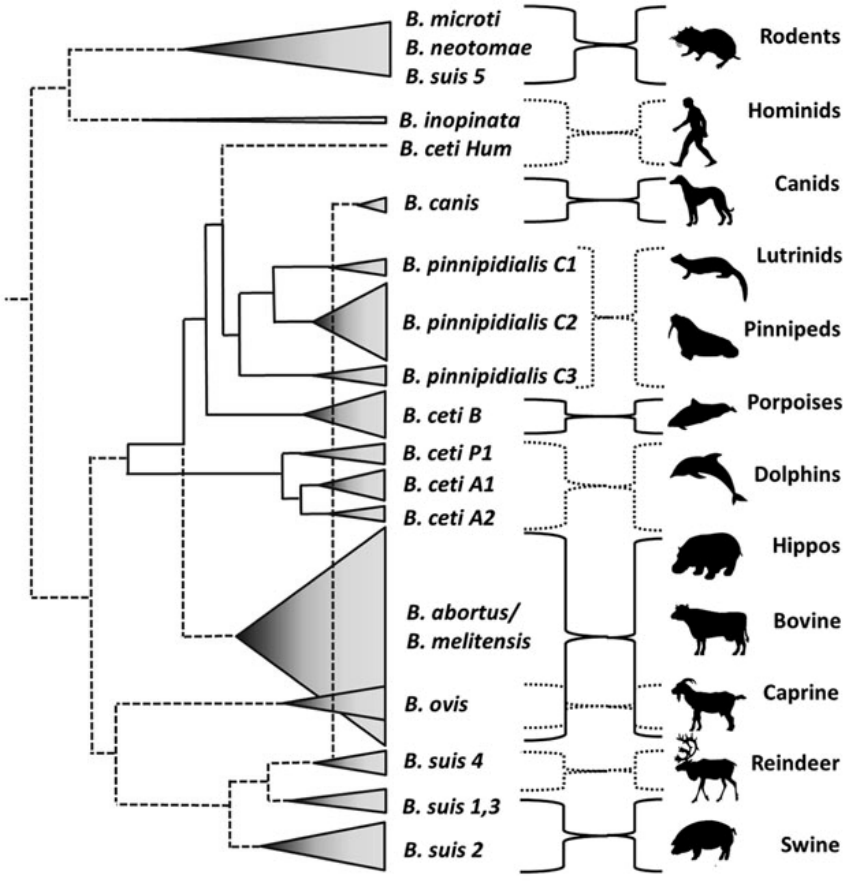

Figure 6: Dispersion of *Brucella* species according to their preferred host mammal. The dispersion of the various *Brucella* species is depicted as cones proportional to the number of strains analyzed (adapted from Moreno, 2014).

Table I: Nomenclature and characteristics of *Brucella* species (from Pappas *et al.*, 2005; Whatmore, 2009; Whatmore *et al.*, 2014)

Species	Biovar	Animal Hosts	Human virulence*	Species discrimation
B. melitensis	1-3	Goats, sheep, camels	++++	Fushin, positive; thionine, positive; safranin inhibition, negative; H_2S production, negative; urease, positive in 24 hr; CO_2 growth, negative;Tbilisi phage lysis, negative; Weybridge phage lysis, negative
B. abortus	1-6, 7**, 9	Cows, camels, yaks, buffalo	++ to +++	Fushin, positive (except biovar 2); thionine, negative (Biovar 1,2 and 4); safranin inhibition, negative; H2S production, positive (except biovar 5) urease, positive in 24 hr; CO2 growth, positive (biovar 1-4);Tbilisi phage lysis, positive; Weybridge phage lysis, positive
B. suis	1-5	Pigs (biovars 1-3), wild hares (biovar 2), caribou (biovar 4), reindeer (biovar 4), rodents (biovar 5)	+	Fushin, negative (except biovar 3); thionine, positive; safranin inhibition, positive; H_2S production, positive (biovar 1); urease, positive in 15 min; CO_2 growth, negative;Tbilisi phage lysis, negative; Weybridge phage lysis, positive
B. canis	-	Canines	+	Fushin, positive or negative; thionine, positive; safranin inhibition, negative; H_2S production, negative; urease, positive in 15 min; CO_2 growth, negative;Tbilisi phage lysis, negative; Weybridge phage lysis, negative
B. ovis	-	Sheep	-	Fushin, negative for some strains; safranin inhibition, negative; H_2S production, negative; urease, negative; CO_2 growth, postive;Tbilisi phage lysis, negative; Weybridge phage lysis, negative
B. neotomae	-	Rodents	-	Fushin, negative; safranin inhibition, negative; H_2S production, positive; urease, positive in 15 min; CO_2 growth, negative;Tbilisi phage lysis, positive or negative; Weybridge phage lysis, positive
B. pinnipidialis and B. ceti	-	Minke whales, dolphins, porpoises (pinnipediae), seals (cetaceae)	+	Fushin, positive; thionine positive; safranin inhibition, negative; H_2S production, negative; urease, positive; CO_2 growth, negative for *B. pinnipidialis* and positive for *B. ceti* ;Tbilisi phage lysis, negative; Weybridge phage lysis, positive for *B. pinnipidialis* and negative for *B. ceti*
B. inopinata	-	Unknown but isolated from human	?***	Fushin positive; thionine , positive; H_2S production, positive; urease, positive; CO_2 growth, negative;Tbilisi phage lysis, positive or negative; Firenze phage lysis, negative
B. papionis sp. nov.	-	Unknown but isolated from baboon	?	Fushin positive; thionine , positive; H_2S production, negative; urease, strongly positive; CO_2 growth, negative; Tbilissi phage lysis, partially positive; Weybridge phage lysis, positive; Berkeley phage lysis, positive; Firenze phage lysis, positive;

** Virulence is graded on a scale from no virulence (–) to the highest degree of virulence (++++); **The status of this B. abortus biovar 7 in under review.***Not known*

1.2. Importance and distribution of brucellosis

1.2.1. Socio-economic and public health importance

Brucellosis is an infectious disease with both socio-economic and public health importance. When present, the disease may have serious impact on animal production and productivity. It may also represent a severe hazard for human health. Brucellosis is also an important disease because of its potential to be weaponized for bioterrorism as it was the case during the 1950s (Cutler *et al.*, 2012).

With more than 500,000 new human cases recorded yearly, brucellosis is a major bacterial zoonotic disease of global importance (Cutler *et al.*, 2005; Pappas *et al*., 2006; Corbel, 2006). Human brucellosis (or Malta fever or undulant fever) is responsible for an acute to chronic or severe debilitating and disabling disease with a wide range of clinical signs. These clinical signs include an "undulant" fever, sweating, weakness, headache, anorexia, weight loss, pain in joints and generalized pain (McDermott and Arimi, 2002; Dean *et al.*, 2012). Human brucellosis is rarely fatal but tends to be chronic if not treated. Thus, complications such as endocarditis, meningitis (also called neurobrucellosis) and orchitis may occur (Corbel, 2006). The severety of the disease in human depends on the type of *Brucella* involved and the source of infection. Most severe clinical cases are commonly associated with *B. melitensis* (Benkirane, 2001; Corbel, 2006). Data on the actual incidence of the disease in humans are scare or lacking especially in sub-Saharan African countries. Available data suggest a higher incidence in low to middle-income countries where effective diagnosis or treatment is lacking or where programs for detecting and preventing infection in both humans and animals are not adequately implemented (Cutler *et al.*, 2005; Corbel, 2006; Dean *et al.*, 2012). In developing countries the infection rate was estimated to be above 10% (USDA and ILRI, 2013) and in the Republic of Chad, an incidence of 34.8 per 100,000 person-year was reported in nomadic communities (Dean *et al.*, 2012).

In animals, brucellosis is also recognized as a major pathological constraint to the development of livestock in sub-Saharan African countries (Camus, 1980a; Domenech, 1987; Akakpo, 1987). As a major constraint, brucellosis needs to be especially accounted for in developing countries, where about 70% of rural poor depend on livestock as part of their livelihood (LID, 1998). In addition to its public health importance, brucellosis has a negative impact on animal health and productivity. It primarily affects the reproductive system of the

host resulting in economic losses on productivity through late term abortions, calf mortality, reduced milk production and infertility (Pilo-Moron *et al.*, 1979; Domenech, 1987; Corbel, 1997). The disease is reported to be responsible for about 20 to 25% of milk yield reduction (Timm, 1982; Acha and Szyfres 2005). A prevalence of about 30% infected cows within a herd is argued to cause a loss of the herd productivity of about 6 % (Domenech *et al.*, 1982). Economic impact of brucellosis may also be indirect through the costs for veterinary interventions, investment for prevention and control measures (including vaccination and compensation), investment for restocking (in countries where culling is practiced) and losses related to consecutive exportation restrictions.

Despite these known consequences, estimation of the actual economic impact of the disease in animal remains difficult. Mangen *et al.* (2002) estimated the losses of the annual value produced per animal between 6 and 10% (Mangen *et al.*, 2002). Similarly, Camus (1980) reported a loss of about 10% of the annual income of cattle breeders in Ivory Coast due to brucellosis (Camus, 1980a). In Latin America, the annual losses related to bovine brucellosis were estimated to approximately 600 million USD[3] (Acha and Szyfres, 2003). Similarly, estimation of the burden of the disease in humans is difficult, but it is expected to be high. For an average of 13 days spent in a hospital, Colmenero-Castillo *et al.* (1989) estimated an overall cost of 8,000 USD[4] for human brucellosis per case in Spain. In addition, the total number of work absence days was 102 days per patient (Colmenero-Castillo *et al.*, 1989). For the case of bioterrorist attack with *B. melitensis*, the economic impact for 100,000 persons exposed is expected to be about 478 million USD[5], related to 82,500 human cases of brucellosis requiring extended therapy and leading 413 deaths (Kauffman *et al.*, 1997). Therefore, given the serious consequences of brucellosis for public health and the economy, the disease needs to be considered carefully, especially in low-income countries such as Ivory Coast (McDermott and Arimi, 2002; Mangen *et al.*, 2002; McDermott *et al.*, 1013).

[3] *About 473 millions euros or about 310 billions FCFA*

[4] *About 6,308 euros or about 4,138,000 FCFA*

[5] *About 377 millions euros or about 248 billions FCFA*

1.2.2. Distribution of brucellosis

1.2.2.1. Disease in animals

Considering the wide range of potential animal hosts, brucellosis is one of the most widespread diseases in the world. Since the beginning of the 1900s, animal brucellosis has been commonly reported in sub-Saharan countries, where bovine brucellosis remains the most widespread form (Akakpo and Bornarel, 1987, Corbel; 1997; Mangen *et al.*, 2002; McDermott and Arimi, 2002; Bronvoort *et al.*, 2009). The disease is endemic in most of the African countries (Corbel, 1997; Akakpo and Bornarel, 1987) with different prevalences (McDermott and Arimi, 2002). An overall apparent seroprevalence rate based on Rose Bengal testing was estimated to range from 10.2 to 25.7% in cattle populations of sub-Saharan Africa (Mangen *et al.*, 2002). In West Africa, evidence of the disease was found in all the countries where research was conducted 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). In Ivory Coast, brucellosis is among the dominant pathologies affecting cattle population (Angba *et al.*, 1987). Since the first evidences in this country, the prevalence of the disease has been investigated throughout the years and at different geographical scales. These investigations mainly covered the northern and central regions, the main breeding areas of the country (**Table II**). Results from a national survey conducted between 1975 and 1977 reported a seroprevalence in cattle of 11.3% (Angba *et al.*, 1987). More recently, Thys *et al.* (2005) estimated a true prevalence of 3.6 and 4.2% respectively in dairy farms and their neighbours' traditional farms in peri-urban area of Abidjan (Thys *et al.,* 2005). Recently, using sera collected during the serosurveillance of Rinderpest in Ivory Coast, the true prevalence of the disease in traditional cattle was estimated to range between 5 and 16% in the central part of the country (Sanogo *et al.,* 2008). The different species and biovars of *Brucella* are distributed heterogenously throughout the world but *B. abortus* remains the most prevalent worldwide so far (Corbel, 1997; Robinson, 2003; Acha and Szyfres, 2005). The presence of biovars 1 and 6 of *B. abortus* has also been confirmed in Ivory Coast from hygroma fluid samples (Pilo-Moron *et al.*, 1979). No isolates of *B. melitensis* have yet been reported in this country neither in cattle nor in the small ruminants, despite evidence of the presence of the disease in small ruminants (Gidel *et al.*, 1974; Chartier, 1982).

Table II: Studies on prevalence of bovine brucellosis in Ivory Coast, 1970-2008

Author (s), year of publication	Study area	Type of herd	Number of tested animals	Tests applied	Average infection rate (%)
Anomynous, 1970	Bouaké	Not specified	24	BPAT [a]	75% (53.3-90.2) [b]
Böhnel, 1971	Korhogo	Not specified	554	MRT	11.7% (9.2-14.7)
Coulibaly, 1973	Bouaké	Not specified	281	Not specified	23% (18.3-28.5)
Gidel *et al.*, 1974	Korhogo, Bouaké Katiola, Odienné, Man	Traditional and sedentary farms	1327	MRT	42,9 (23,0-51,0)
			749	SAW and CFT	15,5 (2,6-25,8)
Pilo-Moron *et al.*, 1979	Korhogo Bouaké, Abengourou Abidjan	Traditional and sedentary farms	12.343	SAW, RBT	10,1 (1,0 -39,3)
Camus, 1980a	Korhogo, Boundiali Odienné, Ferkessédougou, Bouna, Touba	Sedentary herds	1.214	RBT	28,3 (9,1-37,7) [c]
Angba *et al.*, 1987	National survey	Not specified	Not specified	SAW, RBT	11,3 (9,5-14,0)
Thys *et al.*, 2005 [d]	District of Abidjan (Bingerville, Azaguié)	Dairy farms	244	SAW-EDTA RBT, CFT, iELISA	3,6 (1,2-7,1)
		Traditional farms	137	SAW-EDTA, RBT, CFT, iELISA	4,3 (1,3-8,8)
Sanogo *et al.*, 2008 [d]	Bongouanou, Dimbokro, Tiébissou, Toumodi, Yamoussoukro	Traditional farms	660	SAW-EDTA, RBT, CFT, iELISA	8.8 (5.0-16.4)

[a] *The antigen used was a B. melitensis strain*
[b] *The prevalence range is presented within brackets*
[c] *Only cows were used for this estimation*
[d] *Infection rate presented by these authors are true prevalence*
MRT(Milk Ring Test) ; SAW (-EDTA)(Slow Agglutination of Wright(with EDTA) ;RBT(Rose Bengal Test), CFT(Complement Fixation Test) ; iELISA (indirect ELISA)

1.2.2.2. Disease in human

Brucellosis is among the most neglected zoonotic disease in the world (WHO, 2012; Mableson *et al.*, 2014). As any other zoonotic diseases, the occurrence of brucellosis in human in a given geographical region is related to the infectious status of animals (Godfroid *et al.*, 2005; Saegerman *et al.*, 2010). Infection of a given host by *Brucella* may occur either directly via ingestion, via inhalation of infected products or indirectly (**Figure 7)**.

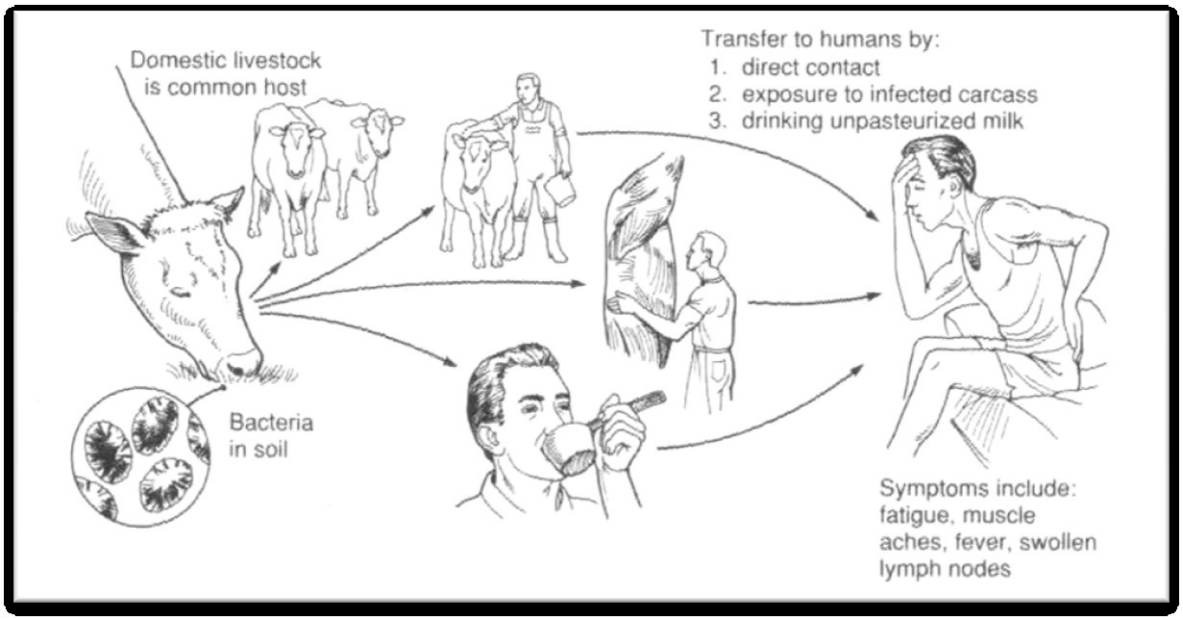

Figure 7: Main transmission routes of brucellosis from livestock to humans (by Sir David Bruce, 1855-1931) (from http://m2002.tripod.com/brucellosis.jpg)

Even if direct or close contact with aborted material or infected animal is required for transmission of *Brucella*, indirect transmission is possible through contaminated pasture, vehicles, feed or water (Roop *et al.*, 2003). Most of the time, the transmission of *Brucella* to human mainly occurs via the consumption of raw animal products and direct contact with infected animals, aborted tissues and discharges (Marcotty *et al.*, 2009; Saegerman *et al.*, 2010). Human brucellosis is mainly an occupational disease, affecting people who have contact with infected animals or their tissues such as farm workers, veterinarian, ranchers, and meat picking employees (OIE, 2009). Therefore, consumers of unpasteurized dairy products and hunters who unknowingly handle infected animals may also get brucellosis (Ocholi *et al.*, 2004; Arimi *et al.*, 2005). To a lesser extent, sexual transmission was also reported (Meltzer *et al.*, 2010) but not confirmed (Ron-Román *et al.*, 2012). So far, human

brucellosis is known to be caused by *B. melitensis, B. suis, B. abortus* and to a lesser extent by *B. canis* (Acha and Szyfres, 2005). A zoonotic potential has also been alleged for newly reported strains in marine mammals but further investigations are needed (Godfroid *et al.*, 2005). Humans are known to be more sensitive to infections caused by *B. melitensis and B. sui*s, especially biovars 1 and 3 for the later (Godfroid *et al.*, 2005; Saegerman *et al.*, 2010). *Brucella abortus* infections are relatively low pathogenic and usually develops an insidious subclinical form. The incidence of the disease in human is not well known but higher prevalences are reported in the Mediterranean region of Africa, Middle East, Latin America and Asia (Samartino *et al.*, 2005, Pappas *et al.*, 2006; Aznar *et al.*, 2014) (**Figure 8**).

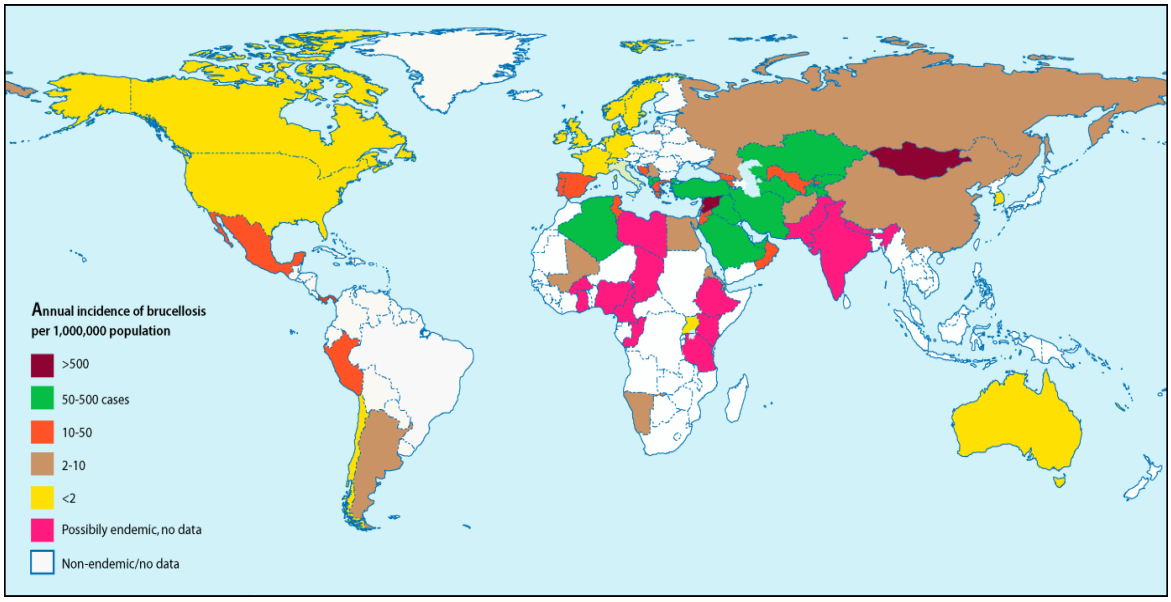

Figure 8: Global Incidence of Human Brucellosis (from Pappas *et al.*, 2006)

In West Africa, knowledge on the actual impact of the disease in humans remains limited (**Figure 8).** However, even if the actual incidence of the disease is not known, the presence of human brucellosis cannot be excluded since brucellosis stays neglected and under-reported in many African countries. This might be related to the non-specific clinical signs of human brucellosis, which could also be confused with other endemic febrile illness such as malaria and typhoid fever (McDermott and Arimi, 2002). Nevertheless, serological evidence of the presence of *Brucella* in humans has already been documented for many Western African countries like Benin, Burkina Faso, Ivory Coast, Guinea, Guinea Bissau, Mali, Togo and Nigeria (Pappas *et al.*, 2006; Akakpo *et al.*, 2010, Dean *et al.*, 2013). Available data on human brucellosis in Ivory Coast are scarce. In the 1970s, serological evidence of the presence of the disease in human was reported in northern and central region

of the country despite a low infection rate in animals (Gidel *et al.*, 1974). In 1985, brucellosis infection in human was estimated to be 6.52 % and 0.45% using respectively the intradermo-reaction with *Brucella* melitine as allergen and serology (Angba *et al.*, 1987).

1.3. Prevention and control of brucellosis

1.3.1. Diagnostic tools

Brucellae are facultative intracellular bacteria with a special tropism for the host reproductive system and may affect a wide range of hosts including human, domestic and wild animals. When transmission occurs, *Brucellae* initially invade regional lymph nodes. Then, after a brief bacteremia, they spread to other tissues and organs of the body, with a particular tropism for the reproductive tract (Olsen and Tatum, 2010). This results in an increased excretion of *Brucellae* during parturition particularly in aborted fetal fluid, vaginal discharges, placenta and milk. Due to the tropism and their proliferation in the reticulo-endothelial cells of the reproductive tract, *Brucellae* induce various clinical signs from birth of a viable but weak calf, placenta retention, metritis, subclinical mastitis, reduced milk production, infertility, orchitis or epididymitis with or without sterility to late term abortion at the first gestation (Acha and Szyfres, 2003). Among these clinical manifestations, abortion is the cardinal clinical sign commonly associated with brucellosis (Acha and Szyfres, 2003; Godfroid *et al.*, 2010). Joint colonization by *Brucella* may occur resulting in articular and peri-articular hygroma's. In Africa, the presence of hygroma in a herd is commonly associated with brucellosis (Thienpont *et al.*, 1961; Ocholi *et al.*, 2004; Bankole *et al.*, 2010; Saegerman *et al.*, 2010). However, none of these clinical signs is specific for brucellosis. Therefore, the use of laboratory techniques remains essential to improve the accuracy of the diagnosis. Two main types of laboratory techniques could be used for the diagnosis of brucellosis: on the one hand, tests which allow a direct detection of the presence of *Brucella* (bacteriology, molecular methods) and on the other hand, tests which detect this presence indirectly through detection of the immune response of the host to *Brucella* antigens (mainly serological and intradermic methods).

Diagnostic tests are useful to determine the disease status at individual level or group of individual within a population of interest. They are also crucial for studying the epidemiology of the disease, to assess the actual impact of disease (Greiner and Gardner, 2000). Diagnostic assays for brucellosis were thoroughly described and reviewed in the OIE manual of

diagnostic tests and vaccines for terrestrial animals (OIE, 2009) and by several authors (Nielsen, 2002; Saegerman *et al.*, 2010; Godfroid *et al.*, 2010; Yu and Nielsen, 2010). A brief overview of the different methods is presented in the following sections.

1.3.1.1. Direct diagnostic methods

1.3.1.1.1. Bacteriological examination

Morphological, staining and cultural characteristics of *Brucella* may be used for a direct identification of its presence. Despite its lack of specificity, examination of stained smears from abortive material or suspicious organs or fluid can provide valuable information. However, definitive diagnostic is made by culture (Yu and Nielsen, 2010). According to clinical signs, a range of samples is available including fetal membranes, vaginal secretions, milk, semen, arthritis or hygroma fluids, lymph nodes, spleen, uterus, udder, testes, epididymes, joint exudates, abscesses and other tissues of infected cattle. The stomach content, spleen and lungs of aborted fetuses may also be used for bacteriological examination (Alton *et al.*, 1988; Corbel, 2006, OIE, 2009; Godfroid *et al.*, 2010). In case of abortion caused by brucellosis, concentrations of *Brucella* in fetal fluids or placenta may reach 10^9 to 10^{10} colony-forming units (CFUs)/g compared to an estimated minimum infectious dose of 10^3 to 10^4 CFU (Olsen and Tatum, 2010; Saegerman *et al.*, 2010). *Brucellae* may also be spread from infected udders and supramammary lymph nodes into milk at concentrations going from a few hundred up to $2x10^6$ organisms/ml of milk (Corbel, 1988). Therefore milk, mammary glands and associated lymph nodes can be used as samples (Xavier *et al.*, 2009; O'Grady *et al.*, 2014). In Africa, the use of hygroma fluid as sample for *Brucella* isolation and identification is common (Ocholi *et al.*, 2004; OIE, 2009; Bankole *et al.*, 2010). Isolation of *Brucella* is considered as the "gold standard" reference test to determine the status of the animal regarding brucellosis (Godfroid *et al.*, 2010; Yu and Nielsen, 2010). Nevertheless, implementation of bacteriological methods is laborious, time consuming, costly and requires enhanced biosafety and biosecurity measures. The risk for human health implies that handling of *Brucella* be done careful and restricted to laboratories with appropriate containment facilities like biosafety level 3 (BSL3) laboratories (OIE, 2009). Thus, despite their usefulness for the detection of *Brucella*, the implementation of these methods is limited in developing countries. The main different characteristics used to differentiate among *Brucella* species using bacteriological examination are summarized in **Table III**.

Table III: Characteristics for differentiating the different *Brucella* (*from OIE, 2009*)

Species	Colony morphology[b]	Serum requirement	Lysis by phages[a]					Oxidase	Urease activity	Preferred host
			Tb		Wb	Iz_1	R/C			
			RTD[c]	10^4RTD	RTD	RTD	RTD			
B. abortus	S	–[d]	+	+	+	+	–	+[e]	+[f]	Cattle and other Bovidae
B. suis	S	–	–	+	+[g]	+[g]	–	+	+[h]	Biovar 1: swine Biovar 2: swine, hare Biovar 3: swine Biovar 4: reindeer Biovar 5: wild rodents
B. melitensis	S	–	–	–	–[i]	+	–	+	+[j]	Sheep and goats
B. neotomae	S	–	–[k]	+	+	+	–	–	+[h]	Desert wood rat[l]
B. ovis	R	+	–	–	–	–	+	–	–	Rams
B. canis	R	–	–	–	–	–	+	+	+[h]	Dogs
B. ceti	S		+[m]		+[n]	+[o]	–	+	+	Cetaceans
B. pinnipedialis	S		+[m]		+[n]	+[o]	–	+	+	Pinnipeds
B. microti	S	–	–	+	+	+	–	+	+	Common vole

From refs 1, 39.

a Phages: Tbilisi (Tb), Weybridge (Wb), Izatnagar1(Iz_1) and R/C
b Normally occurring phase: S: smooth, R: rough
c RTD: routine test dilution
d *B. abortus* biovar 2 generally requires serum for growth on primary isolation
e Some African isolates of *B. abortus* biovar 3 are negative
f Intermediate rate, except strain 544 and some field strains that are negative
g Some isolates of *B. suis* biovar 2 are not or partially lysed by phage Wb or Iz_1
h Rapid rate
i Some isolates are lysed by phage Wb
j Slow rate, except some strains that are rapid
k Minute plaques
l *Neotoma lepida*
m Some isolates are lysed by Tb
n Most isolates are lysed by Wb
o Most isolates are lysed by Iz

1.3.1.1.2. Molecular methods

Compared to bacteriological methods, molecular based methods to detect *Brucella* are considered less fastidious, less time consuming and with reduced risk of manipulation. Even if detection of antibodies produced against *Brucella* is indicative of the presence of brucellosis, identification and typing of the disease-causing agents provides the ultimate evidence of the actual presence of the disease (Nielsen, 2002). Molecular methods are based on the detection of *Brucella* DNA and therefore provide actual evidence of the latter (Yu and Nielsen, 2010). Since 1987, many Polymerase Chain Reaction (PCR) based methods were developed throughout the years for the diagnosis and identification of *Brucella* (Bricker and Halling, 1994; Bricker, 2002; Whatemore, 2009; Yu and Nielsen, 2010; Godfroid *et al.*, 2011, Scholz and Vergnaud, 2013). It includes i.e., Abortus-Melitensis-Ovis-Suis (AMOS) PCR, conventional multiplex PCR, single nucleotide polymorphism (SNP) analysis and multilocus sequence typing (MLST) or multilocus sequence analysis (MLSA) . They can be used either for diagnostic purposes or for biotyping. Molecular methods are useful and convenient to characterize circulating biovars of *Brucella* and for epidemiological investigations particularly when a higher discriminatory power is needed (Bricker *et al.*, 2003; Cutler *et al.*, 2005; Le Flèche *et al.*, 2006). Molecular methods can also be used as a complementary test for other tests (Adone *et al.*, 2001; Bricker, 2002). Yu and Nielsen (2010) published a broad overview on molecular methods for detection of *Brucella*, including highly discriminative methods such as Multiple loci Variable number tandem repeats Analysis (MLVA). More discriminative molecular methods are under development with recently available methods like the whole genome sequencing (WGS) and the global genome-wide Single Nucleotide Polymorphism (SNP) analysis.

1.3.1.1.3. Maldi-TOF Mass Spectrometry (MALDI-TOF-MS)

The Matrix-assisted laser desorption/ionization time-offlight mass spectrometry (MALDI-TOF-MS) is recognized as a reliable method for identification of *Brucella* at genus level from culture plates samples and directly from blood culture bottles (Ferreira *et al.,* 2010; Lista *et al.,* 2011, Kasymbekov *et al.*, 2013). It is considered as a fast, cost-effective and accurate method, which is suitable for the high-throughput identification of bacteria by less-skilled laboratory personnel (Seng *et al.*, 2009). Identification of bacteria is done by comparing the obtained MS spectra to the MS spectra or profiles from a reference library,

which constitutes the main limiting factor of the enhanced identification of *Brucella* using this method so far (Lista *et al.*, 2011).

1.3.1.2. Indirect diagnostic methods

1.3.1.2.1. Serological methods

When a host is exposed to *Brucella*, the immune system induces the production of different types of immunoglobulins (Ig) regardless the *Brucella* species. This immune response is induced by the presence of surface lipopolysaccharides (LPS) on the outer cell membrane of *Brucella*, which contains the O chain, the immunodominant antigen[6] (Alton *et al.*, 1988). In cattle, the first antibody response is the production of IgM at a larger (or less) and persistent amount (2-3 weeks post-exposure) according to the dose of bacteria, the route of infection and the status of the animal infected. However, IgM may disappear after a few months (Godfroid *et al.*, 2002; Saegerman *et al.*, 2004; Godfroid *et al.*, 2010). Then, the production of IgG_1 arrives very shortly after the IgM, followed later by IgG_2 and IgA (Nielsen, 2002; Yu and Nielsen, 2010). The presence of these different antibodies can therefore be useful to evidence of the presence of *Brucella* and serological diagnostic tools can be use to detect them. Compared to bacteriological examination, detection of antibodies appears to be a more convenient approach for the diagnosis of brucellosis, since they are less fastidious, easier to implement and more suitable for large-scale investigation.

Because the immunodominant epitope on the *Brucella* LPS is the basis of the serodiagnosis of brucellosis, most of the conventional serological tests may suffer from some limitations since the immunodominant antigen is also present in many other Gram-negative bacteria. Like *Brucella,* these bacteria induce the production of identical antibodies resulting in cross-reactions or false positive serological reactions (FPSR) when testing. These false positive serological reactions (FPSR) were reported with Gram-negative bacteria such as (e.g.) *Yersinia enterocolitica O: 9, Escherichia coli O: 157, Francisella tularensis,*

[6] *The immunodominant O-polysaccharide (OPS) which has been chemically defined as a homopolymer of 4,6-dideoxy-4-formamide-alpha-D-mannose linked via glycosidic linkages is common in Smooth Brucella strains but is lacking in Rough strains (i.e., B. ovis and B. canis). As a result, B. abortus antigen in the form of whole cells, SLPS or OPS is used as antigen for serological detection of the smooth strains while RLPS is commonly used as the main antigen for detection of antibody for the latters (Yu and Nielsen, 2010).*

Salmonella urbana O: 30, Vibrio cholera. The presence of possible FPSR bacteria become an important issue especially in free brucellosis areas, countries with low incidence or in the last stages of an eradication program (Saegerman *et al.*, 2004; Munoz *et al.*, 2005; Cutler *et al.*, 2005; Corbel, 2006; Yu and Nielsen, 2010). In addition, vaccination with strain S19 (also named B19) may also cause interference in serological reactions. Consequently, the application of serological tests has to be related with the incidence of the disease and vaccination status in order to prevent misinterpretation (Corbel, 2006). The use of a given serological test for the diagnosis of brucellosis should therefore take into account of the epidemiological context (Robinson, 2003). A description of the different types of antibodies detected by the different serological assays is presented in **Table IV**. Different serological methods for the diagnosis of brucellosis have been developed over the years. These include the agglutination tests, complement fixation tests, primary binding tests and hypersensitivity tests (Nielsen, 2002, Cutler *et al.*, 2005).

Table IV: Types of antibodies detected by conventional serological assays for the diagnostic of brucellosis (from Quinn *et al.*, 1999 and Saegerman, 2004)

Type of Sample	Tests	Type of antibodies			
		IgM	IgA	IgG_1	IgG_2
Blood	SAW (with EDTA)	+	-	±	+
	RBPT	+	-	+	-
	CFT	+	-	+	-
	iELISA[a]	-	-	+	+
Milk	MRT	+	+	±	±
	iELISA	-	-	+	+

+/-/± : serological response ; MRT (Milk Ring Test) ; SAW (-EDTA)(Slow agglutination test of Wright (with EDTA) ; RBPT (Rose Bengal Plate Test), CFT (Complement fixation test) ; iELISA (Indirect Enzyme linked immunosorbent assays); [a] Detection of IgG_1 and/or IgG_2 would depend on the conjugate used

1.3.1.2.1.1. Sero-agglutination of Wright (SAW)

The Sero-agglutination of Wright (SAW) or Slow Agglutination Test (SAT) is the oldest diagnostic test of brucellosis. It is an inexpensive and relatively easy to implement semi-quantitative test. Different types of SAW exist, from the simple SAW to the SAW-EDTA where EDTA is added to enhance the specificity of the test by inactivating some non-

specific IgM (Alton *et al.*, 1988; Corbel, 2006). It is susceptible to false positive reactions by cross-reacting antibodies.

1.3.1.2.1.2. Rose Bengal Test (RBT)

The Rose Bengal Test (RBT) is an agglutination test between agglutinating IgM immunoglobulins and a colored antigen suspended in an acid buffer (pH 3.65) to prevent reaction of non-specific agglutinins. It can be used for diagnosis at the herd level and particularly for the sero-surveillance of cattle herds in brucellosis free areas. It is a quite sensitive and relatively simple test to apply (OIE, 2009). Although, it is a good screening test for brucellosis, it is unable to distinguish vaccinated from infected animals (Corbel, 2006) and can produce false positive serological reactions. It is a prescribed test for trade by OIE (OIE, 2009).

1.3.1.2.1.3. Complement Fixation Test (CFT)

The Complement Fixation Test (CFT) is based on the activation of the complement in the presence or not of the antigen-antibody complex. This presence of the complex is detected using a hemolytic system. This test is quite specific even if false-positive reactions may still occur. Despite its status of prescribed test by the OIE for trade (OIE, 2009), it is a difficult and complicated test to implement (Alton *et al.*, 1988). Moreover, it is not well standardized and anti-complementary reactions (due to bacterial contamination of the sera, or other factors) sometimes make the interpretation of the results difficult (Corbel, 2006).

1.3.1.2.1.4. Enzyme Linked Immunosorbent Assays

The indirect Enzyme Linked Immunosorbent Assays (iELISA) use a purified Smooth LPS coated on a polystyrene matrix as an antigen (Nielsen, 2002; Saegerman *et al.*, 2004; Godfroid *et al.*, 2010). It allows an indirect detection of antibodies against the *Brucella* antigen using various conjugates with enzymes. Indirect ELISA techniques are considered as very sensitive tests but do not allow to distinguish between post-vaccination and post-infective antibodies (OIE 2009). Therefore, competitive ELISAs were developed to improve the test specificity (Nielsen *et al.*, 1996a; Portanti *et al.*, 2006) but they are less sensitive compared to indirect ELISAs (Nielsen *et al.*, 1995; Samartino *et al.*, 1999a). Competitive ELISAs are helpful to eliminate false positives and to discriminate between post-vaccination

antibodies due to the vaccine strain S19 and post-infection antibodies (Weynants *et al.*, 1997). These tests are prescribed for trade by the OIE (OIE, 2009).

1.3.1.2.1.5. Fluorescence Polarization Assay (FPA)

The FPA is a highly sensitive, specific, rapid and easy to implement test (Gall and Nielsen, 2004). This method consist in the blank reading of the diluted sample in a fluorescence polization analyser, addition of an antigen labeled with a fluorochrome and final reading after two minutes of incubation (Samartino *et al.*, 1999b). It is based on the rotational differences between molecules in solution, the smaller rotating randomly at a rapid rate and resulting in a rapid depolarization of light while the larger will rotate slower and depolarize light at reduced rate (Nielsen and Gall, 2001). Depending on the presence or absence of an antigen-antibody complex, the size of the molecules in the solution will increase (or not) and therefore, its rate of rotation will be proportionally reduced (or not). For brucellosis, a small subunit of the O Polysaccarhide (OPS) of *B. abortus* strain 1119-3 smooth Lipopolysaccharide (sLPS) conjugated with fluorescein isothiocyanate is added to diluted serum, milk or whole blood sample. The test result is obtained by measuring the time of rotation in a given angle with a polarized light after incubation (OIE, 2009). The specificity of the FPA is quite high even in cattle herds vaccinated with strain 19 (Nielsen *et al.*, 1996b; Gall *et al.*, 2000; Gall *et al.*, 2001). It is a prescribed test by OIE for trade (OIE, 2009).

1.3.1.2.1.6. *Brucella* Immunochromatographic Lateral Flow Assays (LFA)

The LFA is a rapid test initially developed for the diagnostic of human brucellosis (Smits *et al.*, 2003; Irmak *et al.*, 2004, Franco *et al.*, 2007). It was later adapted for the serodiagnosis of brucellosis in different livestock species including cattle, sheep, goat and pigs (Abdoel *et al.*, 2008). It is a simple test also based on the detection of IgM/IgG against *Brucella* LPS. As in other serological tests, cross-reaction may also occur. LFA does not require a specific expertise, expensive equipment, electricity and refrigeration, or a specific training. It is also argued to be suitable for remote areas where access to adequate laboratory facilities is problematic or when testing animals from nomadic or other migratory livestock keepers (Abdoel *et al.*, 2008, Bronsvoort *et al.*, 2009).

1.3.1.2.1.7. Milk testing assays

Serological diagnostic tests applied to milk are good and practical means for screening in the dairy sector. They can be easily implemented in milk collection centers. In this case, when the test is positive, it implies that all cows which are in production and were milked, be tested using other serological tests. The "milk iELISA" is a very sensitive and specific test. The Milk Ring Test (MRT), an adaptation of the agglutination test for milk, is also a good alternative test in the absence of ELISA because it is very cheap (OIE, 2009). False positive reactions are common with MRT especially in brucellosis free areas (Corbel, 2006).

1.3.1.2.2. Cellular methods

1.3.1.2.2.1. Skin Delayed-type Hypersensibility Test or Skin Test

The “Skin Test” or intradermal test with brucellin is based on the hypersensitivity or allergic inflammatory reaction of the host after an intradermal injection with a protein extract of a rough strain of *Brucella* (Saegerman *et al.*, 1999; Godfroid *et al.*, 2010). This test is based on the specific cell-mediated immunity against *Brucella* and is able to identify latent carriers and to discriminate false positive serological reactions due to *Yersinia enterocolitica O: 9*, when associated with other serological tests (Saegerman *et al.*, 1999; Bercovich, 2000). It is highly specific with some disadvantages such as the inability to discriminate between infected and vaccinated animals, and the need for at least two visits with an interval of 2 to 3 days to get the result (Weynants *et al.*, 1995; Cutler *et al.*, 2005). It is prescribed as an alternative test by the OIE (OIE, 2009).

1.3.1.2.2.2. Antigen-Specific Gamma Interferon Production Test

The antigen-specific Gamma interferon production test is an *in vitro* test developed in order to improve the diagnostic specificity of bovine brucellosis. It is based on the quantification of the Gamma interferon (γ-IFN) produced in response to antigenic stimulation. It is a delayed-type hypersensitivity test similar to the "skin test" but with a lower specificity. It can be used as a complementary test with others serological assays (Weynants *et al.*, 1995).

1.3.1.3. Identification and typing methods

Results of identification and typing of *Brucella* are useful to have a better knowledge of the epidemiology of the disease to manage disease outbreaks, to identify appropriate antigens and to test and set up efficient preventive and control measures (Crawford *et al.*, 1979; Ica *et al.*, 1998; Saegerman *et al.*, 2010; Godfroid *et al.*, 2010). At national and at regional levels, identification and typing results from infected animals are helpful to assess potential threats for public health since animal hosts are the source of human brucellosis infections. Despite their high genetic relation, application of both bacteriological and molecular typing methods may be used for identification and typing of *Brucella* (Scholz and Vergnaud, 2013) as discussed above. However, differentiation among species and biovars is sometimes complicated because of the existence of strains showing atypical characteristics (Acha and Zsyfres, 2003; Scholz and Vergnaud, 2013). Handling and biotyping of *Brucella* also requires facilities, equipment and technical skills that are not always available in diagnostic laboratories in Africa, limiting the availability of data on prevailing strains of *Brucella* (Samartino *et al.*, 2005).

1.3.2. Prevention and control measures

Given that brucellosis is a zoonotic disease, there is a correlation between human and animal brucellosis. Prevention of brucellosis in human mainly depends on the control of the disease in the animal hosts (Godfroid *et al.*, 2005; Pappas *et al.*, 2006; Saegerman *et al.*, 2010). Different strategies for controlling brucellosis exist and have been applied in different part of the world (Benkirane, 2001; Godfroid and Kasbohrer, 2002; Ragan, 2002; Samartino, 2002; Poester *et al.*, 2002; Rivera *et al*., 2002; McDermott and Arimi, 2002; Saegerman *et al.*, 2010, FAO, 2013). The aim of these strategies is to prevent the spread of the infection, to reduce the risk of abortion and to increase the herd or population immunity. Strategies for controlling brucellosis could include measures as appropriate herd management (Samartino *et al.*, 2005), vaccination of the susceptible population, slaughtering of the animals recognized positive to testing (Benkirane, 2001), and increase of public awareness and education of population at risk (Robinson, 2003). All these measures could be applied separately or in combination but need to be backed up by appropriate regulations or legislation. An efficient control strategy need to consider some key elements like the true prevalence of the disease (**Table V**), livestock management system, organization of the veterinary services, implication of policymakers and communities (stakeholders), availability of resources to sustain control

measures, and the intersectoral collaboration between veterinary services and public health actors (Benkirane, 2001; Saegerman *et al.*, 2010). In many developed countries, control programs including measures such as test-and-slaughter with compensation for farmers, accreditation and financial incitation for disease-free herds have been successfully applied to control brucellosis (Saegerman *et al.*, 2010). Many countries such as Australia, Belgium, Canada, Cyprus, Denmark, Finland, the Netherlands, New Zealand, Norway, Sweden, and the United Kingdom have been declared free from bovine brucellosis. In developing countries, despite its known endemicity, its socioeconomic impacts and the beneficial effect of possible control measures, resources allocated to control of brucellosis are declining or absent. Most of the time, vaccination is the only mean applied for the control of animal brucellosis in these countries (McDermott and Arimi, 2002).

Table V : Control strategies of brucellosis according to the epidemiological status (adapted from Benkirane, 2001; Saegerman *et al.*, 2010)

Epidemiological status	Control strategy and associated measures	Monitoring method	Outcome/ next step
A: High prevalence in animals; high clinical incidence in humans	- Mass vaccination - Support to veterinary services - Rational use of available resources - Movement control	- Serology - Bacteriology - Monitoring the incidence of human cases	Go to **B**
B: Moderate prevalence	- Combined prophylaxis	- Counting and identification of animal - Serology control - Bacteriological monitoring - Communication/sensitization/education - Intersectoral collaboration with human health services	Go to **C**
C: Low prevalence (<1%)	- Sanitary prophylaxis	- Monitoring in farms and slaughter houses - Serological monitoring - Survey in target groups	Reach **D**
D: Absence of the disease	- Movement control	- Monitoring of risk indicators	Preserve this status

1.3.2.1. Communication and education for prevention

Communication and education of public at risk are considered as a key component to increase the awareness of the disease, to prevent its spread and to reduce occupational and food-borne risk linked to zoonotic diseases such as brucellosis (Robinson, 2003). Education and sensitizing should be undertaken to prevent the consumption of unpasteurized milk and milk derivatives (Samartino *et al.*, 2005). Populations with cultural habits encouraging the consumption of milk and the use of its products raw or poorly cooked are highly at risk and should be sensitized in priority. Since the disease is likely to be transmitted in a context where people have close contact with the animal host, hygienic and biosecurity measures during handling and disposal of afterbirths- especially in cases of abortion - should also be taught and encouraged, particularly among professionals at higher risk like hunters, farmers, butchers, and veterinarians (Corbel, 2006; Saegerman *et al.*, 2012).

1.3.2.2. Prophylactic measures

Since *Brucellae* are facultative intracellular organisms, the effectiveness of antibiotherapy is limited. Furthermore, the use of antibiotics for the treatment of brucellosis would require the use of massive doses, increasing the risk of antibiotic residues and resistance dissemination to humans through the food chain. Implementation of preventive medical measures, e.g. vaccination, is therefore a key component for the prevention/control of brucellosis. Vaccination is used to increase the resistance of susceptible animals to infection, to reduce the expression of clinical signs and to diminish the excretion of *Brucella* by infected animals (Corbel, 2006). In many countries, it was adopted as the most practical and economical way for controlling animal brucellosis (McDermott and Arimi, 2002; Aznar *et al.*, 2014).

In Ivory Coast, vaccination was used between 1978 to 1982 during a control program conducted by the SODEPRA. Females from 1 to 10 years of age were vaccinated at primo-vaccination. Then, non-pregnant females of one to two years old were vaccinated every year. About 300,000 females have been vaccinated in the north and the centre of the country (Angba *et al.*, 1987) using mostly H38, but also B19 vaccine strains. The campaign led to the reduction of abortion and mortality rate up to more than 37% (Camus, 1980a; Camus, 1980b; Angba *et al.*, 1987). Because of the resurgence of brucellosis, female calves of 4 to 8 months of age and non-pregnant cows in dairy farms were vaccinated again in 1992 using a single

dose sub-cutaneaous injection as previous years (Camus, 1995). With the privatization of SODEPRA in 1993, vaccination activities were transferred to the private sector and were progressively abandoned, farmers being henceforth asked to pay for vaccination. Currently, there is no official control program or official vaccination against brucellosis in Ivory Coast.

When vaccination is applied for the control of brucellosis, there might be some disadvantages such as its possible interference with most diagnostic tests (serological and hypersensitivity). In cattle, the use of S19 vaccine (smooth attenuated strain of *B. abortus*) is recommended but is not effective in protecting animals against infections with *B. melitensis* (Corbel, 1997). The RB51 vaccine (rough attenuated strain of *B. abortus*) also gives satisfaction and seems to interfere very little with serological tests (Schurig *et al.*, 2002). Despite a lower efficiency compared to the S19 strain, RB51 vaccine is preferred over the S19 in several Latin American countries (Corbel, 2006).

In addition to medical prophylactic measures, sanitary measures can also be used to prevent the introduction and the spread of the disease in a given population. For brucellosis, these include hygiene, containment and animal movement control. In addition, the use of appropriate and accurate diagnostic tests, allow to identify and eliminate infected or test-positive individuals. Aiming to prevent the spread of the disease, elimination may imply the slaughtering of positive tested animals (test-and-slaughter). The efficiency of these methods depends on the epidemiological context, the availability of sustainable resources and appropriate regulation. In many developed countries, test-and-slaughter was applied in a control strategy for brucellosis, in combination with compensation of farmers, accreditation and financial incentives for disease-free herds (Saegerman *et al.*, 2010, Godfroid *et al.*, 2013). Also known as stamping out method, test-and-slaughter is generally implemented in association with vaccination (Corbel, 1997). In effect, vaccination is first used to prevent or control the infection among infected host, and then it is gradually restricted while test-and-slaughter is implemented to eliminate the infection. Availability of an appropriate financial compensation scheme is the main limiting factor of the implementation and the success of any control program including test-and-slaughter policy (Seleem *et al.*, 2010; Godfroid *et al.*, 2013), particularly in low resource countries. The success of the application of this measure is unlikely if the herd level prevalence is more than 2% (Corbel *et al.*, 2006).

1.3.2.3. Intersectoral collaboration for control

The efficiency of prevention and control measures of zoonotic diseases like brucellosis requires the implication and the collaboration of both animal and human health sector. It is

expected to ensure joint administrative arrangements, facilitate cross-notification of cases, as well as coordinated investigations, surveillance and prevention/control activities, and public health education programs (Corbel, 2006). Such collaboration should be encouraged at both national and regional level, in order to put together limited resources and capabilities for an efficient control. Thus, emerging concepts such as the "*One health approach*" can be considered as an opportunity to improve human health and well-being through an integrated management of pathogens as *Brucella spp* in both humans and domestic animals (Saegerman *et al.*, 2010; Saegerman *et al.*, 2012; Marcotty *et al.*, 2013). This approach is expected to be particularly beneficial in low resource societies where different disciplines could be combined to improve the strength of the surveillance and the control of infectious diseases like brucellosis.

CHAPTER 2:

EPIDEMIOLOGICAL CONCEPTS AND METHODOLOGIES

CHAPTER 2: EPIDEMIOLOGICAL CONCEPTS AND METHODOLOGIES

This chapter describes the epidemiological tools used in the thesis.

2.1. Systematic review and meta-analysis

Reliable and good quality data or information are essential to support decision-making and for answering urgent questions. Most of the time, such data or information are provided by systematic review or meta-analysis. They gather data or results from several studies into a single synthesis (Montori and Guyatt, 2003, Leeflang *et al.*, 2008). Synthesizing results from several studies can be done in many ways but not all of these are scientifically robust (Honest and Khan, 2002).

Systematic reviews allow a synthesis of relevant studies by applying scientific strategies that limit biases (Wright *et al.*, 2007). A systematic review is a review of a clearly formulated question that uses systematic and explicit methods to identify, select, and critically appraise relevant research, and to collect and analyze data from the studies that are included in the review (Moher *et al.*, 2009). Thus, when doing a systematic review, the author(s) should *i*) address a defined question; *ii*) conduct a detailed and exhaustive search for relevant studies; *iii*) include studies of high methodological quality; and *iv*) use reproducible approaches to assess the limitations in the methodological quality of the studies on which they focus (Montori and Guyatt, 2003). When similar individual studies are summarized, they can be pooled together and analysed statistically using a meta-analysis (Deeks, 2001; Gatsonis and Paliwal, 2006; Wright *et al.*, 2007). Applications of recommended guidelines are useful to ensure good quality of both systematic reviews and meta-analysis (Moher *et al.*, 2009).

2.2. Logistic regression analysis

A range of statistical methods is available to analyze data from epidemiological studies according to the objectives. When epidemiological studies are aiming to demonstrate or identify relationships between different factors or variables of interest, regression methods are mostly used (Lewis and Michael, 2013). These methods are helpful to identify and describe potential associations that might exist between variables of interest such as the serological status of an animal (known as dependent response or outcome variable) and the sex and the age of this animal (independent predictive variables or explicative variables). When the outcome or the response variable is dichotomous such as disease status

(seropositive or seronegative), the logistic regression modeling was applied. It is therefore used to identify statistical associations among variables of interest and to identify variables that might be relevant for disease control (Lewis and Michael, 2013).

Logistic regression is among the most important regression techniques in epidemiology (Stephen, 2001). It is the most appropriate modeling approach to describe and to test hypotheses about relationships between a categorical outcome and one or more categorical or continuous predictor variables. Dohoo *et al.* (2003) provided more discussions on logistic regression modeling. Briefly, estimations in logistic regression modeling are obtained through a Maximum Likelihood Estimation process. In this later approach, the coefficients in the model represent the amount of the logit of the probability of the outcome changes with a unit increase in the predictor variable. Since these coefficients are hard to interpret, they are commonly expressed as odds ratios (OR). When, the predictor variable is continuous, the OR represents the factor by which the odds of outcome variable are increased (or decreased) for each one-unit change in the predictive variable. When, the predictor is a categorical variable, the coefficient for each level of the variable represents the effect of that level compared to the category (i.e. the 'baseline') not included in the model (Dohoo *et al.*, 2003).

2.3. Determination of disease status

Determining the status of a given individual regarding a given condition could be helpful for many purposes. Knowledge of a disease status can be used to support decision for diagnosis or for treatment. Identification of the status of an individual in a given population could also be required to assess a new diagnostic test. Determining a disease status implies the use of appropriate diagnostic tools or tests. When the diagnostic test is able to determine the disease status of an individual with 100% accuracy, it is considered as the gold standard test. In practice, gold standard tests are seldom available due to many factors including the biological variability of each individual. Therefore, disease status has to rely on so-called “bronze” test or another test closest to the standard test. As an alternative to the absence of a gold standard test, a combination of tests could also be used to improve the diagnostic performance or obtain a gold standard effect (Black and Craig, 2002). This is often the case with brucellosis for which an unequivocal diagnosis can be made only with the isolation and identification of *Brucella* (OIE, 2009). However, isolation and identification methods as *Brucella* culture are not always available or feasible in common diagnostic conditions contrary to serological tests (Nielsen, 2002; Godfroid *et al.*, 2010). In addition, the

probability of finding *Brucella* spp. can decrease one month after the parturition as previously assessed (Saegerman *et al.*, 2004).

When a combination of tests is used, results can be interpreted in series or in parallel according to the objectives of testing. With serial interpretation, only animals that give positive results to both tests are considered positive. Consequently, this increase the diagnostic specificity (Sp) and decrease the diagnostic sensitivity (Se). With parallel interpretation, animals that give a positive result to one of the tests or to both tests are considered positive. Conversely to serial interpretation, parallel testing increases *Se* and decreases *Sp*. Depending on the fact that diagnostic tests target the similar biological phenomenon or not, combined tests could be considered as either dependent or independent. The combined tests may be correlated if they measure or target a similar biological phenomenon such as immunoglobulins (Gardner *et al.*, 2000; Dendukuri and Joseph, 2001). When two tests are combined, the presence of a positive dependence would respectively reduce the test sensitivity value in a parallel interpretation scheme and the test specificity value in a serial interpretation scheme compared with values expected if tests were conditionally independent (Gardner *et al.*, 2000). More discussions on test dependence issues are provided in further sections.

2.4. Estimation of disease true prevalence and performance of diagnostic tests

2.4.1. Estimation of disease true prevalence

Disease prevalence is a key parameter to assess the impact of a disease in the population of interest and for estimating the disease burden (Speybroeck *et al.*, 2012a). To determine the actual level of a disease in a population of interest, the true prevalence needs to be estimated (Dohoo *et al.*, 2003). Accuracy of true prevalence is related to performance parameters of tests to be applied (Ihorst *et al.*, 2007). Assuming that the sensitivity (Se) and the specificity (Sp) of a given diagnostic test are known, and AP being the apparent prevalence resulting from the application of the test in the population of interest, the true prevalence (P) can be determined using the following formula (Rogan and Gladen, 1978):

$$P = \frac{AP + Sp - 1}{Se + Sp - 1}$$

The estimation of the disease true prevalence is then straightforward with this formula when a gold standard test is available or fixed and known values are assumed for test characteristics (Dohoo *et al.*, 2003; Berkvens *et al.*, 2006). In practice, fixed and known values assumption may be unrealistic. Moreover, a straightforward application of this formula may result into estimates exceeding 1 (one) or may yield negative values (Spybroeck *et al.*, 2012a; Lewis and Torgerson, 2012). In addition, perfect reference tests are hardly ever available since diagnostic performance of any test is known to be influenced by several endogenous and exogenous factors and should be considered as context-specific parameters (Saegerman *et al.*, 2004; Berkvens *et al.*, 2006; Rutjes *et al.*, 2007). All these elements imply that imperfect tests should be used for disease prevalence estimation. Additionally, the use of multiple imperfect tests for estimation is suggested to reduce misclassification errors. So, appropriate methods and assumptions should be used to get unbiased estimates (Enøe *et al.*, 2000; Berkvens *et al.*, 2006).

2.4.2. Assessment of performance of diagnostic tests

The performance and the accuracy of all diagnostic assays need to be determined under routine conditions. This includes the estimation of parameters measuring the accuracy and the diagnostic performance of assays. While the reproducibility of a test measures the degree of agreement between test results when the conditions for testing or measurement change (e.g., two operators or laboratory technicians or two laboratories), the repeatability expresses the similarity of the test results in the same conditions (OIE, 2013). The robustness of an assay is another parameter referring to the assay's capacity to remain unaffected by minor variations (e.g., pH, temperature of reagents, brand of microtiter plates) while using an assay in the single laboratory conditions.

During this thesis work, agreement between test results was assessed using indexes of agreement as indicator and sensitivity and specifity as diagnostic test performance parameters.

2.4.2.1. Indicators of agreement between tests

In order to increase the diagnostic performance, to assess a new diagnostic test or to evaluate test characteristics, two diagnostic tests can be used in combination. Different indicators can be used to assess the agreement between the results of the different tests. The most commonly applied is the kappa coefficient of agreement (K). It is the corrected index of

the agreement between the results of two diagnostic tests. It is calculated as the ratio of the observed excess over chance agreement to the maximum possible excess over chance. The kappa coefficient is equal to 1 (100%) when there is perfect agreement and it takes the value of zero when the observed agreement is equal to the chance agreement (Dohoo *et al.*, 2003). However, the kappa coefficient is under the influence of the prevalence. Moreover, it was noticed that despite a high concordance between two tests, the kappa coefficient may paradoxally be low (Feinstein and Cicchetti, 1990; Cicchetti and Feinstein, 1990). To solve paradoxes with kappa coefficient, two indexes, e.g., the positive and negative index of agreement were proposed to measure the level of agreement between two tests (Cicchetti and Feinstein, 1990; Graham and Bull, 1998). These indexes represent respectively the observed agreement proportion for positive and negative results. Using the contingency table (**Table VI**), the two indexes of positive agreement (P_{pos}) and negative agreement (P_{neg}) are respectively:

$$p_{pos} = \frac{2a}{2a+b+c} \qquad \text{and} \qquad p_{neg} = \frac{2d}{2d+b+c}$$

Where P_{pos} and P_{neg} are respectively the indexes of positive agreement and negative agreement; a, b, c and d are given in the contingency table.

Table VI : Contingency table showing results for two diagnostic tests (Test 1 and Test 2)

		Test 1		
		pos	**neg**	**Total**
Test 2	**pos**	a	b	a+b
	neg	c	d	c+d
	Total	a+c	b+d	N

pos: positive result; neg: negative result

2.4.2.2. Performance parameters of diagnostic tests

In both human and animal health, diagnostic tests are useful tools to determine the true disease status of an individual or a group of individuals in a population of interest. For a given disease, the accuracy of information on individual's status depends on the performance of the applied diagnostic tests. In fact, the performance of a diagnostic test indicates its ability to correctly identify truly diseased from non-diseased individuals when applied in a randomly

chosen sample from a population of interest (Lewis and Torgerson, 2012). This ability is also an important point when evaluating a new diagnostic test and for implementing disease control programs since a correct classification of herds and individual animals regarding their status is looked-for (Greiner and Gardner, 2000). The actual level of a disease in a population of interest, i.e. the true prevalence, is also an essential parameter (Dohoo *et al*., 2003). Its estimate is helpful to assess the impact of a disease in the population of interest and to avoid biased estimation of disease burden (Speybroeck *et al.*, 2012a). Accuracy of true prevalence depends on the performance parameters of the tests to be applied (Ihorst *et al.*, 2007). The performance of a diagnostic test may be evaluated through several quantitative parameters including predictive values, likelihood ratios (LR), the area under the Receiver-Operating-Characteristic (ROC) curve (AUC), the diagnostic odds ratio (DOR), Youden's index (J), and Se and Sp (Greiner and Gardner, 2000; Glas *et al.*, 2003). These parameters are helpful to support decision-making while selecting a diagnostic test for a given context or purpose. Based on the contingency table below **(Table VII)**, a summary of these different parameters of performance and their definitions are given in **Table VIII** (Glas *et al.*, 2003).

The predictive values express the probability of diseased animals (PPV) or non-diseased animals (NPV) among positive and negative results respectively. The likelihood ratios indicate the ratio of the expected result between animals with a disease and animals without that disease. The area under the ROC curve (AUC) is a plot of sensitivity against one minus the used specificity and is applied to measure the discriminative power of a diagnostic test. The Diagnostic Odds Ratio (DOR) is referred as the ratio of the odds of positivity in diseased animals compared to the odds of the same test result in non-diseased animals. Both DOR and the Youden's index, which is a combination of sensitivity and specificity minus one, are significantly influenced by these two parameters. Among the indicators of performance, sensitivity and specificity are the most employed. Test Se (or Sp) indicates the probability that a truly infected (or non-infected) individual yields a positive (or a negative) test result. Similarly, when the epidemiological unit of concern is the herd, Se corresponds to the probability that an infected herd yields a positive herd-test result, and herd-level Sp (HSp) is the probability that a non-infected herd yields a negative herd-test result (Martin *et al.*, 1992). Positive herd result might refer to the presence of at least one animal testing positive within this herd while negative herd result corresponds to absence of positive animals. However, the threshold number of positive animals that denotes the herd as positive needs to be determined.

Table VII: Contingency table showing results between a reference test and a given imperfect test (Test)

		Reference test	
		Diseased	Not diseased
Test	Positive	TP	FP
	Negative	FN	TN

Where TP, FP, FN, TN are respectively the True positive, the False positive, the False negative and the True negative.

Table VIII: Definitions of commonly used performance indicators of diagnostic test (Glas *et al.*, 2003)

Test performance Parameters	Formula	Definition
Accuracy	(TP+TN)/(TP+TN+FP+FN)	Proportion of correctly identified subjects
Sensitivity (Se)	TP/(TP+FN)	Proportion of positive test results among diseased (true positive rate)
Specificity (Sp)	TN/(TN+FP)	Proportion of negative test results among the "healthy" (true negative rate)
Positive predictive value (PPV)	TP/(TP+FP)	Proportion of diseased among subjects with a positive test result
Negative predictive value (NPV)	TN/(TN+FN)	Proportion of non-diseased among subjects with a negative test result
Likelihood ratio of a positive test result (LR+)	Se/(1-Sp)	Ratio of a positive test result among diseased to the same result in the "healthy"
Likelihood ratio of a negative test result (LR-)	(1-Se)/Sp	Ratio of a negative test result among diseased to the same result in the "healthy"
Youden's index (J)	Se +Sp-1	Difference between the true positive rate and the false positive rate

2.4.3. Methods for estimating disease true prevalence and test sensitivity and specificity

2.4.3.1. Estimation at individual-level

As mentioned above, diagnostic tests are useful to detect the presence or evidence of the presence of an infection or a disease. This ability of a diagnostic test to detect a condition of interest is crucial for selecting appropriate control strategies. So, the performance of a diagnostic test is of key importance. However, since this performance might be influenced by several variables, appropriate methodologies are needed to get better estimates of performance parameters. Estimation of test performance parameters and the true prevalence are two mathematically identical situations, even if the parameter of interest might change according to study objectives (Lewis and Torgerson, 2012).

When a gold standard test is available, the true status of an epidemiological unit of interest regarding a disease can be determined. As a result, performance parameters and true prevalence can be easily deduced from the Rogan-Gladen equation, putting into relation apparent prevalence (AP) and true prevalence (P) with test sensitivity (Se) and specificity (Sp), as described previously (Rogan and Gladen, 1978).

However, assuming a test is a "gold standard" test would mean that no classification errors exist and no false positive or negative result is possible. In practice, such a perfect reference test is hardly ever available since diagnostic performance of any test is known to be under the influence of several endogenous and exogenous factors (Rutjes *et al.*, 2007). As an alternative to the absence or the unavailability of a gold standard test (Black and Craig, 2002), the use of multiple imperfect tests was suggested to facilitate estimation. With multiple tests, misclassification errors are reduced and expected to be lower compared to the application of a single imperfect test. Over the years, several authors have attempted to provide options or solutions to get better and unbiased estimates of the disease prevalence and test Se and Sp in different settings and particularly in absence of a gold standard reference test. These solutions were inspired by frequentist and Bayesian concepts, the two statistical approaches through which inference to the population is made (see section 2.4.3.3.).

2.4.3.2. Estimation at herd-level

Test properties and prevalence estimation could also be considered at group or herd level. Indeed, for many diseases, control programs include groups of individuals or herd testing. Like for individual testing, herd level test performance parameters are crucial. Christensen and Gardner (2000) and Martin *et al.* (1992) discussed the evaluation of diagnostic tests at herd level. Assuming known individual test characteristics and a cut-off of at least one animal testing positive for a herd to be considered positive, herd Se (HSe) and Sp (HSp) are computed as in Thrusfield *et al.* (2005). Dohoo *et al.* (2003) provided a general formula covering the cases with more than one positive animal to consider the herd as positive. Group or herd level test characteristics were shown to be under the influence of different factors. These factors include individual level test Se and Sp, sample size, threshold number (or the percentage of positive tests that denote the herd, or group as test positive) and the within-herd prevalence (Martin *et al.*, 1992). The threshold number of positivity or minimum within-herd prevalence is usually determined according to the epidemiology of the disease or specific national or international rules (Wagner and Salman, 2004). Usually, the presence of one animal testing positive within a herd would be sufficient to classify it as positive but more than one positive result could be necessary for some diseases (Pfeiffer, 2002). Herd size is also known to have a significant influence on herd test performance (Pfeiffer, 2002). Herd-level test performance estimation is comparatively less complicated when herd size in the population of interest is constant. Estimation becomes more challenging when the herd size varies. Then, the procedure for estimating the herd-level test performance needs to account for this variability by weighting estimated values using herd size. As for individual testing, a single test or a combination of test could be used for herd level testing to improve testing performance. Similar requirements as for individual Bayesian modeling also applies when herd is considered, with also the need of good quality prior information[7].

[7] *When prior knowlegde provides information on the uncertainty of a parameter to be estimated, it is considered as an"informative prior". Conversely, prior knowledge might be unavailable implying an absence of information on the uncertainty of a parameter to be estimated. This lack of knowledge can be included in the modelling process as a"non-informative prior".*

2.4.3.3. Bayesian versus frequentist methods for estimating the disease true prevalence and diagnostic test performance

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Personal View

Bayesian versus frequentist methods for estimating true prevalence of disease and diagnostic test performance

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Introduction

As the two main schools of statistical reasoning through which inference to the population is made by analysing data and incorporating uncertainty of measures, Bayesian and frequentist philosophies have been used to estimate diagnostic test performance and the true prevalence of diseases. However, controversies exist between the two philosophies, such as the use of fixed parameter values in the frequentist approach or the inclusion of prior information in the Bayesian approach. So, is the philosophical debate between these two approaches still relevant for such practical questions?

The Bayesian philosophy arose from a statement made by the Reverend Thomas Bayes (1702–1761), a British mathematician and theologian, who was the first to apply statistical probability inductively. According to Bayes, *'all forms of inference are based on the validity of their premises'* and *'no inference can be known with certainty'* (Thrusfield, 2005). In 1814, the French mathematician, Simon-Pierre Laplace published a mathematical description based on Bayes's idea (Gelman et al., 2004). In the Bayesian philosophy, scientific observations do not exist in a vacuum and information available prior to making a series of observations influences the interpretation of those observations (Thrusfield, 2005).

Bayesian analysis can be regarded as a process of adjusting and updating the likelihood of an event based on data. Thus, population parameters, such as sensitivity (Se) and specificity (Sp), are assumed to have a probability distribution representing our prior knowledge of their values. This information is combined with observed factual field data in a model for estimation (Speybroeck et al., 2012a). For Bayesians, a parameter is assumed to have an intrinsic probability distribution with a 95% credibility interval (Gardner, 2002). Thus, Bayesian principles are often applied in order to estimate disease prevalence and test characteristics, especially when there is no gold standard (Enøe et al., 2000; Branscum et al., 2005; Rutjes et al., 2007; Meyer et al., 2009).

The frequentist philosophy emerged in the 20th century with the works of Fisher (1922) and Neyman and Pearson (1928a,b), who enunciated the concept of relative frequency (Vallverdú, 2008). This concept sustains the idea that a probability is a frequency determined from an experiment repeated a large number of times. Frequentist statisticians attempt to draw conclusions by focussing primarily on results obtained from experiments or samples. In the frequentist reasoning, a parameter is a fixed value with a 95% confidence interval derived from the sample. It is assumed that this 95% confidence interval would contain the true value of the parameter 95% of the time if the estimation were repeated a large number of times.

Therefore, Bayesian philosophical methods are based on the idea that unknown quantities, such as population means or proportions, have a probability distribution that expresses our prior knowledge or belief about such quantities, *before* we add the knowledge gained from observational data. Bayesian inference considers the data to be fixed and parameters to be random, because they are unknown. In frequentist

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methods, prior knowledge is apprehended differently and population means or proportions are considered as fixed values (Bland and Altman, 2002). Frequentist inference considers the unknown parameters to be fixed and the data to be random.

Bayesian and frequentist methods have been published to handle a variety of situations in which diagnostic tests are evaluated. In this article, we consider the requirements, limitations and controversial points of the methods proposed for estimating test performance and the true prevalence of disease, using the case where one test or a combination of two imperfect diagnostic tests is applied in the absence of an appropriate gold standard.

Estimating the true prevalence of disease and diagnostic test performance with imperfect tests

The ability of a diagnostic test to distinguish correctly truly diseased from non-diseased individuals, when applied to a randomly chosen population, is necessary so as to understand the epidemiology of the disease, to implement disease control programmes and to evaluate new diagnostic tests (Greiner and Gardner, 2000; Lewis and Torgerson, 2012). Mathematically, the estimation of test performance parameters is essentially the same question as estimating true prevalence (Lewis and Torgerson, 2012). The true prevalence (the proportion of truly diseased individuals in the population of interest) is also an essential parameter required to appraise the impact of a disease in a population of interest and to prevent biased estimations of disease burden (Dohoo et al., 2003; Speybroeck et al., 2012a).

The accuracy of estimation of true prevalence depends on the performance parameters of the test(s) to be applied (Ihorst et al., 2007). Among performance indicators of a diagnostic test, Se and Sp are the most commonly used. Test Se or Sp indicates the probability that a truly infected or uninfected individual yields a positive or negative test result, respectively. Ideally, Se and Sp values for a given test should be estimated from a reference population with a clearly identified status determined by historical (accurate) information or, more commonly, by a relevant gold standard ($Se = 1$ and $Sp = 1$) that is able to discriminate infected/diseased individuals from uninfected/non-diseased individuals in a population (Dohoo et al., 2003). When such a perfect test exists, an estimation of performance parameters of the new test, as well as true prevalence, can be done easily (Rogan and Gladen, 1978).

In practice, such a test is hardly ever available, given that the diagnostic performance of a test is influenced by a number of endogenous and exogenous factors (Rutjes et al., 2007). As an alternative, a combination of multiple imperfect tests ($Se < 1$ and/or $Sp < 1$) may be used to estimate disease parameters (Black and Craig, 2002). With multiple tests, overall misclassification errors are reduced and are expected to be lower than with a single imperfect test.

For example, the isolation and identification of *Brucella* spp. is considered as the reference standard method; a positive test result provides an unequivocal diagnosis of a positive case of brucellosis (World Animal Health Organisation, 2009). However, these methods are not always feasible in diagnostic investigations. Therefore, diagnosis must be based on imperfect serological methods, such as the Rose Bengal test (RBT) and the indirect ELISA (iELISA), which are the two OIE prescribed tests for trade and are commonly used in combination for the diagnosis of brucellosis (Nielsen, 2002; Saegerman et al., 2004; World Animal Health Organisation, 2009; Godfroid et al., 2010; Sanogo et al., 2013).

Estimation of true disease prevalence and test characteristics with combined imperfect tests poses challenges, including (1) potential misclassification errors, (2) possible dependence between tests, and (3) sparseness of data (Cowling et al., 1999; Dohoo et al., 2003; Messam et al., 2008). Both Bayesian and frequentist approaches have been proposed to tackle these challenges.

Estimation with a single test

In the simple case, where a single imperfect diagnostic test is applied in a population of interest, a total of three parameters must be estimated, whatever the method, namely, Se, Sp and true prevalence. In this case, the apparent prevalence (the proportion of positive test results) is the only information given by the data. From a frequentist perspective, estimation can be done only if fixed external information is provided on the values of Se and Sp, but this is difficult, since test properties are known to be context-specific and cannot realistically be assumed to be fixed and known in advance, such as the values given by the manufacturer of a test (Thrusfield, 2005).

As far as external information has to be included for estimations, Bayesian methods seem to be more helpful in obtaining acceptable and realistic results, since they offer the possibility of including the known uncertainty with respect to diagnostic test characteristics, while testing whether data conflict with prior information (Joseph et al., 1995; Berkvens et al., 2006; Speybroeck et al., 2012b). However, the accuracy of Bayesian estimates is dependent on the availability and quality of prior knowledge, which may be a limiting factor and may also conflict with frequentist philosophy.

Estimation with more than one test

When a combination of at least two tests is used, the test results for a given individual could be interpreted either in series (only animals that test positive to both tests are considered to be test positive) or in parallel (animals that test positive to one test, to the other test or to both tests are considered to be test positive) (Black and Craig, 2002). A combination of tests may also result in dependence or correlation between the test results. As a consequence, either conditional independence or conditional dependence assumptions need to be made for accurate estimation of disease prevalence and test properties (Jones et al., 2010).

Conditional independence implies that the results of the second test (T2) do not depend on whether the results of the first test (T1) are positive or negative among infected (or uninfected) individuals (Enøe et al., 2000; Gardner et al., 2000). If we consider the skin test or the iELISA, two tests referred to above for the diagnosis of brucellosis, conditional independence is likely to exist in relation to their respective targets (cellular response for the skin test and humoral response for iELISA), especially in a low prevalence context (Saegerman et al., 1999). In this case, calculation of test Se and Sp will depend mainly on the testing strategy adopted (in parallel or in series) (Dohoo et al., 2003).

Mathematically, assumptions such as conditional independence and a constant prevalence over sub-populations are needed to estimate prevalence (Enøe et al., 2000). These assumptions are necessary so as to reduce the number of unknown parameters to be estimated (Berkvens et al., 2006). Gart and Buck (1966) and Staquet et al. (1981) proposed frequentist methods assuming conditional independence between a new test and a reference test with known Se and/or Sp. However, test Se (stage of infection) and Sp (similar immunogenic component) values are influenced by the characteristics of the population in which the test is applied (Saegerman et al., 2004; Berkvens et al., 2006) and cannot be considered as intrinsic constant and known parameters (Thrusfield, 2005). Moreover, assuming fixed values might not be realistic, since many factors, such as the presence of cross-reacting agents (Saegerman et al., 2004) and low infection pressure, may influence test parameter values (Speybroeck et al., 2012b).

Hui and Walter (1980) proposed another major frequentist method to deal with the case where Se and Sp values of the reference test are unknown. In addition to an assumption of conditional independence, this approach required testing at least two populations with distinct prevalences of disease, but constant Se and Sp (Hui and Zhou, 1998; Enøe et al., 2000; Dohoo et al., 2003). The approach was extended to

cover other settings, including cases with more than two tests and multiple populations (Walter and Irwig, 1988; Johnson et al., 2001). The accuracy of estimates made with these methods also relies on the assumption of a large sample size (Enøe et al., 2000; Pouillot et al., 2002; Berkvens et al., 2003; Pouillot, 2003). Toft et al. (2005) provided a useful overview of possible pitfalls when using this paradigm, especially the assumption of conditional independence, which is not always satisfied in practice (Gardner et al., 2000; Dendukuri and Joseph, 2001; Branscum et al., 2005; Berkvens et al., 2006).

Testing situations handled by the frequentist models of Gart and Buck (1966), and the case of unknown Se and Sp already covered by the model of Hui and Walter (1980), have also been examined under the Bayesian framework. Joseph et al. (1995) proposed a Bayesian model for estimation with no constrained parameters and assuming conditional independence. Numerically, this model appeared to be approximately equivalent to the frequentist approach (Dendukuri and Joseph, 2001). Nevertheless, even if estimation was possible with this latter model, inclusion of information on the uncertainty of parameters to be determined is required to get realistic and meaningful estimates (Enøe et al., 2000).

Conditional dependence particularly occurs when combined tests target a similar biological phenomenon, such as the presence of immunoglobulins (Igs) (Gardner et al., 2000; Dendukuri and Joseph, 2001). Thus, conditional dependence is likely to exist between the RBT and iELISA, two assays ostensibly targeting similar anti-*Brucella* antibodies. However, the RBT detects the presence of IgG_1 (IgG_2 and IgM also have some agglutination activity), while the iELISA targets IgG_1 and/or IgG_2, depending on the conjugate used (Nielsen, 2002; Saegerman et al., 2004, 2010; Sanogo et al., 2013). In this scenario, calculation of test Se and Sp under conditional independence is adjusted by the inclusion of the covariance factor expressing the extent of the dependence among positive and negative results, and by taking the testing strategy into account (Dohoo et al., 2003).

When dependence is present, estimates should be adjusted by considering biological and technical mechanisms giving rise to the test results and by including the extent of the dependence between them (Pepe and Janes, 2007). With two correlated tests, a total of seven parameters have to be estimated instead of five under conditional independence (e.g. two sensitivities, two specificities, two covariances and the true prevalence), and the dependence needs to be accounted for (Berkvens et al., 2006; Praet et al., 2006). Some frequentist methods require the application of at least two tests to allow estimation of parameters of interest (Dendukuri and Joseph, 2001). Such an approach might be impractical when tests are expensive, time consuming or invasive.

Instead of using results from at least two tests in order to allow estimation of disease parameters, Bayesian modelling offers an alternative option to obtain estimates of the true prevalence of disease, and test Se and Sp, while accounting for conditional dependence (Qu and Hagdu, 1998; Gardner et al., 2000; Dendukuri and Joseph, 2001; Georgiadis et al., 2003; Sanogo et al., 2013). However, informative priors are needed for at least four of the parameters of the model: two sensitivities, two specificities, two covariances and the true prevalence.

Bayesians versus frequentist methods

Previously, Bayesian approaches were difficult to apply because of major mathematical and computational requirements, but have been facilitated by the application of Markov chain Monte Carlo (MCMC) methods and the availability of high quality statistical software packages, including JAGS (Plummer, 2003), WinBUGS (Lunn et al., 2000) and OpenBUGS (Lunn et al., 2009). These are now the tools of choice in many areas of application and appear to offer practical advantages over their frequentist counterparts (Greiner and Gardner, 2000; Dunson, 2001; O'Hagan, 2004).

Bayesian methods facilitate estimates of population parameters by combining additional knowledge and likelihood in the same model. Uncertainties of reference tests with respect to Se and/or Sp, expressed as probability distributions, are combined with observed field data to produce posterior probability distributions of true prevalence and test performance (Speybroeck et al., 2012b). Compared to frequentist methods, Bayesian methods also offer more options and flexibility so as to derive the best possible estimates of parameters in realistic settings. When two imperfect tests are used, conditional dependence can be addressed in a Bayesian framework by running both models with conditional independence between tests given true disease status, and those with conditional dependence, then checking the robustness of the parameters or using model selection criteria, such as the deviance information criterion (DIC) (Berkvens et al., 2006; Dendukuri et al., 2010). Robustness of estimates should also be checked systematically across a range of plausible values based on the evidence to date (Enøe et al., 2000; Speybroeck et al., 2012a).

A systematic review and/or quantitative reviews summarising data using appropriate meta-analytical methodologies are preferred in order to obtain informative priors on diagnostic test performance (Irwig et al., 1995; Dohoo et al., 2003; European Food Safety Authority, 2009). Application of evidence-based medicine and the quality assurance of the process used to obtain prior information are important in assessing the quality of the approach. In the case of emerging infectious diseases, where prior information may not be available, non-informative priors might be used. When no informative prior knowledge is included in the estimation process, the results of frequentist and Bayesian analyses are similar (Enøe et al., 2000; Dendukuri and Joseph, 2001).

Whatever the priors, a sensitivity analysis of prior information should be undertaken to assess its potential influence on estimates (Menten et al., 2008; Sanogo et al., 2013). Special care should be given to the selection of available information in order to obtain unbiased estimates (Spiegelhalter et al., 2002; Berkvens et al., 2006). The procedure for incorporating available knowledge or prior information into the model, and the mathematical issues, have been described previously (Enøe et al., 2000; Gardner et al., 2000; Dendukuri and Joseph, 2001; Branscum et al., 2005; Berkvens et al., 2006).

In addition to the challenges relating to misclassification bias, the representativeness of data regarding the population of interest and the quality assurance of the process (traceability) are two key issues to be considered in both approaches. Thus, different stages of the disease and the age of the animals should be considered, and an appropriate sampling strategy used to compose the reference population and, consequently, to minimise sampling error and biased posterior estimates. Consequences of using imperfect tests should be accounted for at the analysis stage (as well as the planning stage) of the estimation process.

Conclusions

Controversies between the frequentist and Bayesian approaches are more a philosophical than a practical issue. Although originating from different statistical philosophies, Bayesian and frequentist approaches are two methodological options to deal with test performance and true prevalence estimation. While the frequentist approach concentrates only on likelihood-based estimation, the Bayesian approach uses the likelihood and prior information for estimation. Both approaches have proposed solutions to address challenges related to estimation of test performance and true prevalence, taking into account field conditions. Whatever the approach used, it is necessary to ensure that appropriate assumptions related to the application of a given approach hold.

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CHAPTER 3: OBJECTIVES OF THE THESIS

CHAPTER 3: OBJECTIVES OF THE THESIS

In many developing countries such as Ivory Coast, the development of livestock and the improvement of their health environment is part of the fight against poverty and for food security of populations. Developing livestock production and productivity imposes dealing with many constraints including pathological ones such as brucellosis. In addition to its impact on animal's health, brucellosis is also one of the widespread zoonotic diseases. In developing countries and more precisely in Africa, this disease is endemic and known to be among the pathologic constrains to the development of livestock. Despite its known negative socio-economic impact and zoonotic potential, the disease is not considered yet as a priority disease and therefore remains neglected, underreported and uncontrolled in many countries (Mableson *et al.*, 2014).

In Africa, bovine brucellosis is the most widespread form among animals. In Ivory Coast, this form was recognized as one of the dominant pathologies and is argued to be responsible for the loss of about 10% of the annual income of the livestock breeders (Angba *et al.*, 1987). In this country, investigations were conducted on bovine brucellosis throughout the years to determine its incidence and for a better knowledge of its epidemiology (Gidel *et al.*, 1974; Pilo-Moron *et al.*, 1979; Camus, 1980a; Thys *et al.*, 2005). But similarly to many low-resource countries, these investigations are still few and their results are outdated particularly on the actual distribution of the disease, on the transmission within and across species and the impact on human and animal health, precluding the development of prevention and control strategies (Marcotty *et al*., 2013).

The general objective of the research presented in this thesis is to improve the knowledge on the epidemiology of bovine brucellosis in Ivory Coast permitting future strategic actions. In this respect, different aspects of the disease were studied including prevailing strains causing brucellosis in cattle, performance of diagnostic test for brucellosis, estimation of true disease prevalence and identification of risk factors associated with brucellosis seropositivity in cattle from Ivory Coast (Figure 9).

The specific objectives of the research are as follows:

1) Identification and isolation of the causal agent remains the ultimate evidence of the presence of the disease. The demonstration of *Brucella* as causal agent of brucellosis may

be done through various methods including bacteriological and molecular methods. Knowledge on prevailing field strains of *Brucella* in the particular context of Ivory Coast is useful to elaborate and set up appropriate preventive and control measures against brucellosis. In effect, data on prevailing field strain would be useful and critical to select the appropriate antigen for serological assay, to determine intra-species and interspecies transmission and to assess the potential risk of human infection. Consequently, one of the specific objective of this research was to investigate circulating species and biovars of *Brucella* associated with cattle in Ivory Coast (**Chapter 4 and 5**) aiming to provide an overview, to determine their geographical distribution and discuss public health implications (**Chapter 4**).

2) Diagnostic test are key components for disease-control programs since they are useful for classifying individuals according their serological status. Estimates of performance parameters are also useful to assess the impact of a disease in a given population, through accurate estimates of true prevalence. Thus, since the performance of diagnostic tests are under the influence of the population in which they are applied (including prevailing disease-causing agent), the diagnostic sensitivity, specificity and the true prevalence were estimated for bovine brucellosis in the Ivorian context (**Chapter 5 and 6**). The performance of the diagnostic tests was also discussed in light of circulating field species and biovars of *Brucella*, regarding particularly the appropriateness of the antigen used in serological assays. The true prevalence of brucellosis was estimated in Ivory Coast using a Bayesian approach, a statistical methodology allowing the combination of many testing results for accurate estimates.

3) Identification of risk factors related to the presence or the spread of the disease are useful to adjust preventive and control measures. So, another specific objective of this research was to investigate the potential risk factors associated with bovine brucellosis in Ivory Coast (**Chapter 7**).

Figure 9: Schematic summary of the main objectives of the thesis

PART TWO:

EXPERIMENTAL SECTION

CHAPTER 4:

DETERMINATION OF SPECIES AND BIOVARS OF BRUCELLA INFECTING CATTLE POPULATION IN IVORY COAST

4.1. Introduction

Identification and characterization of the causative agent of an infectious disease is important to consider for epidemiological studies, management of outbreaks and to identify potential source of human infection (Saegerman *et al*., 2010; Godfroid *et al.*, 2010). Additionnaly, it allows to obtain data on possible interspecies and intra-species transmission of *Brucella*. Knowledge of prevailing species and biovars of *Brucella* infecting the livestock is a crucial prerequisite to the formulation of strategies for the prevention and the control of brucellosis in animal populations (Ocholi *et al.*, 2004). Aiming to contribute to the knowledge on prevailing strains of *Brucella* in Ivory Coast and in West Africa, a summary and some updates were provided in this thesis.

4.2. Prevailing species and biovars of *Brucella* in cattle and their implications

This section constitutes the following original paper published in *Veterinary Microbiology*.

ARTICLE 2:

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Review

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

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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.

Contents

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1. 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; Bronsvoort 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, 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 subtypes (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, 2003; 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., 2008; 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.

2. Methodology

2.1. 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 km^2 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

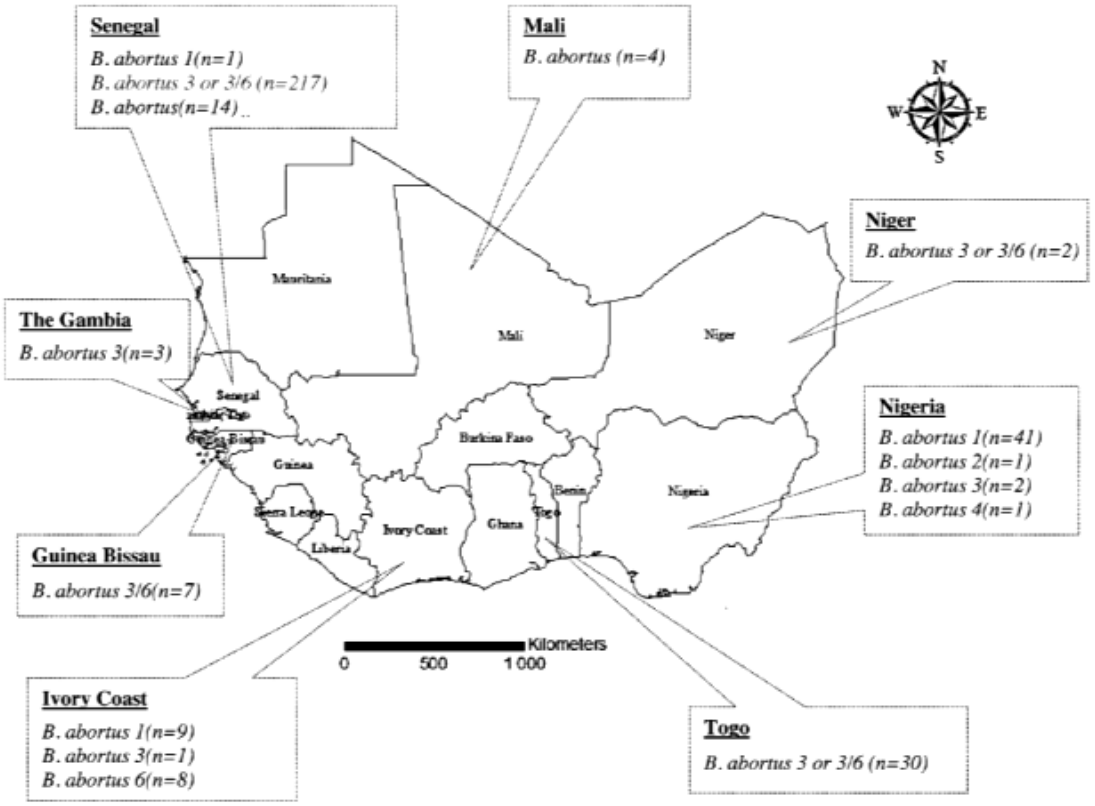

Fig. 1. A map showing Western African countries and the geographical distribution with cumulative total number of species and biovars of *Brucella* reported through years in cattle, period 1960–2009.

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).

2.2. 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.

2.3. 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.

3. 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

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. (2013)[a]	Ivory coast (Dimbokro)	2009	H	Alton et al. (1988) and Le Flêche et al. (2006)	*B. abortus 3*	1
Bankole et al. (2010)[a]	The Gambia (Kombo East District)	NA	H	Alton et al. (1988) and Le Flêche 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	*B. abortus 3/6*	7
	Niger		H	McCullough (1974)	*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) and 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

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.
[a] Except these studies where molecular methods were also used for typing, all the results reported in this review were obtained using bacteriological methods.
[b] These studies were not included in this review.
[c] Heart blood from aborted fetuses was used. Hygroma fluid and milk samples from aborted cows were negative to bacteriological examination.

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Table 2
Summary of growth characteristics reported for isolates of *Brucella* of cattle origin in West Africa, period 1977–2013.

Country	Authors (year)	Species and biotypes	Growth characteristics											
			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. (2013)	*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) Verger and Grayon (1984)	*B.abortus 3 or 3/6*	–	+	+	+	A(+); M(–)	+	+	+	+	+	+	–
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	+	+[a]	–(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) Verger and Grayon (1984)	*B. abortus 3 or 3/6*	+	+	+	+	A(+); M(–)	+	+	+	+	+	+	–

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.
[a] 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.

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 3/4 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 Figs. 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 ($P = 0.006$).

4. Discussion

4.1. 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; Sanogo et al., 2013). 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 10^3–10^4 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,

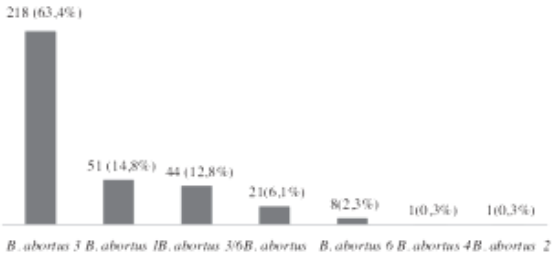

Fig. 2. Number and proportion of isolates of *Brucella* of cattle origin per species and/or biovar in West Africa, period 1960–2009.

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., 2013). 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).

4.2. 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., 2013). 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, 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 Grayon, 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.

4.3. 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-Román 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 and Arimi, 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.

5. 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.

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CHAPTER 5:

PERFORMANCE OF DIAGNOSTIC TESTS FOR BOVINE BRUCELLOSIS IN IVORIAN EPIDEMIOLOGICAL CONTEXT

5.1. Introduction

Sufficient knowledge on species and biovars of *Brucella* at national and regional scales are important to set up and implement efficient control measures against brucellosis. From data on circulating field strains, the appropriateness of the antigen used in serological tests can be verified. This appropriateness is of key importance since the detection of the presence or evidence of the presence of an infection or a disease such as brucellosis is dependent on diagnostic tests. The ability of a diagnostic test to detect a condition of interest can be measured through performance indicators such as sensitivity and specificity. Since test sensitivity and specificity are known to be under the influence of several variables, an appropriate methodology is needed to get accurate and unbiased estimates and to get knowledge on the actual impact of the disease among a population of interest. In this chapter, typing results were put in relation with the epitope used in the serological tests applied to assess their appropriateness. Then, a Bayesian approach was implemented to determine the performance of two commonly used diagnostic tests for the diagnostic of bovine brucellosis in Africa, the Rose Bengal Test and the indirect enzyme linked immunosorbent assay. For representativeness, data from two surveys were combined for the analysis as a single population. Indigenous cattle of *Bos indicus* type*, Bos taurus* type and their crossbred, more than one year old were included in this study. Hygroma fluid collected from a carpal hygroma was used as sample for biotyping.

5.2. Bayesian estimation of true prevalence, sensitivity and specificity of Rose Bengal Test and indirect ELISA for the diagnosis of bovine brucellosis in Ivory Coast

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Bayesian estimation of the true prevalence, sensitivity and specificity of the Rose Bengal and indirect ELISA tests in the diagnosis of bovine brucellosis

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ABSTRACT

Serology is the most convenient method for detecting brucellosis but the efficient use of such tests in disease control requires evaluation of diagnostic performance and discriminative ability. The objective of this study was to assess the performance of the Rose Bengal test (RBT) and an indirect ELISA (iELISA) in diagnosing brucellosis in 995 serum samples collected from cattle in the Ivory Coast between 2005 and 2009. A Bayesian approach was used to evaluate the two tests by estimating their sensitivities and specificities.

The correlation-adjusted sensitivity of the iELISA was estimated to be 96.1% (credibility interval [CrI], 92.7–99.8), whereas that of the RBT was 54.9% (CrI, 23.5–95.1). High correlation-adjusted specificities were found for both tests (95.0%; [CrI, 91.1–99.6] for the iELISA and 97.7%; [CrI, 95.3–99.4] for the RBT, respectively). The true prevalence of brucellosis was estimated from the serum samples to be 4.6% (95%; [CrI, 0.6–9.5]). The level of agreement between the two tests was evaluated using indices of agreement ($n = 995$). Good agreement was found for negative results (96.6%; confidence interval [CI], 95.7–97.4), a finding supported by an estimated significant correlation of 0.37 (95%; CI, 0.01–0.73) within the sera testing negative. Agreement was lower for sera testing positive (52.2% CI: 41.9–62.5). The findings highlight the importance of using these two tests in combination as part of any brucellosis control programme.

Introduction

Brucellosis causes considerable economic loss in livestock production in many developing countries through abortion, premature birth and retained placentae (Corbel, 2006). In Africa, brucellosis is more widespread in cattle (Akakpo and Bornarel, 1987; Corbel, 1997; McDermott and Arimi, 2002), and is caused by one of seven biovars of *Brucella abortus*, namely, 1, 2, 3, 4, 5, 6 and 9.

Infected animals are detected using serological tests based on the detection of antibodies against lipopolysaccharide (LPS), the dominant antigen of the outer membrane of the organism (Alton et al., 1988). When used for confirmatory or screening purposes, or when choosing between their serial or parallel interpretation, knowledge of test sensitivity (Se) and specificity (Sp) is essential. Although considered 'intrinsic' parameters, Se and Sp are often estimated following the application of the test to a limited number of animals that may not necessarily match the scale of the populations on which the test will subsequently be used, so results can vary between test populations (Saegerman et al., 2004; Berkvens et al., 2006). When used in a specific, representative sample of a population, it is therefore essential that the test's performance in that sub-population is properly assessed.

Bovine brucellosis is endemic in the Ivory Coast, as in many other developing countries. Although studies have estimated the prevalence of the disease (Gidel et al., 1974; Pilo-Moron et al., 1979; Camus, 1980; Angba et al., 1987; Thys et al., 2005; Sanogo et al., 2008), few have focussed on the prevailing field strains of *B. abortus* involved, or evaluated the diagnostic tests used. Such information would be valuable in establishing an official brucellosis control programme as part of the overall development of the dairy industry.

The objectives of the present study were: (1) to estimate the prevalence of bovine brucellosis in Ivory Coast, and the characteristics of the two widely used diagnosis tests, the RBT and an

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(iELISA); and (2) to verify, a posteriori, the appropriateness of the antigen used in these tests by identifying the field strain(s) of *B. abortus* circulating within cattle.

Materials and methods

Data selection

The data came from cattle herds of the Southern and Central regions of the Ivory Coast, located between 3–9°W and 5–11°N. Three agro-ecological zones exist within the country: (1) the Guinean zone in the south; (2) the Soudanian zone in the north; and (3) the Soudano-Guinean zone in the centre. Sedentary herds managed extensively in different localities of the Soudano-Guinean and Guinean regions were sampled during two serological surveys.

The first survey was conducted in 2005 when 660 serum samples were obtained in the Soudano-Guinean region from cattle herds randomly selected using a two-staged sampling strategy (Sanogo et al., 2008). Briefly, 314 herds were randomly selected as part of a sero-surveillance campaign for rinderpest from an established sampling frame of 1265 herds and taking into account their geographical distribution. Of the herds selected, a fraction of 44 herds covering two administrative regions in the Soudano-Guinean region were used. A second step in the process involved the blood sampling of >15 cattle/herd. The sera obtained were combined with 568 sera collected from 13 conveniently chosen herds from both the Soudano-Guinean and Guinean zones in 2009 during a second survey. Indigenous cattle >1 year old were sampled and were *Bos indicus* or *Bos taurus* types or cross-breeds of these. Fig. 1 illustrates the geographical area.

Samples were collected from the jugular or coccygeal veins into 5–10 mL tubes without anticoagulant. After clotting at ambient temperature and centrifugation, sera were separated and stored at −20 °C until tested. Sera were pooled given the absence of an official brucellosis control programme. Bacterial biotyping was performed on 5–10 mL of fluid collected from a carpal hygroma from a 6-year old cow with a history of abortion. The animal came from a herd from Ebimolossou (6°37′N, 4°38′W), a village in the centre of the country. The fluid collected was stored at −20 °C until typing was performed.

Serology

Sera were tested using the RBT and by iELISA as described by Alton et al. (1988). Briefly, serum samples were mixed and rotated on a glass plate for 4 min with a *B. abortus* biotype 1 antigen (Strain Weybridge 99, A epitope), and the presence or absence of agglutination was recorded as positive or negative reaction, respectively. The iELISA was used as described by Limet et al. (1988) using *B. abortus* biotype 1 antigen (Strain Weybridge 99, A epitope), protein G-horseradish peroxidase as the bovine anti-immunoglobulin G conjugate, and serum No. 1121 as a positive control. Samples were diluted 1:50 on wells of previously sensitised plates with antigen and incubated for 1 h at room temperature. Wells were then washed and filled with conjugate to reveal antigen–antibody binding. Following a further 1 h incubation at room temperature, conjugate activity was demonstrated using a citrate buffer with 0.4% O-phenylenediamine and 2 mM hydrogen peroxide as substrate.

Optical densities (OD) were read at 492 nm with a differential filter of 620 nm. In addition to negative and positive controls, six dilutions of the reference serum (1/270–1/8340, corresponding to 2–60 units), were included in the assay to build a

Fig. 1. Map of the Ivory Coast illustrating the sampled area and the location of the selected herds within this region.

Table 1
Contingency table showing results for two diagnostic tests (T1 and T2).

	T1		
	Positive	Negative	Total
T2			
Positive	*a*	*b*	*a* + *b*
Negative	*c*	*d*	*c* + *d*
Total	*a* + *c*	*b* + *d*	*N*

N, total of samples tested by both tests (*a* + *b* + *c* + *d*).

standard curve. The average OD of the tested sera was corrected with the buffer OD and compared to the standard curve using a cut-off of 2 units as the threshold of seropositivity. The RBT was performed at the Central Veterinary Laboratory, Bingerville, Ivory Coast, and the iELISA at the Veterinary and Agrochemical Research Centre, Brussels, Belgium.

Estimation of true prevalence, test sensitivity and specificity

In the absence of a 'gold standard', a Bayesian approach was used to evaluate the performance of the RBT and iELISA by estimating Se and Sp (Branscum et al., 2005; Berkvens et al., 2006). In addition, given both tests are based on antibody detection, they can be considered conditionally dependent, i.e. the results of the two tests for a given animal are correlated. A Bayesian model was thus developed taking account of the correlation between the tests on infected and non-infected animals. The approach facilitated the inclusion of both field data and prior expert information in the same model to estimate test characteristics and prevalence. Using prior information relating to prevalence (0–0.2) (Mangen et al., 2002; Shey-Njila et al., 2005; Sanogo et al., 2008), iELISA data (Se, 0.925–1; Sp, 0.906–1), RBT data (Se, 0.210–0.983; Sp, 0.688–1) (Nielsen, 2002), and covariance (Branscum et al., 2005), the model was established within 'Winbugs' (Spiegelhalter et al., 2003) (see Appendix A, Supplementary material).

Additional calculations during the analysis were carried out using 'R'.[1] Three parameters were monitored during the analysis: (1) the deviance information criterion (DIC), (2) the effective number of estimated parameter (p_D), and (3) the Bayesian *P* value. In brief, the DIC and *P* value were used to check if the prior information was in conflict with testing data results. The p_D of the model was used to assess the impact of the constraints. The model used three chains, a 'burn-in' of 10,000 iterations, and an additional 50,000 iterations to obtain the posterior distributions. Trace plots were simultaneously combined with autocorrelation plots to explore the convergence of the model. If the trace plot indicated good mixing and the autocorrelation plot little or no correlation among samples, then convergence was claimed. Where autocorrelations were still high after the first few lags, 'thinning' was applied where the thinning coefficient was determined by the number of lags at which the autocorrelations significantly dropped to zero. A more formal test for convergence, the Brooks–Gelman–Rubin (BGR) statistic was also used (Gelman and Rubin, 1992). A good fit of the model was indicated by a Bayesian *P* value tending towards zero under the constraints (Berkvens et al., 2006).

Prior sensitivity analysis

The parameter estimates obtained using the Bayesian model with conditional dependence between tests vary with the prior distributions (Branscum et al., 2005). Therefore, in order to assess the influence of the proposed prior distributions on the estimated parameters, a sensitivity analysis was performed which consisted in using non-informative 'priors' (Enoe et al., 2000; Branscum et al., 2005). For each set of alternative prior distributions considered for the parameters, the model was run with the same number of chains and similar diagnostics were performed.

Assessment of agreement between the tests

The two tests were compared using concordance analysis in order to assess their agreement with our results. The level of agreement was expressed in terms of indices of positive and negative agreement (Cicchetti and Feinstein, 1990), respectively, the observed agreement proportion for positive and negative test results. Confidence intervals were calculated according to the method of Graham and Bull (1998). Calculations of the different parameters were carried out in 'R'. Using a 'two-by-two' contingency table (Table 1), the two indices of positive agreement (P_{pos}) and negative agreement (P_{neg}) were respectively:

$$P_{pos} = \frac{2a}{2a+b+c} \quad \text{and} \quad P_{neg} = \frac{2d}{2d+b+c}$$

[1] See: http://www.r-project.org.

where P_{pos} and P_{neg} were the indices of positive and negative agreement, respectively (parameters *a*, *b*, *c* and *d* are detailed in Table 1).

Strain identification and typing

Fluid was collected from a carpal hygroma, a common lesion associated with chronic brucellosis in cattle in Africa (Thienpont et al., 1961; Saegerman et al., 2010), and a potentially good source of bacteria (OIE, 2009). Hygroma fluid is thus useful for the isolation and typing of *Brucella* spp. in an African context (Musa and Jahans, 1990; Ocholi et al., 2004). Bacteriological culture employed selective media (Alton et al., 1988), and the fluid was cultured on two plates with Farrell's selective media. The plates were incubated at 37 °C, in 5–10% CO_2 and examined for suspicious colonies after 3 days. Such colonies were screened microscopically and evaluated for their catalase, urease and oxidase activities, their CO_2 requirement, H_2S production, growth in the presence of thionin (at 10 and 20 μg/mL), fuchsin (20 μg/mL), safranin (100 μg/mL), and their agglutination response to monospecific antisera 'A' and 'M'. Identification to biovar level was based on morphological, cultural and biochemical characteristics.

In addition to bacteriological typing, a multiple loci variable number tandem repeats analysis (MLVA) was used for both strain and species identification (Le Flèche et al., 2006; Maquart et al., 2009). In brief, a combination of two complementary sets of 16 loci (MLVA-16) was used to determine the MLVA profile of the isolate. The first set of markers (panel 1) was composed of eight minisatellite loci (bruce06, bruce08, bruce11, bruce12, bruce42, bruce43, bruce45, bruce55) useful in species identification. This set is associated with a second panel of markers (panel 2) consisting of three highly discriminative microsatellite loci (bruce04, bruce07, bruce09) and five additional, more variable loci, with a lower weight in clustering analysis (bruce16, bruce18, bruce21). Using these loci, a PCR was performed and the number of repeated units for each locus determined. These numbers were converted into a string of integers and referred to as the MLVA profile. This unique profile was compared within the Brucella 2010 MLVA database (MVLABANK, 2010), and a clustering drendrogram constructed (Fredslund, 2006).

Results

Serology

Of the 1228 samples collected, only 995 gave comparable and interpretable results in both tests and were considered suitable for further analysis. Considering the two-by-two contingency table (Table 1), cross-classified test results of these 995 samples resulted in *a* = 35, *b* = 9, *c* = 55 and *d* = 896. A total of 44 (4.4%) of serum samples tested positive on the RBT, whereas 90 (9.0%) were positive using the iELISA. Both tests gave the same results in the case of 931 samples (93.6%). In addition, except for the iELISA, statistical analysis of the serological results from the two sub-datasets demonstrated insufficient evidence of difference ($P > 0.05$, Fisher's Exact Test), considering either the RBT results separately or in serial and parallel interpretation.

Indices of agreement between tests

The cross-classified test results (*a* = 35, *b* = 9, *c* = 55 and *d* = 896) were used to calculate the indices of agreement between the two assays. Both results were the same for 52.2% of the positive results to both tests (P_{pos}), whereas the agreement on negative test results (P_{neg}) was estimated to be 96.6%. The 95% confidence intervals for P_{pos} and P_{neg} were 41.9–62.5% and 95.7–97.4%, respectively.

Estimated true prevalence, test sensitivity and specificity

An observation of the trace plots suggested that the chains were mixing poorly and the autocorrelation plots indicated that there were significant autocorrelations up until lag 40. Following application of a thinning coefficient of 40, the model appeared to converge as the chains were now properly mixing and the autocorrelations were zero. The BGR plots also corroborated these findings. The estimated Bayesian *P* value of our model was 0.406, indicating no particular problems with model fit. The p_D estimated from the multinomial probabilities in 'R' was 2.82 and the DIC was 21.12. The estimated p_D and DIC values based on our model were

2.67 and 20.8, respectively, which were quite close to optimal values.

The estimated values of Se and Sp for both the RBT and iELISA are summarised in Table 2. As an outcome, the model also estimated an overall true prevalence of brucellosis to be 4.6% (95%; CrI, 0.6–9.5). The estimated correlation between the two tests within the non-infected population was 0.37 (95%; CI, 0.01–0.73) and for the positive test results was 0.03 (95%; CI, −0.27 to 0.48). The significant correlation of 0.37 provides evidence that the test outcomes for non-infected animals are correlated.

Following Se analysis, the selected set of priors and their corresponding posterior estimates were summarised (Table 2). The results indicated that using: (1) a non-informative prior for the prevalence, and informative priors for the Se and Sp of the iELISA and RBT, (2) non-informative priors for the Se and Sp of the iELISA, and informative priors for the prevalence and Se and Sp of the RBT, and (3) non-informative priors for the Se and Sp of the RBT and informative priors for the prevalence and Se and Sp of the iELISA: had no major influence on the estimated parameters except for the estimated dependency coefficient between the iELISA and RBT among animals seropositive on both tests (magnitude similar but signs opposite). However, when an informative prior was only used for the prevalence and non-informative priors for the Se and Sp of the two tests, the estimated Se of the iELISA decreased to 0.65 and the dependency coefficient between the iELISA and RBT amongst this population changed sign (Table 2). The use of non-informative priors for all parameters led to a non-identifiable model.

Strain typing and identification

Round (1–2 mm diameter), convex colonies with entire edges and smooth shiny surfaces, with a honey colouration that was transparent when viewed by transmitted light, grew following 3 days incubation. Gram negative coccobacilli were observed microscopically. Colonies required CO_2 for growth, produced H_2S and grew in the presence of basic fuchsin, thionin and safranin. These bacteria agglutinated the A monospecific antiserum and demonstrated positive reactions for catalase and urease, but no reaction for oxidase. The biotyping results were in agreement with the strain type profile of *B. abortus* biovar 3 except for oxidase activity.

The MLVA-16 assay classified the isolate as *B. abortus* biovar 3. The number of tandem repeats for each locus is shown in Table 3. Considering only the first panel, this profile appeared to be close to that of two *B. abortus* biovar 3 originating from Africa (reference strain Tulya and dromedary strain BCCN 93_26). Our isolate and its closest MLVA 'neighbours' in clustering analysis are illustrated (Fig. 2).

Table 2
Sensitivity (Se) and specificity (Sp) estimates for the Rose Bengal test (RBT) and indirect enzyme-linked immunosorbent assay (iELISA) using a Bayesian approach.

Test	Parameter	Uniform prior	Posterior estimates (CrI)
	Prevalence	[0, 0.2]	0.047 (0.005–0.095)
RBT	Se	[0.210, 0.983]	0.547 (0.235–0.950)
	Sp	[0.688, 1]	0.978 (0.954–0.994)
iELISA	Se	[0.925, 1]	0.962 (0.927–0.998)
	Sp	[0.906, 1]	0.951 (0.912–0.995)
	Prevalence	[0, 0.2]	0.047 (0.002–0.139)
RBT	Se	[0, 1]	0.469 (0.034–0.960)
	Sp	[0, 1]	0.973 (0.947–0.998)
iELISA	Se	[0, 1]	0.653 (0.091–0.982)
	Sp	[0, 1]	0.937 (0.899–0.989)

CrI, credibility interval; Se, sensitivity; Sp, specificity.

Discussion

The objectives of this study were to estimate the true prevalence and the diagnostic characteristics of the RBT and an iELISA for brucellosis and to verify, a posteriori, the appropriateness of the antigen used in these assays (same epitope of the O-chain of the LPS of *Brucella* spp.). Data from two surveys carried out within a 4-year period were combined for analysis as a single population since insufficient statistical evidence of absence of homogeneity was found between the two sub-datasets built from these surveys. In both cases, samples came from the same target population and laboratory testing was performed under similar conditions. In addition, the combination of the two surveys provided a large sample size and consequently more serological results. Moreover, this combination appears to be logical in the absence of an official brucellosis control programme in the Ivory Coast.

By definition, the estimation of the Se and Sp of a diagnostic test requires knowledge of the true disease status of the animals on which this assay is applied (ideally provided by a 'gold standard' test). In the absence of such a standard, a Bayesian approach is helpful to estimate test Se, Sp and prevalence (Dorny et al., 2004; Branscum et al., 2005; Geurden et al., 2006; Praet et al., 2006; Brochier et al., 2007). This approach has also been used in the case for bovine brucellosis (Fosgate et al., 2002). We used prior information given by Nielsen (2002) on the Se and Sp of the iELISA in our estimation process. Prior knowledge may help to reduce the number of parameters to be estimated by the model, but it must be appreciated that the posterior estimates resulting from the Bayesian analysis were a combination of the data and of prior knowledge. Although helpful for the analysis, prior information influenced posterior estimates.

In this study, priors of test characteristics used were ranges of values built by summarising estimates obtained in various epidemiological conditions and using methodologies that might not necessarily be relevant to our context. Nevertheless, the findings from the Se analysis suggested that the data influenced the posterior estimates more than the prior information, indicating that the results can be considered robust regarding the choice of priors. Where prior information on Se and Sp is lacking, as is the case in many developing countries, it is recommended that a sufficient representative sample is used to accurately estimate the true prevalence and test characteristics. In addition, a Se analysis should be systematically performed.

In addition to the primary focus of this study, the model estimated a relatively moderate true brucellosis prevalence compared to the previous estimate of 8.8% (Sanogo et al., 2008). The prevalence estimated was therefore close to the 3.6% reported by Thys et al. (2005). Nevertheless, comparisons of these results have some limitations related to the difference in sampling design and strategy.

Posterior estimates given by the Bayesian model are based on serological test results of the RBT and iELISA. As it is the case with most of the serological assays for brucellosis, both tests could also cross-react with antibodies produced by Gram-negative bacteria antigenically related to *Brucella* spp., thus influencing test Sp (Alton et al., 1988; Saegerman et al., 2004). Considering estimates of test characteristics, the best combination of Se and Sp was obtained for the iELISA. A previous Bayesian analysis in the Ivory Coast gave comparable results. Thys et al. (2005) estimated a Se of 92.7–99.8% and a Sp of 96.4–99.7%. Also, this iELISA is considered superior to other serological tests for brucellosis screening (Tounkara et al., 1994; Saegerman et al., 2004). Despite a relatively low Se, the RBT remains useful from a practical perspective, especially when cost is considered, and continues to plays a major role in brucellosis diagnosis worldwide (Corbel, 2006).

Table 3
The multiple loci variable number tandem repeats analysis (MLVA) profiles illustrating the number of variable tandem repeats (VTR) for a *Brucella abortus* biovar 3 isolate from the Ivory Coast (IVC_isolate) and its closest 'MLVA neighbour' in the MVLABANK (2010). *B. abortus* biovar 3 strain BCCN 93_26 from Sudan, *B. abortus* biovar 3 reference strain Tulya from Uganda, and *B. abortus* biovar 6 strain BfR7 from Chad.

Variable tandem repeats	MLVA profile			
	Reference strain Tulya	Strain BCCN[a] 93_26	Strain BfR7[b]	IVC_isolate
Panel 1				
bruce06	3	3	3	3
bruce08	5	5	5	5
bruce11	5	5	5	4
bruce12	11	11	11	11
bruce42	2	2	2	2
bruce43	2	2	2	2
bruce45	3	3	3	3
bruce55	3	3	3	3
Panel 2				
bruce18	8	6	6	7
bruce19[c]	–	–	–	21
bruce21	8	8	8	8
bruce04	6	6	6	4
bruce07	5	8	4	5
bruce09	3	3	3	3
bruce16	11	7	8	7
bruce30	5	7	4	3

[a] Brucella culture collection.
[b] Federal Institute for Risk Assessment.
[c] Additional locus within MLVA-16.

The level of agreement between the two diagnostic tests was measured through P_{pos} and P_{neg}. Our results suggest a good level of agreement when negative results for both tests were considered. Similarly, a significant correlation between the two tests for the non-infected population was found which could be related to the high Sp estimated for both tests. A good level of agreement was also reported for tests with high Sp by Dohoo et al. (1986). The agreement was only 52% for sera which tested positive on both assays. The status of seropositive animals can vary depending on whether the infection is acute or chronic (Saegerman et al., 1999, 2004). Moreover, the estimated sensitivity of the iELISA was almost twice that of the RBT so that seronegative animals in our context would have a higher probability of being false negatives if only the RBT was used.

Both results were concordant on almost 94% of the results from which 52% were seropositive. While the RBT detects the presence of IgM and IgG_1, the iELISA detects both IgG_1 and IgG_2 (Saegerman et al., 2010). As a result, the level of agreement for positive results correlated with the relatively high sensitivity observed for the iELISA, possibly due to the high proportion of IgG-positive sera and higher prevalence of chronically infected cases. This might also explain the relatively low sensitivity of the RBT. Given these characteristics, the combined use of these assays appears the most effective strategy in the diagnoses and the control of this disease in our setting.

Another objective of this study was to investigate the strains of *Brucella* spp. found in cattle in the Ivory Coast, and to verify the appropriateness of the antigen used in the serological tests (Saegerman et al., 2004). Pilo-Moron et al. (1979) identified *B. abortus* 1 and 6 as the two biovars present in the Ivory Coast. Of the 17 isolates collected throughout the country, four were identified as *B. abortus* 1, whereas seven were classified as *B. abortus* 6 in isolates from cattle in the centre of the country. In our case, an isolate obtained from a carpal hygroma was classified as biovar 3. Biovars 1, 3 and 6 were characterised by the same 'A' epitope used in serological tests in this study. This observation highlights the appropriateness of the testing strategy used (Saegerman et al., 2004).

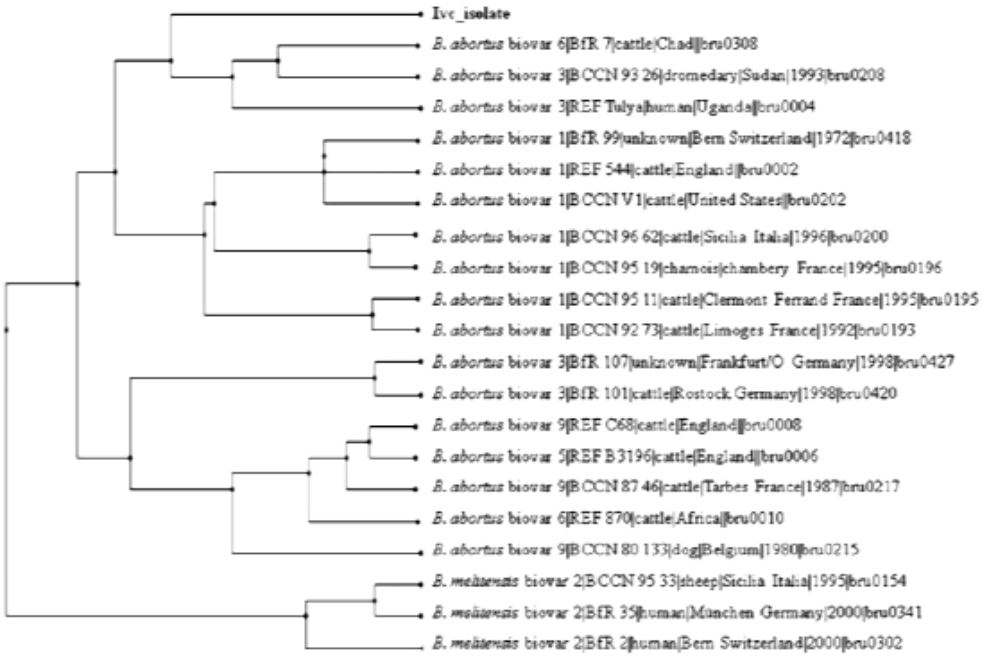

Fig. 2. Clustering analysis of a field strain of *Brucella abortus* 3 from the Ivory Coast (IVC-isolate) with field and reference strains in the *Brucella* multiple loci variable number tandem repeats analysis (MLVA) database (MVLABANK, 2010) using panels 1 and 2. The data are given in columns from left to right: species and biovar; strain reference (BCCN, *Brucella* Culture Collection, Nouzilly, INRA, France; BfR, Federal Institute for Risk Assessment, BfR, Berlin, Germany; REF, 21 reference and marine mammal strains prepared by BCCN); host; geographical origin; and year of isolation and 'alias'.

This is the first time biovar 3 has been isolated in the Ivory Coast. Biochemical characteristics led to this identification despite a negative oxidase reaction. A positive oxidase reaction is a typical feature of *Brucella* spp. except for *B. ovis* and *B. neotomae*. However, atypical characteristics such as a negative oxidase reaction may be encountered with some strains (Verger et al., 1979, 1982; Alton et al., 1988). In fact, some biovars of *B. abortus* from cattle in Africa are known to grow slowly, to be sometimes negative on the oxidase test and to have a specific oxidative pattern (Verger et al., 1979). Such atypical characteristics should be considered when typing *B. abortus* strains. Biovar 3 has also been typed in other West African countries such as Senegal and Togo. In Senegal, all but 1/181 isolates strains were reported to be biovar 3 with a negative oxidase reaction (Verger et al., 1979). In Togo, 30 strains were identified as biovar 3, with unusual growth characteristics and oxidative pattern (Verger et al., 1982).

The MLVA-16 method has been shown to be highly relevant and efficient in typing and clustering strains from both animal and human origin (Maquart et al., 2009), and our MLVA result was concordant with classical typing. Our isolate profile was biovar 3, a MLVA profile close to that of two strains isolated in Uganda and Sudan, respectively. These two African strains are known to exhibit some distinct phenotypical characteristics compared to other strains in the biovar 3 group (Le Flèche et al., 2006). The profile also appeared to share some genes with biovar 6 isolated in The Republic of Chad. Comparison with historical isolates was not possible as these were no longer available. An organism with a very similar MLVA profile was recently identified in hygroma fluid in Gambia (Bankole et al., 2010). This classification appears to be in agreement with the fact that isolates originating from Africa could significantly differ from those elsewhere and confirms the heterogeneity within this group (Le Flèche et al., 2006).

Conclusions

The iELISA had good performance parameters and consequently might be a valuable screening assay under the epidemiological conditions pertaining in the Ivory Coast. Our results highlight the need to use combined serological tests to obtain more reliable results and to ensure optimal disease diagnosis and control in the absence of a gold standard test.

Conflict of interest statement

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

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tvjl.2012.06.007.

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Appendix A. Supplementary material

WinBugs code for estimating prevalence and test characteristics for Rose Bengal Test (RBT) and indirect enzyme-linked Immunosorbent assay (iELISA).

```
model
{
r[1:4] ~ dmulti(p[1:4], n)
p[1] <- pi*(SeElisa*SeRBT+covDp) + (1-pi)*((1-SpElisa)*(1-SpRBT)+covDn)
p[2] <- pi*(SeElisa*(1-SeRBT)-covDp) + (1-pi)*((1-SpElisa)*SpRBT-covDn)
p[3] <- pi*((1-SeElisa)*SeRBT-covDp) + (1-pi)*(SpElisa*(1-SpRBT)-covDn)
p[4] <- pi*((1-SeElisa)*(1-SeRBT)+covDp) + (1-pi)*(SpElisa*SpRBT+covDn)
ls <- (SeElisa-1)*(1-SeRBT)
us <- min(SeElisa,SeRBT) - SeElisa*SeRBT
lc <- (SpElisa-1)*(1-SpRBT)
uc <- min(SpElisa,SpRBT) - SpElisa*SpRBT
pi ~ dunif(0, 1)
SeElisa ~ dunif(0.925,1)
SpElisa ~ dunif(0.906,1)
SeRBT ~ dunif(0.21, 0.983)
SpRBT ~dunif(0.688,1)
covDn ~ dunif(lc, uc)
covDp ~ dunif(ls, us)
rhoD <- covDp / sqrt(SeElisa*(1-SeElisa)*SeRBT*(1-SeRBT))
rhoDc <- covDn / sqrt(SpElisa*(1-SpElisa)*SpRBT*(1-SpRBT))
r2[1:4] ~ dmulti(p[1:4],n)
for ( i in 1:4)
{
d[i] <- r[i]*log(max(r[i],1)/(p[i]*n))
d2[i] <- r2[i]*log(max(r2[i],1)/(p[i]*n))
}
bayesp <- step(sum(d[]) - sum(d2[]))
}
list(r=c(35,55,9,896), n=995)
```

CHAPTER 6:

TRUE PREVALENCE OF BOVINE BRUCELLOSIS IN IVORY COAST

6.1. Introduction

The actual level of a disease in a population of interest, i.e. the true prevalence, is an essential parameter to assess the impact and importance of a disease in the population of interest and to avoid biased estimation of disease burden. Accuracy of true prevalence is related to performance parameters of tests to be applied. Estimation of test performance parameters and the true prevalence are two mathematically identical situations, even if the parameter of interest might change according to study objectives. By definition, estimation of sensitivity and specificity of a diagnostic test requires knowledge of the true disease status of animals on which this test is applied. This status is given by a reference test, which might be a "gold standard" test. In the absence of a "gold standard" test, a combination of available imperfect tests may be used for estimation. An appropriate methodology being needed for accurate estimation, a Bayesian approach was used to estimate the true prevalence of bovine brucellosis in the centre of Ivory Coast.

6.2. Prevalence of bovine brucellosis in Ivory Coast

This section constitutes the following original paper published in *La Revue d'élevage et de médecine vétérinaire des pays tropicaux*.

ARTICLE 4:

SANOGO M., CISSE B., OUATTARA M., WALRAVENS K., PRAET N., BERKVENS D., THYS E. Etude la prévalence de la brucellose bovine dans le centre de la Côte d'Ivoire. *Rev. Elev. Med. vet. Pays trop.,* 2008, **61 (3-4)**, 147-151.

Prévalence réelle de la brucellose bovine dans le centre de la Côte d'Ivoire

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Mots-clés

Bovin – *Brucella* – Brucellose – Technique immunologique – Morbidité – Côte d'Ivoire.

Résumé

Lors de l'enquête de sérosurveillance active de la peste bovine organisée en Côte d'Ivoire de juillet à août 2005, 660 sérums ont été collectés dans les régions administratives du N'Zi Comoé et des Lacs, au centre du pays. Ces sérums ont été utilisés pour faire une estimation de la prévalence réelle de la brucellose bovine en utilisant une approche statistique bayésienne avec quatre tests de diagnostic sérologiques : la séro-agglutination lente de Wright (SAW), le test du rose bengale (TRB), l'Elisa indirect (iElisa) et le test de fixation du complément (TFC). Les résultats de ce dernier n'ont pas été pris en compte dans cette étude. Les analyses sérologiques ont donné des séroprévalences individuelles de 6,2 p. 100 à la SAW, 5,1 p. 100 au TRB et 7,0 p. 100 à l'iElisa. L'analyse bayésienne faite avec ces mêmes résultats sérologiques a permis d'obtenir une estimation de la prévalence réelle de la brucellose de 8,8 p. 100 (intervalle de crédibilité à 95 p. 100 : 5,0–16,4). Ces résultats constituent une bonne indication de l'importance de la brucellose dans cette partie de la Côte d'Ivoire et confirment l'attention qui doit être accordée à cette maladie.

■ INTRODUCTION

L'élevage occupe une place importante pour les populations africaines. Le développement de cet élevage est cependant sous la contrainte de nombreux facteurs dont les contraintes pathologiques. Outre leur impact sur la santé des animaux, certaines de ces pathologies, peuvent aussi causer des problèmes de santé publique. C'est le cas de la brucellose (1, 6, 10), maladie fortement pathogène, due à des bactéries du genre *Brucella*. Affectant aussi bien les humains que de nombreuses espèces animales domestiques et sauvages, elle est considérée comme l'une des zoonoses les plus répandues dans le monde (8, 17). Chez les animaux, la brucellose bovine, essentiellement due à *Brucella abortus*, reste la plus répandue en Afrique (7, 15). Connue depuis de nombreuses années en Côte d'Ivoire, cette maladie du bétail y est considérée comme une dominante pathologique (4). A ce titre, diverses études épidémiologiques lui ont ainsi été consacrées, mais elles sont relativement peu nombreuses et peu récentes (4, 6, 13, 18). S'inscrivant dans la continuité de l'étude de Thys et coll. en 2005 (24), la présente étude a eu pour objectif de contribuer à l'actualisation des connaissances épidémiologiques sur la brucellose bovine dans le centre de la Côte d'Ivoire et, notamment, dans les régions du N'Zi Comoé et des Lacs.

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■ MATERIEL ET METHODES

Contexte de l'étude

Les régions du N'Zi Comoé et des Lacs sont localisées au centre du pays, à environ 300 km au nord d'Abidjan, la capitale économique du pays. Elles sont subdivisées administrativement en plusieurs départements (figure 1). Le centre de la Côte d'Ivoire est une zone intermédiaire entre le nord (relativement sec) et le sud (humide) du pays, aussi bien au niveau du climat que de la végétation.

Figure 1 : *zone d'étude avec les localités de Bongouanou, Dimbokro, Toumodi, Yamoussoukro et Tiébissou (Côte d'Ivoire).*

Habituellement, cette zone est occupée par des élevages bovins de type sédentaire avec parfois quelques troupeaux transhumants. Le mode d'élevage dominant est de type traditionnel sédentaire avec le recours au pâturage comme principale source alimentaire. Le cheptel est constitué de taurins (*Bos taurus*) (N'Dama, Baoulé) et de zébus (*Bos indicus*) transhumants. On y trouve aussi des métis issus de croisements entre zébus et taurins.

Données de l'étude

Les données utilisées dans cette étude sont issues du N'Zi Comoé et des Lacs. Elles ont été collectées lors d'une enquête de sérosurveillance organisée en 2005 dans le cadre des activités du programme panafricain de contrôle des épizooties en Côte d'Ivoire. Cette enquête a eu pour objectif de détecter la présence de la peste bovine, avec un niveau de confiance de 95 p. 100 en supposant une prévalence de 1 p. 100 au niveau des troupeaux et de 5 p. 100 au sein des troupeaux du pays (14). Un échantillon aléatoire de 15 animaux de plus d'un an d'âge a été sélectionné dans chaque troupeau sélectionné. A chaque fois, des informations complémentaires (race, sexe, âge, localité, nom du propriétaire) ont été collectées.

Ainsi, 660 sérums bovins ont été collectés dans les 44 troupeaux localisés dans les cinq départements de ces deux régions, puis utilisés dans le cadre de cette étude de prévalence (tableau I). Cet échantillon était composé essentiellement de femelles issues de troupeaux traditionnels sédentaires, avec environ deux tiers des bovins métis et zébus, d'une part, et deux tiers des bovins de plus de trois ans, d'autre part (tableau II).

Analyses sérologiques

Les sérums ont été analysés au Centre d'étude et de recherches vétérinaires et agrochimiques (Cerva, Bruxelles, Belgique) en utilisant quatre tests sérologiques de diagnostic de la brucellose : la séro-agglutination lente de Wright (SAW), le test au rose bengale (TRB), le test d'Elisa indirect (iElisa) et le test de fixation du complément (TFC). Ces tests ont été appliqués selon Shey-Njila et coll. (22), et Thys et coll. (24).

Tableau I

Nombre de troupeaux et de sérums collectés par région et par département

Région	Département	Nb. de troupeaux	Nb. de sérums collectés
N'Zi	Bongouanou	15	225
Comoé	Dimbokro	12	180
Lacs	Tiébissou	7	105
	Toumodi	5	75
	Yamoussoukro	5	75
Total		44	660

Tableau II

Composition de l'échantillon par sexe, âge et race

Sexe	Age	Race			Total
		Taurin	Zébu	Zébu x Taurin	
Femelle	1 à 3 ans	67	20	127	214
	> 3 ans	115	20	162	297
Total femelle		182	40	289	511
Mâle	1 à 3 ans	42	14	72	128
	> 3 ans	4	4	13	21
Total mâle		46	18	85	149
Total (femelle + mâle)		228	58	374	660

Analyse statistique bayésienne

Pour l'estimation de la prévalence réelle, une approche bayésienne a été utilisée (5, 19). Cette technique d'analyse statistique a consisté à combiner les résultats des tests sérologiques dans un modèle dit bayésien avec des informations d'experts (information *a priori*) sur la sensibilité et la spécificité des tests utilisés, pour obtenir une estimation de la prévalence réelle *a posteriori*. Cette analyse a été faite à l'aide d'un modèle mathématique multinomial construit à partir des différentes combinaisons possibles de résultats avec les différents tests sérologiques pour chaque sérum. Ce modèle a été basé sur les probabilités conditionnelles (par exemple, la probabilité qu'un individu teste positif au test 2, étant donné qu'il était infecté et positif au test 1) et les éventuelles dépendances conditionnelles entre les tests appliqués dans l'analyse (5). Les informations *a priori*, utiles pour permettre d'avoir des estimations, ont été traduites sous la forme de contraintes sur les valeurs possibles des caractéristiques (sensibilité et spécificité) des tests employés dans ce modèle multinomial (annexe). La concordance de celles-ci avec les résultats d'analyse sérologique ainsi que la validation du modèle utilisé ont été évaluées parallèlement au cours de l'analyse. Les opinions d'expert utilisées dans l'analyse ont été les mêmes que celles utilisées par Thys et coll. (24) et extraites de la synthèse faite par Nielsen sur le diagnostic sérologique de la brucellose (16) (tableau III). Cette analyse a été faite à l'aide du logiciel Winbugs® (5, 19, 23). En raison d'un grand

nombre de résultats ininterprétables au TFC, les résultats de ce dernier test n'ont pas été inclus dans l'analyse.

Tableau III

Informations *a priori* utilisées pour l'analyse bayésienne

Test	Sensibilité (%)	Spécificité (%)
SAW	29,1 – 100	99,2 – 100
TRB	21 – 98,3	68,8 – 100
TFC	23 – 97,1	30,6 – 100
iElisa	92,5 – 100	90,6 – 100

Source : Nielsen, 2002

SAW : séro-agglutination lente de Wright ; TRB : test du rose bengale ; TFC : test de fixation du complément ; iElisa : Elisa indirect

Annexe

Modèle bayésien utilisé pour l'estimation de la prévalence réelle avec les informations *a priori* de Nielsen sur la sensibilité et la spécificité du test de séro-agglutination lente de Wright.

Les p[i] sont les différents résultats possibles en fonction du nombre de tests utilisés.

Les θ[i] sont les probabilités conditionnelles.

```
model
{
res[1:8] ~ dmulti(p[1:8],n)
p[1]<-θ[1]*(1-θ[2])*(1-θ[5])*(1-θ[11])+(1-θ[1])*θ[3]*θ[6]*θ[12]
p[2]<-θ[1]*(1-θ[2])*(1-θ[5])*θ[11]+(1-θ[1])*θ[3]*θ[6]*(1-θ[12])
p[3]<-θ[1]*(1-θ[2])*θ[5]*(1-θ[10])+(1-θ[1])*θ[3]*(1-θ[6])*θ[13]
p[4]<-θ[1]*(1-θ[2])*θ[5]*θ[10]+(1-θ[1])*θ[3]*(1-θ[6])*(1-θ[13])
p[5]<-θ[1]*θ[2]*(1-θ[4])*(1-θ[9])+(1-θ[1])*(1-θ[3])*θ[7]*θ[14]
p[6]<-θ[1]*θ[2]*(1-θ[4])*θ[9]+(1-θ[1])*(1-θ[3])*θ[7]*(1-θ[14])
p[7]<-θ[1]*θ[2] *θ[4]*(1-θ[8])+(1-θ[1])*(1-θ[3])*(1-θ[7])*θ[15]
p[8]<-θ[1]*θ[2]*θ[4]*θ[8]+(1-θ[1])*(1-θ[3])*(1-θ[7])*(1-θ[15])
res1[1:8] ~ dmulti(p[1:8],n)
for (i in 1:8)
{
di[i]<-res[i]*log(max(res[i],1)/p[i]*n))
di2[i]<-res1[i]*log(max(res1[i],1)/p[i]*n))
}
G0<-2*sum(di[])
Gt<-2*sum(di2[])
bayesp<- step(G0-Gt)
θ[1] ~ dbeta(1,1)
θ[2] ~ dbeta(1,1)I(0.291,1)
θ[3] ~ dbeta(1,1)I(0.992,1)
θ[4] ~ dbeta(1,1)
θ[5] ~ dbeta(1,1)
θ[6] ~ dbeta(1,1)
θ[7] ~ dbeta(1,1)
θ[8] ~ dbeta(1,1)
θ[9] ~ dbeta(1,1)
θ[10] ~ dbeta(1,1)
θ[11] ~ dbeta(1,1)
θ[12] ~ dbeta(1,1)
θ[13] ~ dbeta(1,1)
θ[14] ~ dbeta(1,1)
θ[15] ~ dbeta(1,1)
se[1]<- θ[2]
sp[1]<- θ[3]
se[2]<- θ[2]*θ[4]+(1-θ[2])*θ[5]
sp[2]<- θ[3]*θ[6]+(1-θ[3])*θ[7]
se[3]<-θ[2]*θ[4]*θ[8]+θ[2]*(1-θ[4])*θ[9]+(1-θ[2])*θ[5]*θ[10]+(1-θ[2])*(1-θ[5])*θ[11]
sp[3]<-θ[3]*θ[6]*θ[12]+θ[3]*(1-θ[6])*θ[13]+(1-θ[3])*θ[7]*θ[14]+(1-θ[3])*(1-θ[7])*θ[15]
}
list(res=c(542,5,18,8,18,3,2,15), n=611)
```

■ RESULTATS

Résultats sérologiques

Les résultats sérologiques bruts obtenus sont résumés dans le tableau IV. Les séroprévalences par test sont présentées dans le tableau V.

Résultats de l'analyse statistique bayésienne

L'analyse bayésienne à partir des résultats sérologiques a permis d'obtenir une estimation de la prévalence réelle avec un intervalle de crédibilité de 95 p. 100. Les meilleures estimations ont été obtenues en utilisant les informations *a priori* fournies par Nielsen sur la SAW. Les résultats sont résumés dans le tableau VI.

Tableau IV

Résultats sérologiques bruts pour les différents tests inclus dans l'analyse

SAW	TRB	iElisa	Nb. de sérums
0	0	0	542
0	0	1	18
0	1	0	5
0	1	1	8
1	0	0	18
1	0	1	2
1	1	0	3
1	1	1	15
			611

SAW : séro-agglutination lente de Wright ; TRB : test du rose bengale ; iElisa : Elisa indirect

0 : test négatif ; 1 : test positif

Tableau V

Séroprévalences et intervalles de crédibilité (IC) à 95%

Test	Nb. de positifs	Séroprévalence (%)	IC
SAW	38	6,2	4,4 – 8,4
TRB	31	5,1	3,5 – 7,1
iElisa	43	7,0	5,1 – 9,4

SAW : séro-agglutination lente de Wright ; TRB : test du rose bengale ; iElisa : Elisa indirect

Tableau VI

Prévalence réelle et intervalle de crédibilité (IC) à 95%

Test appliqué	Prévalence réelle (%)	IC
SAW TRB iElisa	8,8	5,0 –16,4

SAW : séro-agglutination lente de Wright ; TRB : test du rose bengale ; iElisa : Elisa indirect

■ DISCUSSION

Les estimations de séroprévalence se sont appuyées sur des données collectées dans un autre cadre et avec un objectif différent de l'estimation d'une prévalence. L'utilisation de telles données a permis d'obtenir de façon rapide et à moindre coût des informations sur la brucellose bovine. Cependant, elle peut présenter quelques limites (20). La taille de l'échantillon ainsi que la bonne distribution spatiale des troupeaux sélectionnés ont paru satisfaisant pour utiliser ces données. Par ailleurs, ce type de données a été déjà utilisé dans des études de prévalences (25).

En considérant les différents tests individuellement, la séroprévalence de la brucellose dans la zone d'étude se situait entre 3,5 à 9,4 p. 100, avec respectivement des estimations de 6,2, 5,1 et 7,0 p. 100 pour les tests SAW, TRB et iElisa. Cela confirme la présence de la brucellose dans cette zone. Cependant dans le cas de la brucellose, l'application isolée des tests sérologiques usuels tel que la SAW, le TRB et l'iElisa ne donnent pas d'information suffisante sur le statut réel des animaux et rend difficile l'estimation de la prévalence. Par ailleurs, bien que la vaccination susceptible d'inférer avec ces tests ne soit pas pratiquée en Côte d'Ivoire, d'autres organismes ayant des caractéristiques antigéniques proches, comme *Yersinia enterocolitica* O:9, *Xanthomonas maltophilia* et *Salmonella urbana*, sont capables de donner une sérologie faussement positive (21).

En l'absence de tests parfaits pour le diagnostic de la brucellose, une combinaison de tests sérologiques a été utilisée afin d'obtenir des estimations plus fiables, de réduire les erreurs de classification et d'améliorer la sensibilité et la spécificité du diagnostic. Une prévalence réelle de 8,8 p. 100 (intervalle de crédibilité de 95 p. 100 : 5,0–16,4) a été ainsi obtenue. Cette estimation était supérieure à celles fournies par les tests individuels. Elle était supérieure, voire le double, de celle obtenue par Thys et coll. en 2005 (24) dans le sud de la Côte d'Ivoire (région des Lagunes) en utilisant cette même approche.

Comparativement au sud, la partie centrale de la Côte d'Ivoire est plus favorable à l'élevage des bovins. De plus grands espaces et pâturages favorisent des densités et concentrations de troupeaux plus grandes ainsi que les mouvements d'animaux, facteurs pouvant augmenter le risque de contact et de dissémination de l'infection (1). Par ailleurs, avec la crise sociopolitique que vit la Côte d'Ivoire depuis 2002, les régions du N'Zi Comoé et des Lacs ont accueilli d'autres troupeaux venus du Nord pour se sédentariser dans ces zones, ce qui a pu influencer également la prévalence estimée par la présente étude. Près de deux tiers des animaux inclus dans cette étude étaient âgés d'au moins trois ans, ce qui pouvait avoir une influence ; la prévalence de la brucellose serait plus élevée chez les animaux âgés (2, 12). Cette estimation, bien que proche des valeurs de 11 à 14 p. 100 et de 10,9 p. 100 obtenues respectivement par Angba et coll. en 1987 (4) et Pilo-Moron et coll. en 1979 (18) en Côte d'Ivoire, reste difficilement comparable à celles-ci en raison de la différence d'approche. Ces estimations étaient basées sur un seul test ou tout au plus sur deux tests en complémentarité.

D'autres études de prévalences ont été également faites sur la brucellose bovine en Afrique et dans la sous-région ouest-africaine et ont permis d'obtenir diverses estimations (2, 3, 10, 15, 25). La différence des tests appliqués, les différentes échelles de ces études et surtout la différence de méthodologie rendent difficile toute comparaison avec l'estimation de la présente étude. Dans la plupart des cas précédents, un seul test (TRB ou TFC ou Elisa ou SAW) ou parfois une association de tests a été utilisé. Ainsi, dans des études récentes, Delafosse et coll. (9), et Faye et coll. (12) ont respectivement estimé des prévalences de 2,6 p. 100 au Tchad et de 15,8 p. 100 en Ouganda en s'appuyant sur des résultats du TRB ou du TRB associé au TFC. Shey-Njila et coll. (22) ont également trouvé une prévalence de l'ordre de 10 p. 100 en s'appuyant sur des résultats d'Elisa au Cameroun.

■ CONCLUSION

Cette étude a eu pour objectif d'apporter de nouvelles connaissances sur l'épidémiologie de la brucellose bovine en Côte d'Ivoire. Elle a permis de confirmer la présence de cette maladie dans la zone Centre du pays, avec une prévalence réelle estimée à 8,8 p. 100 (intervalle de crédibilité 95 p. 100 : 5,0–16,4). Cette estimation constitue une bonne indication de l'importance de la brucellose dans cette zone et confirme l'attention qui doit être accordée à cette maladie. Toutefois, il est important de prendre en considération le fait que cette estimation a été faite à partir de sérums déjà existants, dont le mode de collecte pouvait avoir une influence. Il est également important de noter que, dans l'approche bayésienne, les informations *a priori* utilisées ont une influence sur l'estimation de la prévalence réelle, d'où l'intérêt de considérer des opinions d'experts les plus fiables possibles. Toutefois, cette approche, en améliorant la sensibilité et la spécificité de diagnostic, et comparée à l'utilisation d'un test unique imparfait, fournit un résultat relativement fiable.

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Summary

Sanogo M., Cissé B., Ouattara M., Walravens K., Praet N., Berkvens D., Thys E. Real Prevalence of Bovine Brucellosis in the Center of Ivory Coast

During a serosurveillance survey carried out from July to August 2005 in Ivory Coast, 660 sera were collected in the administrative regions of N'Zi Comoe and Lacs, in the central part of the country. The sera were used to determine the real prevalence of bovine brucellosis using a Bayesian framework with four serological diagnostic tests: slow agglutination of Wright (SAW), rose bengale test (RBT), indirect ELISA (iELISA) and complement fixation test (CFT). CFT results were not taken into account in the study. The serological analyses showed individual seroprevalences of 6.2% with SAW, 5.1% with RBT and 7.0% with iELISA. The Bayesian analysis performed with the same serological results helped to estimate at 8.8% the real prevalence of brucellosis (credibility interval of 95%: 5.0–16.4). Results show the importance of brucellosis in this part of Ivory Coast and confirm the attention that must be given to the disease.

Keywords: Cattle – *Brucella* – Brucellosis – Immunological technique – Morbidity – Cote d'Ivoire.

Resumen

Sanogo M., Cissé B., Ouattara M., Walravens K., Praet N., Berkvens D., Thys E. Prevalencia real de la brucelosis bovina en el centro de Costa de Marfil

Durante la encuesta de vigilancia activa de la peste bovina organizada en Costa de Marfil entre julio y agosto 2005, se recolectaron 660 sueros en las regiones administrativas de N'Zi Comoé y de los Lagos, en el centro del país. Estos sueros fueron utilizados para obtener una estimación de la prevalencia real de la brucelosis bovina, utilizando un enfoque estadístico bayesiano con cuatro tests de diagnóstico serológico: la aglutinación serológica lenta de Wright (ASW), el test de rosa bengala (TRB), el Elisa indirecto (iElisa) y el test de fijación de complemento (TFC). Los resultados de este último no fueron tomados en cuenta en este estudio. Los análisis serológicos dieron prevalencias serológicas individuales de 6,2% con la ASW, 5,1% con TRB y 7,0% con iElisa. El análisis bayesiano realizado con estos mismos resultados serológicos permitió obtener una estimación de la prevalencia real de la brucelosis de 8,8% (intervalo de credibilidad de 95%: 5,0-16,4). Estos resultados constituyen una buena indicación de la importancia de la brucelosis en esta parte de Costa de Marfil y confirman la atención que se debe dar a esta enfermedad.

Palabras clave: Ganado bovino – *Brucella* – Brucelosis – Técnica inmunológica – Morbosidad – Cote d'Ivoire.

CHAPTER 7:

RISK FACTORS ASSOCIATED WITH BOVINE BRUCELLOSIS SEROPOSITIVITY IN IVORY COAST

7.1. Introduction

In addition to knowledge on circulating field strains of *Brucella* and availability of adequate diagnostic tests, identification of potential risk factors associated with the disease is also useful for developing and implementing preventive and control measures. Such knowledge might be useful to increase the awareness of farmers, and regulating herd management practices with the ultimate aim of to decrease the prevalence of brucellosis among livestock in Ivory Coast.

7.2. Risk factors associated with brucellosis seropositivity among cattle in the central savannah-forest area of Ivory Coast

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Risk factors associated with brucellosis seropositivity among cattle in the central savannah-forest area of Ivory Coast

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ABSTRACT

Serological results obtained from 907 serum samples collected from unvaccinated cattle of at least 6 months of age in the savannah-forest region of Ivory Coast were used to investigate risk factors associated with bovine brucellosis seropositivity. Serum samples were tested using the Rose Bengal test (RBT) and indirect enzyme linked immunosorbent assay (iELISA). Using a parallel interpretation, RBT and iELISA results showed that 10.3% (95% confidence interval (CI): 8.4, 12.4) of the cattle had antibodies against *Brucella* in our study area. The logistic regression analysis indicated that brucellosis seropositivity was associated with age and herd size. Cattle above 5 years of age were found to have a higher chance of being seropositive (odd ratio (OR) = 2.8; 95% CI: 1.3, 6.4) compared to cattle under 3 years of age. Similarly, the odd of brucellosis seropositivity for herds with more than 100 cattle was 3.3 (95% CI: 1.2, 8.9) times higher compared to those with less than 50 cattle.

1. Introduction

Bovine brucellosis is a widespread infectious disease caused by Gram negative bacteria of the genus *Brucella*. The infection is generally caused by one of the *Brucella abortus* biovars (1, 2, 3, 4, 5, 6 and 9). However, some cases of bovine brucellosis have been occasionally related to *Brucella melitensis* and *Brucella suis* (Verger et al., 1989; Godfroid et al., 2005; Olsen and Hennager, 2010).

As in most African countries, bovine brucellosis has been a problem among livestock for many years in Ivory Coast (Gidel et al., 1974; Pilo-Moron et al., 1979; Camus, 1980a; Angba et al., 1987; Thys et al., 2005; Sanogo et al., 2008). The first evidence of the disease was provided by Bohnel during a serological study in the northern part of the country in 1970 (Pilo-Moron et al., 1979). Thereafter, several studies have been conducted to assess the impact of the disease on livestock production. For example, a national survey estimated the seroprevalence of brucellosis to be around 11.3% (Angba et al., 1987). Estimates obtained during earlier investigations undertaken in the Guinean region were 3.6% and 4.2% in dairy farms and traditional herds, respectively (Thys et al., 2005). In the central savannah-forest region of the country, the true prevalence was found to range between 5 and 16% in traditional herds (Sanogo et al., 2008).

Through its negative impacts such as abortions, decreases in milk production, increases in calf mortality, infertility and veterinary costs, bovine brucellosis is one of the most important pathological constrains for livestock development in Ivory Coast. Angba et al. (1987) estimated the impact of bovine brucellosis to be around 10% of the

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annual income of livestock breeders in Ivory Coast. Consequently, vaccination was practised from 1978 to 1982 and also in the 1990s to reduce abortion and mortality rates of brucellosis and limit its impacts (Camus, 1980b, 1995; Angba et al., 1987). Currently, there is no official control or official vaccination programme against brucellosis in Ivory Coast.

Previous investigations mainly demonstrated the presence of antibodies against *Brucella* in different areas of Ivory Coast and/or characterized circulating strains of *B. abortus* (Sanogo et al., unpublished data) but potential risk factors associated with bovine brucellosis have not been reported. The aim of this study was therefore to assess and describe potential risk factors associated with its seropositivity among cattle in the central Soudano-Guinean regions of Ivory Coast. The results of this study might be helpful for developing and implementing control measures aimed at sensitizing farmers, regulating herd management practices and ultimately on abating the prevalence of brucellosis among livestock in Ivory Coast.

2. Materials and methods

2.1. Study area and data

Ivory Coast is a West African country located between 3–9° West and 5–11° North. It is divided into three main agro-ecological areas: *The Guinean zone*, which is the most humid with annual rainfall generally above 1500 mm, covers almost the whole forest region in the south. *The Soudanean zone* is in the northern savannah regions and registers between 900 and 1200 mm of rainfall per year. *The Soudano-Guinean zone* covers the central savannah-forest regions and serves as a transition zone between the south and the north with annual rainfall of 1200–1500 mm. The northern savannah and central savannah-forest regions are the main livestock breeding areas of the country with nearly 80% of the one million and a half cattle heads of the country (FAO, 2009). Four cattle breeds belonging to two main types are encountered in the country: The *N'Dama*, the *Baoulé*, the *Lagunaire* which are West African humpless shorthorn breeds (*Bos taurus* type) and the longhorn humped zebus of *Bos indicus* type. In addition, various cross-bred of these two main types (*B. taurus* × *B. indicus*) are also met. All types are met in the central Soudano-Guinean zone where samples used in this study were obtained.

The data of this study are composed of two datasets obtained during two surveys undertaken, respectively three (dataset 1) and seven years (dataset 2) after the socio-political crisis of 2002 on sedentarily managed cattle herds in the savannah-forest areas of Ivory Coast. The first dataset comprised data collected in 2005 as part of a sero-epidemiological survey on rinderpest. Forty-four cattle herds out of 214 herds of the savannah-forest area were randomly selected from a list of 1265 herds. Fifteen blood samples were taken in each selected herd. The second dataset was built from data collected in 2009 during a survey of brucellosis. Six cattle herds with a history of abortion were conveniently chosen and sampled. At least 25 cattle were blooded within each herd. In both cases, only cattle above six months of age were included.

A total of 1036 blood samples were collected and submitted to serological assays among which were 660 and 376 samples, respectively for 2005 and 2009. Each blood sample was collected with information on the date of collection, the locality of origin, the type of breed, age, sex and size of the herd of origin. Since the early 1990s, there is no official control program or official vaccination against brucellosis in Ivory Coast.

2.2. Serological analysis

After collection, blood samples were allowed to clot at ambient temperature and centrifuged at 3000 rpm for 10 min. Sera samples were collected and stored at −20 °C until testing. Each serum sample was screened for anti-*Brucella* antibodies by Rose Bengal test (RBT) and indirect enzyme linked immunosorbent assay (iELISA). RBT was applied as described by Alton et al. (1988) by mixing 30 μL of serum with 30 μL of antigen (*B. abortus* strain Weybridge 99) on a glass plate. After 4 min of reaction, the test was recorded as negative when no agglutination occurred and considered positive whenever a level of agglutination was noticed. For the indirect ELISA, the test was done as previously described by Limet et al. (1988) and Godfroid et al. (2002) using *B. abortus* biotype 1 (Strain Weybridge 99, A epitope) as antigen, protein G-Horseradish Peroxidase (G-HRP) as bovine anti-IgG conjugate and serum No. 1121 as positive reference. Briefly, 50 μL of serum were diluted in duplicate on plates and incubated at room temperature. After 1 h, antibodies binding were revealed by protein G-HRP conjugate. Conjugate was also incubated for 1 h at room temperature. Then, protein G activity was revealed by a citrate-phosphate buffer containing 0.4% of O-phenylenediamine and 2 mM of hydrogen peroxide (H_2O_2). Optical densities (OD) were read at 492 nm with a differential wavelength of 620 nm. Seropositivity was determined at a cut-off value of 2 unities, defined as threshold.

Serological status for a given sera was given by a parallel test interpretation of the results of the two tests. Thus, a serum was regarded as serologically positive when a positive result was recorded for one of both RBT and iELISA. Assuming the sensitivity (Se) and specificity (Sp) values for both RBT and iELISA to be: Se (iELISA) = 96.1% (95% Credibility Interval (Cr.I.): 92.7, 99.8), Sp (iELISA) = 95.0% (95% Cr.I.: 91.2, 99.5), Se (RBT) = 54.9% (95% Cr.I.: 23.5, 95.1), Sp (RBT) = 97.7% (95% Cr.I.: 95.4; 99.4) in our context (Sanogo et al., unpublished data), the combined diagnostic sensitivity and specificity for RBT and iELISA interpreted in parallel were estimated to be, respectively 98% and 93% (Thrusfield, 2005).

2.3. Statistical analysis

Serological results (seronegative = 0/seropositive = 1) and information on locality of origin, herd size (<50, 50–100, >100), breed (*B. taurus*, *B. indicus*, cross-bred), age (<3 years, 3–5 years, ≥5 years) and sex (cows or bulls) were recorded for all cattle considered for the study.

Table 1
Serological detection of antibodies against *Brucella* among 907 unvaccinated cattle from the Soudano-Guinean areas of Ivory Coast using Rose Bengal test (RBT) and indirect enzyme linked immunosorbent assay (iELISA).

Serological results	Number of cases (%)
RBT (+)	44(4.9%)
RBT (−)	863(95.1%)
iELISA (+)	84(9.3%)
iELISA (−)	823(90.7%)
RBT (+) iELISA (+)	35(3.9%)
RBT (+) iELISA (−)	9(1.0%)
RBT (−) iELISA (+)	49(5.4%)
RBT (−) iELISA (−)	814(89.7%)
RBT or iELISA (+)	93(10.3%)
RBT or iELISA (+)	814(89.7%)

RBT: Rose Bengal test; iELISA: indirect enzyme linked immunosorbent assay; (+): positive; (−): negative.

Unconditional and conditional logistic regression models were used to assess the association between brucellosis seropositivity and risk factors by setting herds as primary sampling unit and by including weights derived from the sampling fraction in each selected herd. A single stratum was considered. The analysis was conducted in two steps. Firstly, unconditional regression models were used to investigate the association between each risk factor and brucellosis seropositivity. Based on the unconditional analysis, only variables with $p \leq 0.25$ were considered for further analysis. The second stage of the analysis consisted in building a conditional logistic regression model based the potential risk factors identified from the unconditional models. The most appropriate model was selected using the backward stepwise selection approach. The effects of confounding were investigated by observing the changes in the estimated odds ratios of the variables that remained in the model once a non-significant variable was included. When the addition of a variable led to a change of more than 25% in the estimated regression coefficients or odds ratios, that variable was considered as a confounder and was not removed from the model. All pairwise interactions between the variables in the final model were examined for significance.

Goodness of fit of the final model was assessed using the *F*-adjusted mean residual test (Archer and Lemeshow, 2006; Archer et al., 2007). All statistical analyses were performed using STATA, version 12, software (Stata Corp., College Station, Texas). Due to differences in sampling period and strategy across the surveys, the two datasets were analysed separately and the results were compared.

3. Results

3.1. Serological results

A total of 907 out of 1036 blood samples collected gave serological results to both RBT and iELISA and were considered for further analysis. Both combined and separate serological results for RBT and iELISA are presented in Table 1. Together, the two tests identified 93/907 (10.3%) sera as serologically positive. When a single test was used, only 84/93 (90.3%) and 44/93 (47.3%) were, respectively classified as serologically positive by iELISA and RBT. Indirect ELISA identified almost twice more sera (9.3%) as positive compared to RBT (4.9%). Serological results adjusted for the survey design effect for dataset 1 indicated that 11.2% (95% CI: 5.4, 17.1) of the sera tested ($n = 614$) had antibodies against *Brucella* using a parallel interpretation scheme with the two tests. Highest seropositive results were registered among cattle above 5 years of age (17.6%), among cattle sampled from herds with more than 100 heads (14.1%) and among cattle of *B. taurus* type (16.4%). Seropositivity was apparently quite the same among *B. indicus* and cross-bred types, ranging between 3.5 and 13.1% in our study area. Cows represented more than 80% of the study population and showed apparently almost the same proportion of seropositivity cases compared to bulls. Twenty-seven herds out of 44 sampled (61.4%; 95% CI: 45.5, 75.6) had at least one seropositive cattle for brucellosis in our study area.

For the convenient sample ($n = 293$), the seroprevalence of antibodies against brucellosis was estimated to be 15.5% (95% CI: 0.0, 30.6). The number of serologically positive sera among different factors showed the same trends as for dataset 1 except for breeds where cross-bred (21.9%, 95% CI: 0.0, 43.4) had twice more seropositive sera than *B. indicus* (10.5%, 95% CI: 0.1, 11.8). In this sample, four herds out of 6 gave at least one cattle positive to both RBT and iELISA. Serological results per variable are summarized in Table 2 for both datasets.

3.2. Logistic regression analysis

Based on the unconditional regression model, factors as age, herd size, breed and locality showed a p value < 0.25 and were considered as potential risk factors in the conditional logistic regression model. Out of these four potential risk factors, only age and herd size were included in the final model. None of the two-way interaction terms were statistically significant ($p > 0.05$). In addition, there were no confounding factors. The estimated odds ratios and their 95% confidence intervals are presented in Table 3. The *F*-adjusted mean residual goodness of fit test suggested no evidence of lack of fit of the final model using dataset 1 (*F*-adjusted test statistic = 1.08, $p = 0.4$).

These results suggest that cattle above 5 years of age had significantly higher odds of brucellosis seropositivity compared to those younger than 3 years old (OR = 2.8; 95% CI: 1.3, 6.3). Similarly, the odds of brucellosis seropositivity for herds with more than 100 cattle were 3.3 (95% CI: 1.2, 8.9) times higher compared to those with less than 50 cattle (Table 3).

Logistic regression analysis of the convenient sample (dataset 2) led to almost the same conclusion where only age was found to be independently associated with the seropositivity of brucellosis with quite similar odds (OR = 2.5; 95% CI: 1.5, 4.2). In the latter case, herd size was not proven to be a significant risk factor for brucellosis seropositivity.

Table 2
Potential risk factors associated with brucellosis seropositivity among cattle from Soudano-Guinean zone of Ivory Coast using parallel interpretation of Rose Bengal test (RBT) and indirect enzyme linked immunosorbent assay (iELISA). The results are based on data from a simple random sample collected in 2005 and a convenient sample collected in 2009.

Variables	Category	Dataset 1 (2005)		Dataset 2 (2009)	
		n	Seropositivity (95% CI)	*n*	Seropositivity (95% CI)
Locality[a,b]	Bongouanou	210	9.5(2.5,16.6)	–	–
	Dimbokro	177	17.1(7.0,27.1)	104	19.6(0.0,43.7)
	Tiebissou	90	5.5(1.8,9.2)	–	–
	Toumodi	66	6.2(3.3,9.2)	25	0.0(0.0,11.3)
	Yamoussoukro	71	3.8(0.0,8.6)	164	14.9(0.0,34.7)
Herd size[a]	<50	256	6.4(3.3,9.4)	51	0.0(0.0,5.7)
	50–100	282	10.8(4.9,16.7)	–	–
	>100	76	14.1(1.6,26.7)	242	17.7(1.5,33.9)
Breed[a,b]	*B. indicus*	44	8.3(3.5,13.1)	38	10.5(9.1,11.8)
	Cross-bred	354	7.9(3.6,12.2)	117	21.9(0.3,43.4)
	B. taurus	216	16.4(5.4,27.4)	138	12.2(0.0,32.0)
Sex	Bulls	134	10.9(3.8,18.0)	27	13.4(0.0,30.3)
	Cows	480	11.3(5.5,17.1)	266	15.7(0.6,30.8)
Age[a,b]	<3	203	8.8(0.6,16.9)	63	9.7(0.0,24.9)
	3–5	201	8.5(2.5,14.5)	148	14.8(3.8,25.8)
	≥5	210	17.6(10.4,24.7)	82	21.5(0.0,48.0)

CI: confidence interval; *n*: number of sera tested.
[a] Variables with $p < 0.25$ in dataset 1 (2005).
[b] Variables with $p < 0.25$ in dataset 2 (2009).

4. Discussion

The main objective of this study was to investigate the potential risk factors associated with brucellosis seropositivity among cattle reared in Ivory Coast. Thus, serological results obtained from two sero-epidemiological surveys undertaken in Soudano-Guinean savannah-forest region with 4 years interval were used for this purpose. The data came from the same non-vaccinated target cattle population, managed sedentarily in absence of any official control programme.

Without an appropriate gold standard test useful to detect infected animals with certainty, results of RBT and iELISA were used as outcome variable for this risk factor analysis. The two tests are convenient and suitable serological tests for screening of brucellosis antibodies (Saegerman et al., 2004; Corbel, 2006). They are regarded as very sensitive tests but may demonstrate some limitations as other serological tests for brucellosis when used alone for individual testing (OIE, 2009). No single serological test is appropriate for all epidemiological situations. When applied individually in some contexts, RBT or iELISA may suffer from possible false positive reactions due to gram negative bacteria closely related to *Brucella* such as *Yersinia enterocolitica O:9*, *Escherichia coli* 0157:H7, *Xanthomonas maltophilia* and *Salmonella urbana* (Saegerman et al., 2004). Then, RBT was combined with iELISA in a parallel interpretation scheme for this study. This combination is expected to reduce the occurrence of misclassification and increase the chance to detect antibodies against brucellosis when present for a given sera. Even if RBT and iELISA are not absolutely immunologically independent (Nielsen, 2002), their association is also expected to detect antibodies of both acute and chronic cases, improving the testing sensitivity. RBT is known to detect IgG_1 and IgM produced during acute cases of brucellosis while iELISA is more appropriate for detecting IgG dominant in chronic cases (Nielsen, 2002; Saegerman et al., 2004). In our context, iELISA classified twice more sera as serologically positive cases suggesting a possible predominance of IgG, indicating a chronic infection context. Results also indicated that about 10–50% of sera showing antibodies against

Table 3
Conditional logistic regression analysis of risk factors associated with brucellosis seropositivity among cattle from the Soudano-Guinean areas of Ivory Coast.

Variables	Category	Dataset 1 (2005)				Dataset 2 (2009)			
		OR	95% CI	S.E.	*p*	OR	95% CI	S.E.	*p*
Age	<3	1.0	–	–	–	–	–	–	–
	3–5	1.1	(0.6, 1.8)	0.285	0.824	1.6	(0.6, 4.1)	0.580	0.241
	≥5	2.8	(1.3, 6.4)	1.138	0.013	2.5	(1.5, 4.2)	0.496	0.005
Herd size	<50	1.0	–	–	–	–	–	–	–
	50–100	1.9	(0.8, 4.3)	0.770	0.140	–	–	–	–
	>100	3.3	(1.2, 8.9)	1.628	0.023	–	–	–	–

OR: odd ratio; CI: confidence interval; S.E.: linearized standards error; *p*: *p-value*.

brucellosis would have been classified as serologically negative in a single testing approach by iELISA or RBT, underestimating the seroprevalence of brucellosis. Thus, since different status of the disease might coexist, a combination of tests could ensure a more effective diagnosis and control of brucellosis.

The regression analysis identified age of cattle and herd size as risk factors associated with brucellosis seropositivity in our study population. The separate regression built with the two datasets resulted in quite similar odds of brucellosis seropositivity for age. However, the analysis failed to confirm herd size as a risk factor with the convenient sample (dataset 2). This could be related to the difference in sampling strategy. The six cattle herds conveniently chosen during the second survey were less representative of the cattle population of the savannah-forest area than 44 randomly herds selected with a comparatively structured sampling strategy.

Brucellosis seropositivity was found to be higher in herds with more than 100 cattle and among cattle above 5 years of age. These results are in agreement with the findings of Akakpo (1987). A similar observation was made by Kadohira et al. (1997) for whom cows kept over 4 years old, on a large farm and grazing on a community pasture had more chance to be seropositive than younger cows (Kadohira et al., 1997). Association of herd size with the risk of exposure to *Brucella* infection could be related to a high cattle density on pasture and consequently to an increased chance of contact with infection when present (Camus, 1980a; Akakpo, 1987). Since the Soudano-Guinean zone offers more space for livestock breeding and for grazing compared to the south, it has concentrated herds from different regions of the country during the last years subsequent to the socio-political crisis. According to McDermott and Arimi (2002), the incidence of brucellosis decreases when the herd size decreases in pastoral production systems. That statement is in agreement with our findings where small herds count less seropositive cattle. The effect of herd size was also mentioned in different contexts across Africa (Nicoletti, 1984; Muma et al., 2007; Jergefa et al., 2009). In our study area, brucellosis seropositivity was also found to be associated with the age of animals sampled. The influence of age on seroprevalence has already been mentioned in some previous brucellosis studies (Kadohira et al., 1997; Kubuafor et al., 2000; Faye et al., 2005; Muma et al., 2006; Chimana et al., 2010) but conversely in case of false positive serological reaction for which seropositivity is not linked with age (Saegerman et al., 1997; Pouillot et al., 1998). Age is known as one of the intrinsic factors influencing brucellosis seropositivity (Megersa et al., 2011). This influence could be explained by the fact that the older an animal, the higher the likelihood of contact with infected animals and therefore an increased accumulated chance of becoming seropositive. In Ivory Coast, screening and stamping out are not practised. Consequently, animals with infection could be kept in herds for long time periods without being screened for the presence of brucellosis. Absence of culling strategies and the fact of keeping old cattle in herds could result in large herd sizes and might explained the relationship observed between age and herd size with regard to seropositivity in our context.

No statistically significant effect was demonstrated for breed type with our data. Nevertheless, the seroprevalence trends observed are in agreement with some previous studies (Chantal and Thomas, 1976; Akakpo, 1987) where it was argued that *B. taurus* and *B. indicus* crossbred were more susceptible to *Brucella* infection than *B. indicus*. Moreover, as previously observed by Ocholi et al. (1996), our data did not show any significant difference between bulls and cows regarding the seropositivity. It should be noticed that more than 80% of the samples came from cows, limiting comparison with bulls in our context.

5. Conclusion

In this paper, we investigated risk factors for bovine brucellosis seropositivity in the savannah-forest area of Ivory Coast. This investigation revealed that the age of animal tested and herd size were independently associated with seropositive cases of brucellosis, with highest risk of seropositivity among larger herds and among cattle above 5 years of age. Although the presence of antibodies does not necessarily mean cattle are infected, these preliminary results indicate the presence of brucellosis in the country. Further information on risk factors at individual level and also at herd level would be helpful for setting an efficient control programme. However this study should be considered as a contribution to the epidemiology of bovine brucellosis in Ivory Coast.

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.

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PART THREE:

GENERAL DISCUSSION

CHAPTER 8: GENERAL DISCUSSION, CONCLUSIONS AND PERSPECTIVES

CHAPTER 8: GENERAL DISCUSSION, CONCLUSIONS AND PERSPECTIVES

8.1. General discussion

The need to ensure a sustainable development of livestock, to fight poverty and to limit the public health impact of neglected zoonotic diseases as brucellosis, imposes to give consideration to these diseases in low income countries. In West African countries including Ivory Coast, bovine brucellosis is known for many years and evidence was already provided on the benefit to implement control measures against this disease (Camus, 1995; Roth *et al.*, 2003). However, there is still a lack of attention for brucellosis, hindering any evidence-based control measures in most countries. In addition to the need for sufficient and reliable data for a better understanding of its epidemiology, updated knowledge on the disease appeared to be essential in Ivory Coast. Indeed, the country has recently suffered from political instability causing the disorganization and inadequate coverage of veterinary services that are in charge of the animal disease control activities. This situation could have favored the emergence of animal diseases, especially zoonotic ones like brucellosis (Roth *et al.*, 2003).

Our research aimed to improve the knowledge on the epidemiology of bovine brucellosis in Ivory Coast. Through this general objective, the research intended to generate useful information, which could be used to prevent the spread of the disease and to document national or regional (future) preventive and control plans and strategies against brucellosis, especially in cattle. Therefore, this research includes different contributions intending to cover the main aspects of epidemiology of the disease as defined by Carr *et al.* (2007):

- The distribution and frequency of bovine brucellosis and evidence of its presence in Ivorian cattle; involving specifically:
    - The identification and typing of prevailing field strains of *Brucella* in cattle;
    - The assessment of field serological diagnosis test for the diagnostic of bovine brucellosis;
    - The estimation of the true prevalence of the disease in cattle;
- The determination of association of brucellosis with other factors, including the identification of potential risk factors associated with brucellosis seropositivity in cattle.

The following sections of the thesis present a general discussion of the research with emphasis on the limitations. More details discussions are included within the different articles composing the second part of the thesis.

8.1.1. Importance of identification and typing of prevailing strains of *Brucella* in cattle

As recentlty expressed by Godfroid *et al.* (2013), accurate knowledge on *Brucella* spp in both human and the different animal species is needed to identify the source of infection and plan appropriate control measures. In this research, a comprehensive summary of species and biovars of *Brucella* reported in West African countries with their proportion per origin and their geographical distribution was obtained by applying a systematic review approach (See **Chapter 4**). So far, *B. abortus* biovar 3 was found to be the most commonly isolated in cattle in the sub-region while the biovar 1 is considered as the most encountered worldwide (Corbel, 1997), and also in the USA (Bricker *et al.*, 2003) or in Latin America (Acha and Szyfres, 2003). *B. abortus* biovar 3 was also predominantly isolated in both native cattle and buffalo in eastern Africa and China (Timm, 1982, Domenech *et al.*, 1983). Despite the usefulness of the global map provided on what is known so far about the prevailing field strains of *Brucella* in cattle in West Africa, this review cannot be assumed as exhaustive and representative of the actual situation. However, it provides an insight on the status of field strains at both national and regional level what is useful considering the frequent and uncontrolled cattle movement (transhumance) between countries. On West African scale, data on prevailing *Brucella* in cattle have been lately gathered in The Gambia (Bankole *et al.*, 2010), in Niger (Boukary, 2013), in Ivory Coast (Sanogo *et al.*, 2013a) and in Togo (Dean *et al.,* 2014). Similar research initiatives need to be encouraged for more updated and extended data on prevailing field strains of *Brucella* in West Africa. Except for the Ivorian isolate, which appeared to be negative at oxidase test, the five late *B. abortus biovar 3* from West Africa showed identical growth characteristics. Using enhanced molecular typing methods, they showed some dissimilarities despites their classification in the same biovar. The isolates from the Gambia and Niger apparently closer genetically, seem to be more distinct from those of Ivory Coast and Togo (Table IX and Figure 10). This provides some indications on the genetic diversity of circulating strains of *Brucella* in this sub-region (Dean *et al.,* 2014) and the need for further typing results.

Table IX: The Multiple Loci Variable Number Tandem Repeats analysis (MLVA) profiles showing number of variable tandem repeats (VTR) for latest west African isolates of *B. abortus* biovar 3 and their closest MLVA neighbour profile (*B. abortus* biovar 3 strain BCCN 93_26 from in Sudan, *B abortus* biovar 3 reference strain Tulya from Uganda and *B. abortus* biovar 6 strain BfR7 from Chad) in the *Brucella* MLVAbank (from Bankole *et al*., 2010, Sanogo *et al*., 2013a and Boukary *et al.*, 2013, Dean *et al.*, 2014)

	Variable tandem repeats	Reference Strain Tulya	Strain BCCN[a] 93_26	Strain BfR7[b]	IVC_isolate	Niger_isolate	The gambia_isolate	Togo_isolate 1	Togo_isolate 2	Togo_isolate 3
Panel 1	bruce06	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
	bruce08	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**
	bruce11	**5**	**5**	**5**	**4**	**3**	**4**	**3**	**3**	**3**
	bruce12	**11**	**11**	**11**	**11**	**11**	**11**	**11**	**11**	**11**
	bruce42	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
	bruce43	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
	bruce45	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
	bruce55	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Panel 2	bruce18	**8**	**6**	**6**	**7**	**8**	**7**	**10**	**8**	**8**
	bruce19[c]	**-**	**-**	**-**	**21**	**21**	**-**	**41**	**41**	**41**
	bruce21	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**
	bruce04	**6**	**6**	**6**	**4**	**6**	**5**	**4**	**4**	**4**
	bruce07	**5**	**8**	**4**	**5**	**2**	**5**	**2**	**2**	**2**
	bruce09	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
	bruce16	**11**	**7**	**8**	**7**	**12**	**3**	**8**	**5**	**6**
	bruce30	**5**	**7**	**4**	**3**	**7**	**5**	**4**	**4**	**4**

[a]*Brucella* Culture Collection; [b]Federal Institute for Risk Assessment; [c] additional locus comprised in the MLVA-16 and absent in MLVA-15, Isolates from Ivory Coast (IVC_isolate), Niger (Niger_isolate) , The Gambia (The Gambia_isolate) and Togo (Togo_isolate 1 , 2 and 3).

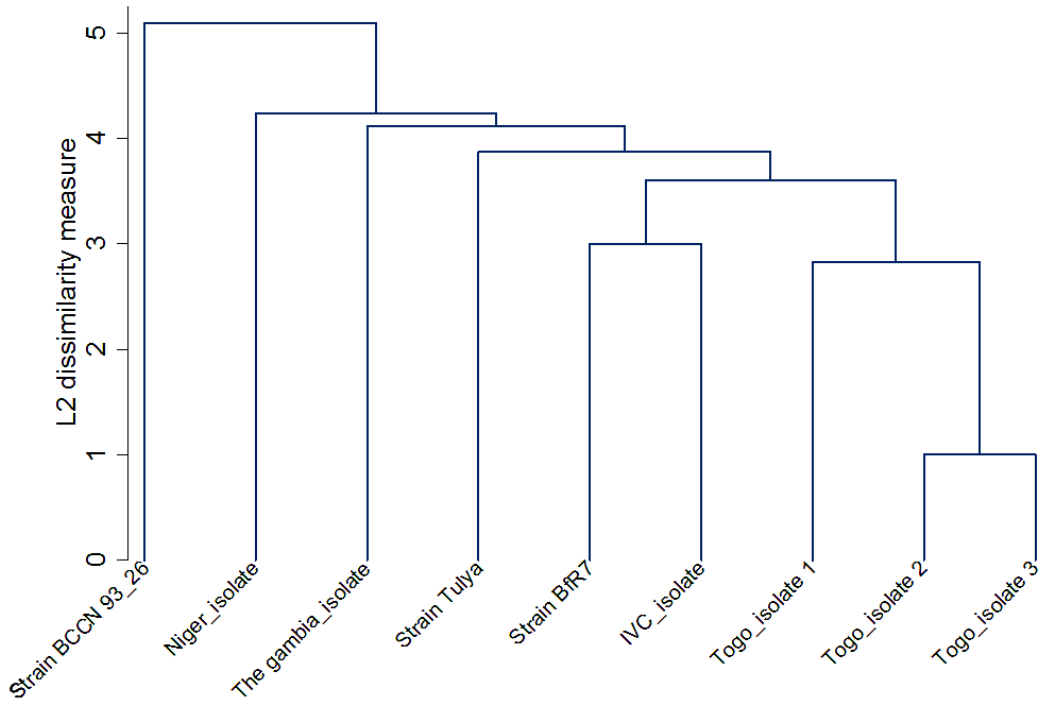

Figure 10: Dendrgram showing the relation between the latest isolates of *B. abortus biovar 3* in West Africa and also with neighbour reference strains in the *Brucella* MLVAbank (*B. abortus* biovar 3 strain BCCN 93_26 from in Sudan, *B abortus* biovar 3 reference strain Tulya from Uganda and *B. abortus* biovar 6 strain BfR7 from Chad) (Bankole *et al.*, 2010; Sanogo *et al.*, 2013a; Boukary *et al.*, 2013, Dean *et al.,* 2014). It is built from results of a simple linkage cluster analysis of the number of variable tandem repeats (VNTR) and the dissimilarities between strains is measured through the eucludian distance between VNTRs (L2 dissimilarity measure).

Regarding Ivory Coast, our study is significant as it was the first report on biovar 3 of *B. abortus* in the country since the first evidence of brucellosis was made (**Figure 11**). In this country, only *B. abortus* biovar 1 and 6 had been isolated from cattle so far. The new biovar was identified from hygroma fluid samples collected from a cow with a carpal hygroma in the central part of the country (**Chapter 5**). This result stresses the importance and the need to continue the efforts to identify circulating field strains in Ivory Coast and at a broader extent, in other West African countries. Until now, only 18 isolates were reported in Ivory Coast since the first report in the early 1970s (**Figure 11**). This is far less compared to the

number of isolates in Senegal (n=232), in Nigeria (n=46), and in Togo (n=30) (**Chapter 4).** All biovars of *Brucella* reported so far in Ivory Coast (*B. abortus* biovar *1, 3 and 6*) are characterized by the same "A" epitope" used in the applied serological tests (i.e., RBT and iELISA). This provides an indication on the appropriateness and the adequacy of the serological tests used so far. However, there is a need for more investigations and more data on prevailing strains of *Brucella* to support this assumption.

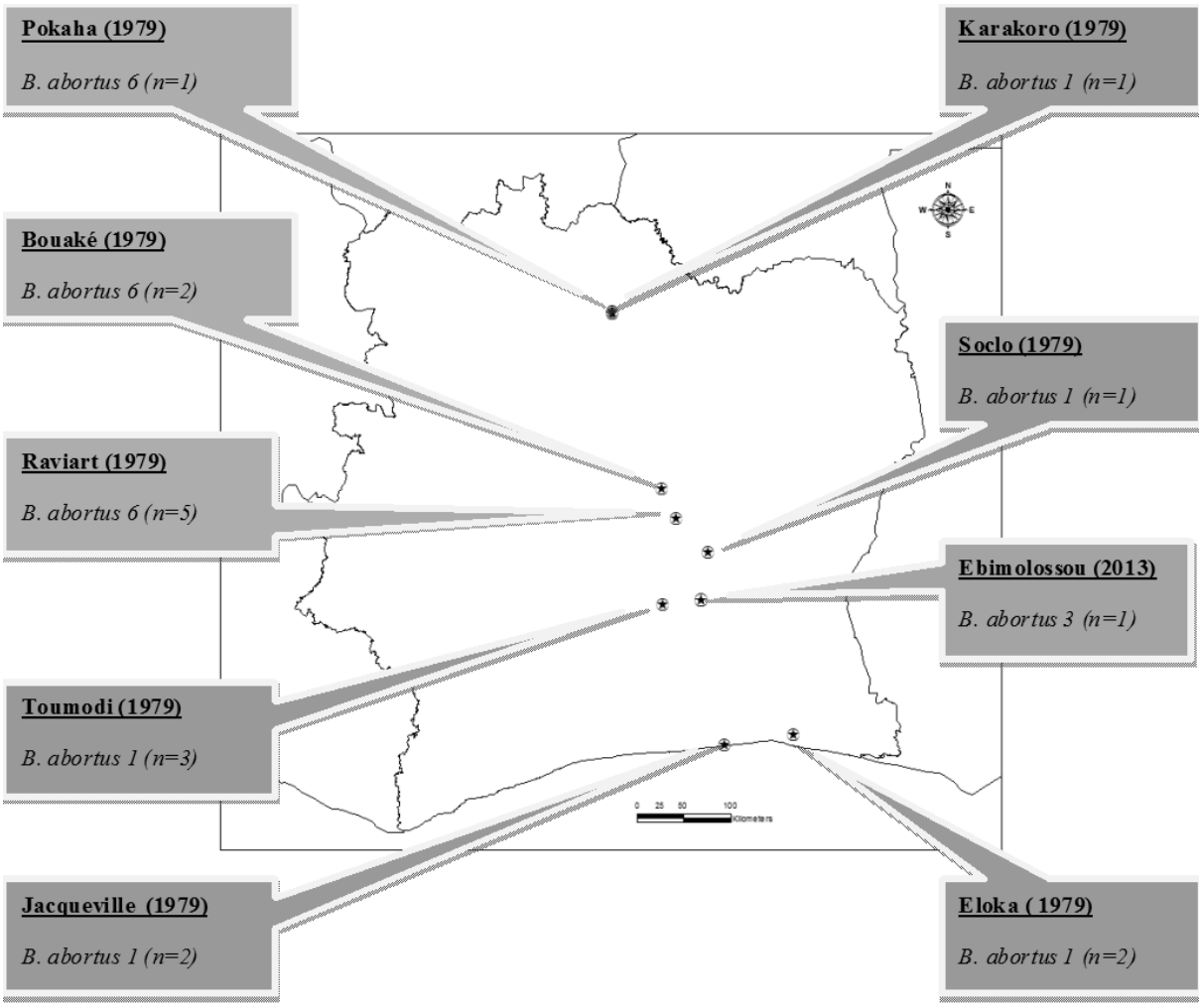

Figure 11: Mapping of field strains of *Brucella* in cattle in Ivory Coast, 2013 (from Pilo-Moron *et al.*, 1979; Sanogo *et al.*, 2013a; Sanogo *et al.*, 2013b). Each bubble contains information on the name of the locality of origin of the strain (e.g. Eloka), the year of publication (e.g. 1979), the biovar (e.g. *B. abortus 1*) and the number of isolates identified (eg. n=2).

The presence of *Brucella* in cattle being confirmed again almost twenty years after the first isolates, it confirms the existence and the persistence of a potential risk for the human population. Indeed, the risk cannot be precluded in the West African epidemiological context where *i*) close contact may occur between animals and people, particularily in urban and peri-urban areas; *ii*) hygienic conditions are usually poor; *iii*) customs often favour consumption of raw milk, and *iv*) where no prevention and/or control strategies are sustainably implemented. However, more data on human cases are needed to clearly establish the public health importance of the disease. As a starting point, seropositivity among slaughterhouse workers and other high-risk professionals might be investigated combined with isolation and characterization of *Brucella*. Reporting of the disease in human could also be improved by considering brucellosis as part of the differential diagnosis for patients with fever of unknown origin (FUO), fever being the most common clinical features in human (Franco *et al.,* 2007).

The comprehensive review of prevailing strains in the field also reveals the frequent isolation of strains of *B. abortus* with unusual characteristics in this sub-region of West Africa (in Senegal, Togo, Niger, The Gambia as well as in Ivory Coast). With conventional typing methods, the differences were not always clear for some of these strains, complicating their classification. The existence of these strains should be considered when typing field strains of *Brucella* in West Africa. This may also justify the need for more typing in the region and, wherever possible, for the application of more accurate discriminative methods (e.g. MLVA) in addition to conventional biotyping (Bankole *et al.*, 2010; Sanogo *et al.*, 2013a, Dean *et al*., 2014). In Ivory Coast the difference between biovar 6 and 3 being not always clear, the availability of more discriminative methods would have been very useful. Identification and typing of *Brucella* strains must continue and be maintained. This type of research will also provide information on possible sources of human infection and on transmission pathways between animals and humans. This is a step needed for an appropriate prevention and control of brucellosis (Adone and Pasquali, 2013). Additionally, the introduction of more advanced methods for identification and typing of *Brucella* such as the Variable number tandem repeat (VNTR) typing or Multilocus Sequence Analysis (MLSA) should be considered in a regional or continental control strategy for cost-effectiveness.

8.1.2. True prevalence, sensitivity and specificity of serological assays for the diagnostic of brucellosis in Ivory Coast

Information on the prevailing field strains of *Brucella* is also important to select the adequate serological diagnosis tests. In addition to information on the actual presence of the disease, serological diagnostic tests are essential to discriminate the status of individuals or group of individuals. Tests are useful for understanding the disease epidemiology and for informing on possible preventive and control programs. However, none of the tests detecting *Brucella* is perfect and sources of interferences exist with many others Gram negative bacteria due to the presence of similarities with immunodominant antigen used (Saegerman *et al*., 2004). Vaccination with strain S19 is also responsible for serological cross-reaction (Corbel, 2006). Therefore, these possible sources of interferences should be taken into account while interpreting serological results (Robinson, 2003). In the epidemiological context of Ivory Coast, vaccination is no more officially practiced since 1992 and no official control program exists so far, excluding therefore interference due to vaccination.

As described in our literature review, most serological assays commonly used for the diagnosis of bovine brucellosis use the *B. abortus* 1 antigen derived from the strain Weybridge 99, epitope A. As a consequence, the performance of assays will also depend on the prevailing field strains in the epidemiological context in which they are applied. The performance of two serological tests commonly used for the diagnostic of brucellosis and also prescribed for trade by OIE (Nielsen, 2002; Saegerman *et al.*, 2004; OIE, 2009; Godfroid *et al.*, 2010; Sanogo *et al.*, 2013a) were assessed in the epidemiological context of Ivory Coast. Ideally, the sensitivity and the specificity of a diagnostic test require knowledge on the true disease status of the population in which the test is applied. This implies, in turn, the availability of a "gold standard reference test" which is absent. Therefore, sensitivity and specificity of Rose Bengal Test and indirect Enzyme Linked Immunosorbent Assay were determined in our study using a Bayesian approach (**Chapter 5**). By offering the possibility to combine prior or expert knowledge on parameters and actual field data in the same model, the Bayesian approach helps to have more accurate and reliable estimates in absence of a gold standard. However, accuracy and validity of Bayesian estimates depend on the availability and the quality of prior information included in the estimation and on the validity of the protocol (e.g. conditional dependence between tests). In developing countries like Ivory Coast where good priors are lacking, their influence has to be checked using a set of prior distributions, as done in this work. An accurate estimation also requires a representative

sample of the target population including all age categories and, ideally the different stages of the disease. In our work, two datasets were combined to improve the representativeness. From a geographical point of view, the representativeness of the aggregated sample for the whole country might be questionable, since only serum collected in cattle herds from the Southern and central regions of the country was used for estimation. However, no association was demonstrated between the origin of sera and the serostatus of cattle included. Moreover, the combined sample included sedentary as well as extensively managed herds with different herd size, age and sex categories, and all types of breed of the country. This allows us to reasonably consider that the aggregate sample was matching the characteristics of the overall cattle population of Ivory Coast. The provided estimates can therefore serve as prior knowledge for future Bayesian estimation of test characteristics and of disease true prevalence in similar conditions in Ivory Coast.

In addition to the provision of estimates of test characteristics, the Bayesian approach also delivered an updated estimation on the true disease prevalence in the sample population. Except the latest studies, most of the previous reports on the prevalence of brucellosis in Ivory Coast, reported only apparent prevalences. Since the prevalence is essential to appraise the impact of a disease in a population of interest, the estimation of the disease true prevalence is of key importance to prevent a biased estimation of disease burden (Dohoo *et al.*, 2003; Speybroeck *et al.,* 2012a). Therefore, the usefulness of methodological options such as the Bayesian approach is obvious. Using a combination of three serological tests, the Bayesian estimates in the central savannah-forest area of the country was 8.8% (credibility interval: 5.0-16.4) (**Chapter 6**). An overall estimate of the true prevalence of brucellosis using aggregated samples from cattle herds from both central and southern parts of the country was 4.6% (credibility interval: 0.6-9.5) (**Chapter 5**). Even if the sampling strategy for the two datasets used in these estimations needs to be taken into consideration, these results provide useful indications on the presence of *Brucella* and the spread of the disease in cattle, justifying the attention that should be given to brucellosis in Ivory Coast. In addition to the sampling bias, the accuracy of the first estimates (**Chapter 6**) is more questionable since the correlation between the tests on seropositive and seronegative cattle was not taken into account in the modeling process. Indeed, the combination of diagnostic tests targeting a similar biological phenomenon -such as immunoglobulins- may result in dependence or correlation between them (Gardner *et al.*, 2000). According to the conjugate used, conditional dependence had to be considered between RBT, detecting the presence of Immunoglobulins

(Ig) IgG_1 and iELISA, targeting IgG_1 and/or IgG_2 (Nielsen, 2002; Saegerman *et al.,* 2004 and 2010; Sanogo *et al.*, 2013a). Moreover, the sensitivity analysis to assess the consistency of estimates was not performed as recommended (Branscum *et al.*, 2005). Therefore, it appears that an appropriate sampling strategy should be designed at the beginning of any study aiming to estimate true prevalence or test characteristics using a Bayesian approach and the dependence between tests and the implementation of a sensitivity analysis on estimates are crucial to facilitate extrapolation of estimates.

Since the performance of RBT and iELISA was evaluated in the Ivorian context, they can be used to support decision making for control and serosurveillance. Different testing strategies can be considered. Following a serial interpretation, RBT positive results have to be confirmed by iELISA while following a parallel interpretation the testing scheme will be expected to detect both acute and chronic infection (Saegerman *et al*., 2004; Godfroid *et al.*, 2013). As demonstrated in this research, the iELISA might also be implemented on its own. However, a combination with other serological tests as the RBT would be a more appropriate strategy. Since most of the serological tests such as RBT and iELISA do not inform on the source of infection, the capabilities of veterinary services for the detection of *Brucella* need to be improved. In the short-term, identification methods such as the Matrix-assisted laser desorption/ionization time-offlight mass spectrometry (MALDI-TOF-MS) might be introduced to identify *Brucella* at a genus level at the central laboratory, with an enhancement of biosecurity and biosafety measures.

8.1.3. Risk factors associated to the seropositivity of brucellosis in cattle from Ivory Coast

Many factors including the density of the animal population, herd size, breed, type of production (dairy or beef), type of husbandry system and environmental factors are thought to be associated with brucellosis. In this research, potential risk factors associated to seropositivity in cattle populations in Ivory Coast were also examined (**Chapter 7**). This is the first work reporting information on risk factors associated with brucellosis in Ivory Coast. Serological data obtained through a survey in the central savannah-forest area of the country were analyzed using a logistic regression. Age of animals and herd size were identified as risk factors associated with seropositivity of brucellosis in Ivory Coast. Specifically, animals above 5 years of age were estimated to be almost 3 times more likely infected than 3 years old cattle. A similar ratio was observed for cattle herds with more than 100 heads compared

to those with less than 50 heads. This information can be used for increasing farmers awareness and regulating herd management practices in order to decrease the seroprevalence of *Brucella* in animals and consequently prevent human infections. Indeed, education on risk factors associated with brucellosis is essential to limit the spread of the disease.

These results were obtained using only data collected in the central soudano-guinean area but could be reasonably extrapolated to the whole country, as there is currently no control strategy against brucellosis at all. In addition to the identification of risk factors at animal level, which was the focus of this work, environmental risk factors need to be investigated as they may also provide information to elaborate prevention and control strategy. The results are in line with the findings of Akakpo and Bornarel (1987) who identified the age of animal, the type of breed and the climate as risk factors for brucellosis. It was also demonstrated that the effect of crowd (e.g. large herd size) together with lower genetic diversity may favor transmission and select fast replicating organisms with major zoonotic potential as *Brucella* (McDaniel *et al.*, 2013). Boukary *et al.* (2013) recently also identified the age of animal as individual risk factor in traditional cattle farms from Niger. Together with the acquired knowledge on the prevailing field strains of *Brucella* (**Chapter 4 and 5**), the data gained on diagnostic tests performance (**Chapter 5**), disease true prevalence (**Chapter 5**), and risk factors (**Chapter 7**) are helpful to develop and implement preventive and control measures.

8.2. Conclusions, implications and perspectives

The development of livestock and the improvement of their health status are an essential part of a pro-poor enhanced food security strategy for the benefit of vulnerable populations in developing countries such as Ivory Coast. This urges to deal with pathologic constrains like brucellosis.

Bovine brucellosis is endemic in many sub-Saharan African countries including Ivory Coast. Its impact on animal production and zoonotic potential are currently well known and the benefits of controlling it was also strongly demonstrated in cattle. However, the disease is still considered as a non-priority disease, i.e. suffering from insufficient knowledge on its epidemiology and public health importance. Therefore, gaining more and accurate knowledge on the epidemiology of brucellosis is required to determine the actual impact of the disease.

It will help to convince the decision makers to implement appropriate and sustainable disease preventive and control measures at national level but also at regional level considering the frequent transboundary herd movements (transhumance).

The current research confirms the presence of bovine brucellosis in Ivory Coast and contributes to the knowledge of the epidemiology of the disease. This research investigates prevailing field strains of *Brucella* in cattle in Ivory Coast but also provides information at West African scale. Biovar 3 of *Brucella abortus* was identified for the first time in Ivory Coast in cattle in this research. Additionally, the performances of Rose Bengal test and iELISA were assessed in the Ivorian epidemiological context, since those tests are of key importance for investigating the epidemiology of the disease as well for planning prevention and control measures. Finally, Estimates of the true prevalence of the disease are now available and some risk factors associated with brucellosis in the country identified for the first time.

Initially, this research intended to cover the different agro-ecological areas of the country including the northern part where the density of cattle population and the presence of transhumant herds are expected to influence the disease epidemiology. Finally, only the southern Guinean and the central Soudano-guinean aeras were covered. This was mainly due to the socio-political context prevailing in the country at the moment of the field study. Data obtained in the accessible areas where combined with previous data collected in the same aeras for true prevalence estimation and for diagnostic performance assessement. The rationale supporting this approach and the consequences on the interpretation of the findings are discussed in **Chapter 5 and 6**. The contribution on prevailing strains of *Brucella* in cattle was done by combining a prospective and a retrospective approach. In the prospective approach, only one isolate was obtained, which was very few despite the added value that it provides. However, this stresses the need for more investigation in field strains of *Brucella* in cattle. Even if hygroma fluid was demonstrated to be a useful sample for strain identification (Sanogo *et al.,* 2013a), other samples such as abortive materials and secretions should be considered. By considering exchanges and movements of cattle within Ivory Coast but also between countries of West Africa, the review provided a more extended picture on the prevailing strains.

Despite its limitations, our research contributes largely to a better knowledge of the epidemiology of brucellosis in Ivory Coast and in West Africa. Additional investigations are needed to obtain a global picture and a reliable understanding of the disease epidemiology.

This is crucial to provide useful evidences to advocate among decision makers for adequate preventive and control measures against brucellosis. It will therefore be crucial to investigate the frequency and the distribution of the disease and the associated risk factors in other regions of Ivory Coast, especially in the northern part where the density of cattle population is higher and where animal movements (transhumance) might influence the disease epidemiology. Compared to the southern and central areas, the distribution and the frequency of the disease in the North might be higher. Information about the presence of *Brucella* in the different livestock breeding areas and systems of the country are essential to implement effective and appropriate prevention and control measures. The role of small ruminants as source of infection for cattle and for human also needs to be addressed. This is important to assess the risk of human brucellosis within the country. Furthermore, other susceptible livestock and wildlife species need to be studied in order to obtain a more extended picture of the disease epidemiology at national and at regional level.

In addition to the need of future research for a better understanding of the epidemiology of bovine brucellosis in Ivory Coast as well as in West Africa, this research inspired some points, which need to be considered for an efficient and sustainable prevention and control of brucellosis as well as other (zoonotic) diseases:

- The diagnostic and surveillance capacities of veterinary services need to be strengthened to provide valuable epidemiological information, especially on prevailing strains of *Brucella*. Hence, improvement of veterinary diagnostic laboratory capabilities, veterinary surveillance and quality and organization of veterinary services are fundamental to provide reliable data, gain of confidence in the veterinary services and disease surveillance, and to ensure the efficiency of the preventive and control programs of brucellosis as well as other zoonoses. So far, RBT is routinely used for screening in Ivory Coast. Additional tests such as indirect or competitive ELISA and FPA also need to be assessed and established as confirmatory tests in the central veterinary laboratory in Ivory Coast. There is also a need to upgrade the laboratory facilities and equipements for safe management of samples possibly contaminated by level 3 pathogens as *Brucella*. In addition, a comprehensive training on biosafety and biosecurity measures and procedures is also important for laboratory workers, for scientists and for all the persons at risk or working with such hazardous pathogens.

- Based on updated information provided on the epidemiological status of bovine brucellosis, a pilot control project covering the savannah-forest sedentary cattle herds and dairy herds in Ivory Coast can be suggested to lower the prevalence (down to 2%). This strategy might include prevention and control measures such as surveillance of dairy herds at national scale through milk testing (at least twice per year), annual vaccination of young calves, seromonitoring of herds (with RBT and iELISA or FPA or competitive ELISA), elimination of infected adult animals from herd and serological control before introduction in the herd. Campaigns of public awareness and education aiming to prevent and reduce risks of transmission from animals to humans are also imperative to sensitize on safe herd management practices and to improve notification of cases of abortion and hygroma. Consideration of the risk factors identified so far in the Ivorian context is helpful to prevent the spread of the disease within cattle population and from cattle to human. This control strategy will be progressively expanded to other livestock breeding areas of the country, but need to be backed by sufficient and updated knowledge on the disease epidemiology (e.g., true prevalence), in line the stepwise progressive approach proposed by FAO (FAO, 2013).

- The maintenance and the improvement of animal health depend not only on financial issues but also on capacity, quality, competence, transparency, expertise and organization of veterinary services. Hence, the results and recommendations of the OIE Performance of Veterinary Services (PVS)[8] assessement are fundamental and need to be considered. Especially, the adequate coverage of the territory with operational veterinary services (both public and private) is required in Ivory Coast after the socio-political crisis of the last ten years. This is of key importance to set up a functional and sustainable surveillance network, for reporting animal diseases and

[8] *The OIE Tool for the Evaluation of Performance of Veterinary Services (OIE PVS Tool) is an evaluation tools, developed initially in collaboration with the Inter-American Institute for Cooperation on Agriculture (IICA) and refined by the OIE, aiming to strengthen the veterinary Services by helping them comply with OIE international standards for quality. It is designed to assist VS to establish their current level of performance, to identify gaps and weaknesses in their ability to comply with OIE international standards, to form a shared vision with stakeholders (including the private sector) and to establish priorities and carry out strategic initiatives for improvement. More information are available on: http://www.oie.int/en/support-to-oie-members/pvs-evaluations/oie-pvs-tool/*

disease related events and for designing appropriate and efficient prevention and control measures. In addition to the effective implication and cooperation with community members (e.g., head of community, paraveterinarians, members of cooperatives, animal owners), the introduction of new tools such as internet and mobile technology might contribute to improve the efficiency of the epidemiological surveillance network especially regarding field data collection, notification of cases of abortion and transmission of reports. The commitment of the government is also essential to guarantee the sustainability of such a system (Ouagal *et al.,* 2008; Ouagal *et al.,* 2012).

- The presence of *Brucella* in most of the West African countries, the existence of cattle movement between countries and the limited resources allocated for disease control in most of African countries are in favour of the creation of a collaborative regional prevention and control strategy to contain brucellosis infection. Such a strategy should adopt the One Health or the Ecohealth principle (Zinsstag, 2013). The approach should take into account the particular ecosystem of West African countries and should ensure more cooperation, and exchange of information and resources between public health and veterinary authorities not only at national level but also at regional level. The One Health approach implies an integrated approach involving both human health and veterinary services for the surveillance of zoonotic diseases such as brucellosis. This approach allows to better understand the epidemiology of zoonotic diseases and induces a more efficient utilization of the limited resources (Saegerman *et al.*, 2010; Dean *et al.*, 2012). Creation of zoonotic disease units should also be promoted to formalize the above-mentioned intersectoral collaboration. A regulatory framework is also needed for a better coordination of control activities in the field within and between countries. In the same spirit, collaboration between researchers, public health and veterinary actors of Ivory Coast and neighbouring countries need to be established and strengthened. The establishment of a reference laboratory at regional level needs to be considered. Finally, the commitment of national authorities and the political support and leadership of regional institutions such as the Ecomonic Community of West African States (ECOWAS) represent a key requirement, beneficial for the sustainability and the development of livestock in the region.

Despite the numerous priorities, more attention and consideration needs to be given to brucellosis as well as other endemic neglected zoonotic diseases, especially in low-income country as Ivory Coast. This is essential to foresee a sustainable development of livestock, to cover the needs of populations in terms of animal protein and to contribute to poverty alleviation.

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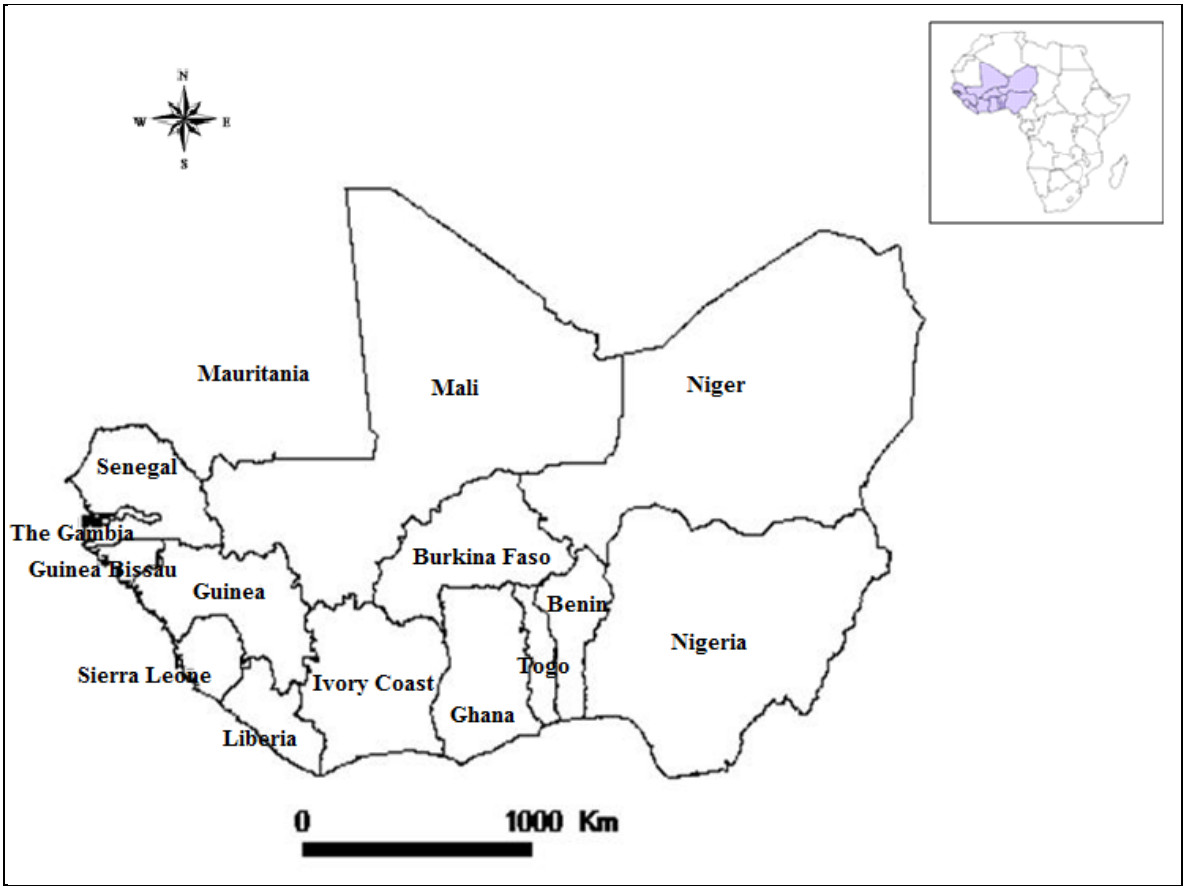
ANNEXES

Annex 1: Map showing the western part of Africa and the neighbour countries of Ivory Coast



Annex 2: Differential characteristics of biovars of *Brucella* species (from OIE, 2009)

Species	Biovar	CO_2 requirement	H_2S production	Growth on dyes[a]		Agglutination with monospecific sera		
				Thionin	Basic fuchsin	A	M	R
B. melitensis	1	–	–	+	+	–	+	–
	2	–	–	+	+	+	–	–
	3	–	–	+	+	+	+	–
B. abortus	1	+[b]	+	–	+	+	–	–
	2	+[b]	+	–	–	+	–	–
	3	+[b]	+	+	+	+	–	–
	4	+[b]	+	–	+[c]	–	+	–
	5	–	–	+	+	–	+	–
	6	–	–	+	+	+	–	–
	9	+ or –	+	+	+	–	+	–
B. suis	1	–	+	+	–[d]	+	–	–
	2	–	–	+	–	+	–	–
	3	–	–	+	+	+	–	–
	4	–	–	+	–[e]	+	+	–
	5	–	–		–	–	+	–
B. neotomae	–	–	+	–[f]	–	+	–	–
B. ovis	–	+	–	+	–[e]	–	–	+
B. canis	–	–	–	+	–[e]	–	–	+
B. ceti	–	–	–	+	+	+	–[e]	–
B. pinnipedialis	–	+	–	+	+	+	–[e]	–
B. microti	–	–	–	+	+	–	+	–

From refs 1, 39.

a Dye concentration in serum dextrose medium: 20 µg/ml
b Usually positive on primary isolation
c Some basic fuchsin-sensitive strains have been isolated
d Some basic fuchsin-resistant strains have been isolated
e Negative for most strains
f Growth at a concentration of 10 µg/ml thionin

Annex 3 : A cow with a carpal hygroma

(Credit picture: M. Sanogo)

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