Rev. sci. tech. Off. int. Epiz., 2014, **33** (3), 957-965

Seroprevalence of brucellosis in sheep and isolation of *Brucella abortus* biovar 6 in Kassala State, Eastern Sudan

M.M. Gumaa [(1)*], H.M. Osman [(2)], M.M. Omer [(1)], E.M. El Sanousi [(2)], J. Godfroid [(3, 4)#] & A.M. Ahmed [(2)#]

(1) Kassala Veterinary Research Laboratory, P.O. Box 237, Kassala, Sudan
(2) Veterinary Research Institute, P.O. Box 8067, Khartoum, Sudan
(3) University of Tromsø – the Arctic University of Norway, Faculty of Biosciences, Fisheries and Economics, Department of Arctic and Marine Biology, 9037 Tromsø, Norway
(4) Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, 0110 Onderstepoort, South Africa
*Corresponding author: mohammedmustafa81@gmail.com
#J. Godfroid and A.M. Ahmed should be considered equally as last authors

Summary

Brucellosis is one of the important zoonotic diseases among livestock. This study was carried out to estimate the prevalence of brucellosis and isolate *Brucella* spp. in sheep in Kassala State in the east of Sudan. Two thousand and five serum samples were randomly collected from nine different localities. All serum samples were examined by the Rose Bengal plate test (RBPT) and the modified RBPT (mRBPT). Forty-three (2.15%, 95% confidence interval [CI]: 1.6, 3.0) and 68 (3.4%, 95% CI: 2.6, 4.2) samples were positive with the RBPT and the mRBPT, respectively. According to a known diagnostic sensitivity of 86.6% and a known diagnostic specificity of 97.6% for the mRBPT, the true prevalence was estimated to be 1.2% (95% CI: 0.3, 2.2). Different tissue samples were collected from 41 mRBPT seropositive animals. *Brucella abortus* biovar 6 was isolated from a pyometra of a seropositive ewe. It is important to note that *B. abortus* biovar 6 cannot be differentiated from *Brucella melitensis* biovar 2 by routine bacteriology. Only phage typing performed in reference laboratories will allow accurate identification of the strain. The fact that *B. abortus* biovar 6 does not require CO_2 for growth, combined with the fact that it has been isolated from a small ruminant in this study, could easily have led to misidentification (as *B. melitensis* biovar 2), to wrong epidemiological inferences and to the implementation of inappropriate control measures. The results presented here suggest that sheep are spillover hosts, as previously described for camels, and that the actual reservoir of *B. abortus* biovar 6 is cattle in Kassala State, Eastern Sudan. This study highlights the importance of isolating and identifying *Brucella* spp. in different livestock species in order to accurately decipher brucellosis epidemiology in sub-Saharan Africa.

Keywords

Brucella abortus biovar 6 – *Brucella melitensis* biovar 2 – Brucellosis – Eastern Sudan – Epidemiology – Livestock – Reservoir host – Spillover host – sub-Saharan Africa.

Introduction

Brucellosis is a contagious disease that infects animals and can be transmitted to humans. This zoonotic disease is caused by different species belonging to the genus *Brucella* (1). In 1887, *Brucella melitensis* was first isolated by David Bruce from the spleen of a hospitalised soldier in Malta and since then brucellosis has been an emerging disease (2). Today, the genus *Brucella* includes ten species: *B. melitensis*, *B. abortus*, *B. suis*, *B. ovis*, *B. canis*, *B. neotomae*, *B. ceti*, *B. pinnipedialis*, *B. microti* and *B. inopinata* (1, 3, 4, 5). Brucellosis in sheep is primarily caused by *B. melitensis*, although *B. abortus* and *B. suis* cause sporadic infections (6). Transmission of brucellosis in sheep occurs via oral, inhalation, conjunctival and venereal routes of exposure, as well as *in utero*. The main sources of contagious material are placenta, fetal fluids and vaginal discharge expelled by infected ewes after abortion or full-term parturition (6). Persistent infection of mammary glands and supra-mammary lymph nodes leads to intermittent or constant shedding of the organism in milk (7).

The transmission of a given *Brucella* species to a non-preferential host (e.g. *B. melitensis* to cattle or *B. abortus* to small ruminants) is facilitated by mixing herds and flocks, purchasing animals from unscreened sources and sharing bulls and rams for breeding. Such practices promote the transfer of pathogens between herds and flocks (6, 8). Although sexually mature animals of both sexes are equally susceptible to the disease, the predominant signs of acute infection are reproductive failure with abortion in the last trimester and birth of weak offspring (7). *Brucella melitensis* infection in sheep is most commonly encountered in countries around the Mediterranean Sea and in the Middle East, Central Asia and parts of South America (6).

The economic impact of brucellosis results from abortions, infertility, a drop in milk yield, veterinary care and the cost of replacing infected animals. In addition, brucellosis is an important public health concern (9, 10). In Sudan, brucellosis in sheep was first reported in 1990 by Musa *et al.* (11) and the brucellosis epidemiology in livestock was reviewed in different parts of the country by Refai in 2002 (12). A study performed by El-Ansary *et al.* in 2001 showed a low frequency of brucellosis among animals in Kassala State compared to other parts of Sudan (13). However, in 2006 the prevalence was reported to have increased in Kassala State (14).

The aim of this study was to estimate the seroprevalence of brucellosis in sheep in different localities in Kassala State, Eastern Sudan, and to isolate and characterise, at the biovar level, the *Brucella* species that induced seropositivity in sheep.

Materials and methods

Area and period of the study

The study was conducted in 2009 and in 2011 in Kassala State, which lies between latitudes 14° and 17°N and longitudes 34° and 37°E.

Collection of samples

Collection of blood samples for serology

The state is divided into nine localities: Halfa, River-Atbara, Refi-Elgirba, Refi-Wadelhilew, Kassala, Refi-Kassala, West Kassala, Refi-Aroma, and North Delta. A random multistage method was used to select samples, with villages as primary sampling units (Step 1) and individual animals as secondary sampling units (Step 2), according to international guidelines for coordinated human and animal brucellosis surveillance published by the Food and Agriculture Organization of the United Nations (FAO) in 2003 (15). Blood was collected by venipuncture of the jugular vein. The data recorded for each sample were serial number, animal sex, age, grazing area and owner's name. The total number of samples was 2,005. Animals were distributed in the following age groups: 409 animals less than one year old, 698 animals between 1 and 2 years old, 586 animals between 2 and 4 years old and 314 animals over 4 years old.

Collection of blood samples for bacteriology

Different tissue samples were collected in separate plastic bags from 41 seropositive sheep. The samples included 38 uteri, 38 mammary glands, 38 supra-mammary lymph nodes, 41 spleens, three testicles, three epididymes, two accessory glands, three scrotal lymph nodes, and three placentas. Twenty mesenteric lymph nodes were also collected. Finally, 38 milk samples, two vaginal swabs, three samples of amniotic fluids, one sample of fetal stomach content and two samples of pus, one from male accessory gland and the other from a purulent pyometra, were collected.

Serology

The sera were screened by Rose Bengal plate test (RBPT) according to the method described by the World Organisation for Animal Health (OIE) (16). The modified Rose Bengal Plate test (mRBPT) was performed on the same samples as described previously with the aim of demonstrating that the mRBPT has a greater sensitivity than the RBPT, as documented for sheep in Portugal and Greece (17, 18). Additionally, in order to control for serum samples classified as false negative by the mRBPT, 400 serum samples negative to RBPT and mRBPT were tested by competitive enzyme-linked immunosorbent assay (cELISA) using a commercial cELISA kit (Animal Health and Veterinary Laboratories Agency [AHVLA], Weybridge, United Kingdom [UK]). The test procedure was carried out according to the manual supplied with the kit.

Bacteriology

The fat around the tissues was first removed. The specimens were then cut into small pieces with sterile scissors and ground with a sterile mortar and pestle using sterile sand and sterile phosphate buffered saline. Smears from these samples were stained with the Modified Ziehl-Neelsen

(MZN) technique. The samples were cultured on tryptose-soy agar (Himedia, India), *Brucella* media (Oxoid, UK), and Columbia agar (Himedia, India), without supplementary antibiotics. The samples were cultured in duplicates, one incubated aerobically at 37°C, and the other under a 5% carbon dioxide atmosphere at 37°C. All cultures were incubated for ten days (1, 16). *Brucella* spp. colonies were initially identified by colony morphology, Gram stain, and MZN stain. Catalase, oxidase, urease, nitrate, citrate, indole and Voges-Proskauer (VP) tests were also performed for further characterisation of the isolates. For final identification and typing the isolate was sent to the AHVLA (an OIE Reference Laboratory for brucellosis).

Results

Serology

The serological results from different localities are shown in Table I. Forty-three out of 2,005 serum samples were positive (2.15%, 95% CI: 1.6, 2.9) by the RBPT. Re-examination of the 2,005 serum samples with the mRBPT resulted in additional positive samples. Sixty-eight samples were classified positive, resulting in an apparent prevalence of 3.4% (95% CI: 2.6, 4.2). Based on the mRBPT results, the apparent prevalence of the disease was highest in Refi-Elgirba and in Refi-Wadelhilew localities and lowest in River-Atbara and Halfa localities.

In the different age groups, the highest prevalence (5.4%, 95% CI: 3.4, 8.4) was observed in sheep older than four years (17/312) while the lowest prevalence (2.4%, 95% CI: 1.1, 4.2) was observed in sheep younger than one year (9/409). These seroprevalences were not statistically different. In this study, the cELISA was used to test selected serum samples that were negative (n = 400) using the mRBPT. Only 0.25% of these samples were classified as positive by cELISA, indicating that only 1/400 samples was false-negative using the mRBPT. According to the diagnostic sensitivity (86.6%) and diagnostic specificity (97.6%) published for the mRBPT (18), the true prevalence in Kassala State was estimated (19) to be 1.2% (95% CI: 0.3, 2.2) in this study.

Bacteriology

One *Brucella* spp. strain was isolated from a purulent pyometra of a seropositive ewe. Colonies were observed after three days of incubation under aerobic conditions at 37°C. The colonies were smooth and shiny with a honey colour. The smears showed Gram-negative and MZN-positive cocci and coccobacilli. The isolate was catalase positive, oxidase positive, urease positive, nitrate positive, citrate negative, indole negative, Voges-Proskauer (VP) negative, and did not utilise glucose. The isolate showed a positive agglutination reaction with anti-A *Brucella* monospecific anti-serum and a negative reaction with anti-M *Brucella* monospecific anti-serum. The isolate was tentatively identified as either *B. melitensis* biovar 2 or *B. abortus* biovar 6. Only additional tests performed in reference laboratories can differentiate these two *Brucella* biovars. Moreover, the isolate was H_2S positive, which is not a common feature for *B. melitensis* biovar 2 or for *B. abortus* biovar 6 (1). The isolate in this study was identified as *B. abortus* biovar 6 by the OIE Reference Laboratory in Weybridge, UK. Interestingly, the isolate also showed an unusual profile with the absence of growth in the presence of thionin (Table II).

Discussion

Brucellosis has been a re-emerging disease since the discovery of *B. melitensis* infection by Bruce in 1887 (2, 20). Brucellosis in small ruminants has been reported in different

Table I
Apparent prevalence in different localities in Kassala State based on the Rose Bengal plate test and modified Rose Bengal plate test results

Locality	No. sampled	RBPT prevalence (CI 95%)	mRBPT prevalence (CI 95%)
River-Atbara and Halfa	788	2.1 (1.3–3.4)	2.7 (1.9–4.2)
Refi-Elgirba and Refi-Wadelhilew	418	2.6 (1.5–4.7)	3.9 (2.4–6.1)
Kassala, Refi-Kassala and Western Kassala	530	2.3 (1.3–4.0)	3.8 (2.4–5.8)
Refi-Aroma and Delta North	269	1.1 (0.4–3.4)	3.7 (2.0–6.8)
Total	**2,005**	**2.15 (1.6–2.9)**	**3.4 (2.7–4.3)**

CI: confidence interval
RBPT: Rose Bengal plate test
mRBPT: modified Rose Bengal plate test

Table II
Identification and typing of *B. abortus* biovar 6 atypical isolate (present study) and *B. abortus* biovar 6 reference strain

Isolate	Test					Agglutination		Lysis with phages at RTD					
	Urease	H_2S	CO_2	BF	Th	A	M	Wb	Tb	B2k	Fi	Iz_1	R/c
B. abortus biovar 6 (atypical)	++	+	–	+	–	+	–	CL	CL	CL	CL	CL	NL
B. abortus biovar 6 (reference)	++	–	–	+	+	+	–	CL	CL	CL	CL	CL	NL

–: Negative to the test
+: Positive to the test
A: *Brucella* monospecific antiserum A
BF: Basic Fuchsin at 20µl/ml (1/50,000 w/v)
B2k: Berkeley 2
CL: Confluent Lysis
Fi: Firenze
Iz_1: Izatnagar1
NL: No lysis
M: *Brucella* monospecific antiserum M
R/c: Rough/canis
RTD: Routine Test Dilution
Tb: Tbilisi
Th: Thionin at 20 µl/ml (1/50,000 w/v)
Wb: Weybridge

parts of the world (6). In sub-Saharan Africa, serological data show that brucellosis is prevalent in livestock on the continent (21). However, isolation is only rarely performed and thus the actual distribution of the different *Brucella* spp. in livestock remains to a large extent unknown. Thus, targeted mitigation strategies are difficult to implement without the identification of the reservoir of *Brucella* spp. (22).

In Sudan, brucellosis in cattle, sheep and goats is endemic throughout the country. As reviewed by Refai (12), in Khartoum State the prevalence in sheep was 14.2%, in South Darfur State the prevalence in sheep was 20.4%, while in South Kordofan State the prevalence in sheep was 5.7% (12). Although the RBPT is recommended by the OIE for the screening of brucellosis in small ruminants (6), the authors used the mRBPT in this study with the aim of increasing the sensitivity without affecting the specificity of detection. This approach has been described in the literature (17, 18) and also suggested by the OIE (6). However, it should be noted that no published information related to the sensitivity and specificity of this test for detecting sheep infected with *B. abortus* is available. With this word of caution, the true prevalence in Kassala State was estimated to be 1.2% (95% CI: 0.2, 2.1), confirming a low prevalence, as reported earlier (12). The prevalence was not significantly different between males and females and not significantly different between young and adult sheep.

Competitive ELISA was initially developed to improve the diagnostic specificity of immunoassays for brucellosis while maintaining a high sensitivity (23, 24). The present study confirms that the mRBPT shows an enhanced sensitivity compared to the RBPT given that only 1/400 samples classified negative by the mRBPT was additionally detected by the cELISA.

In East Africa, *B. melitensis* biovar 3 was isolated from sheep in Sudan in 1990 (10). In South Africa, *B. melitensis* biovar 1 was eradicated in sheep in Kwazulu Natal in 2002 (25). Interestingly, *B. melitensis* has been isolated from cattle in Kenya in 2012 (26) and from camels in Sudan (27). Furthermore, *B. melitensis* infection of cattle has been suggested in Egypt (28) and isolated from cattle in France (29) and Spain (30). It is worth mentioning that in West Africa the only isolation of *Brucella* spp. in small ruminants reported in the international literature was *B. abortus* biovar 1 in Nigeria (31).

This is the first report of the isolation of *B. abortus* in small ruminants in East Africa. In this study, *B. abortus* biovar 6 was isolated from a sheep. The isolate was H_2S positive, which is not a common feature for *B. melitensis* biovar 2 or for *B. abortus* biovar 6; although, this feature had been documented in a previous study in Sudan for *B. abortus* biovar 6 strains isolated from cattle and camels (27, 32). Altogether, the results of these studies suggest that there is a reservoir of *B. abortus* biovar 6 in the cattle population in Kassala State and that the infection spills over to sheep and camels from the cattle reservoir. Given the host preferences of *Brucella* spp., it is unlikely that sheep and camels can sustain *B. abortus* infections without constant influx of *B. abortus* from the cattle reservoir (21). Therefore, the results presented here suggest that the first brucellosis control measures to be taken have to be directed towards the cattle population in Kassala State. Importantly, brucellosis due to *B. abortus* and *B. melitensis* has been described in camels in western Darfur (27), suggesting that *B. melitensis* and *B. abortus* are spilling over to camels from sheep and cattle, respectively.

As highlighted in this study, the recovery rate of *Brucella* spp. may be very low (i.e. only one isolate in the present study). In the authors' opinion this is due to the fact that non-selective culture media are often used rather than the fact that *Brucella* spp. is absent in the analysed samples. The use of non-selective media resulted in a high number of contaminated cultures in this study. The only isolate in this study was obtained from the pus of a pyometra (which very likely consisted of a pure culture of *B. abortus* biovar 6). This illustrates that availability of affordable media to culture *Brucella* spp. is a priority to decipher the global brucellosis epidemiology in sub-Saharan Africa.

Conclusions

By combining serology and bacteriology, this study highlights the main problem encountered in assessing brucellosis epidemiology in most of the sub-African countries, i.e. the impossibility to ascribe which *Brucella* species induced anti-*Brucella* antibodies in a given livestock species; seropositivity only means that animals were likely exposed to *Brucella* spp. (8, 21). The epidemiology of brucellosis throughout the continent is complex due to mixed herding practices. This means that the actual reservoirs of *Brucella* spp. are not identified in the majority of African countries. It is worth noting that a recent study done in Egypt has highlighted the need to identify which *Brucella* species are infecting different livestock species in order to define transmission patterns and adopt the most suitable control strategies (33). Besides *B. abortus* and *B. melitensis* isolation in cattle, *B. suis* was unexpectedly cultured from two cows (33).

Therefore, there is an urgent need to perform bacteriological studies to supplement findings of serological tests in order to identify which *Brucella* species induced seropositivity in one or more livestock species, wildlife and humans (21). Unfortunately, making use of the OIE-recommended selective Farrell's medium for the isolation of *Brucella* spp. is often not affordable in developing countries given the high cost of the selective antibiotic supplement. This results in a very poor recovery rate of *Brucella* spp. Such a problem has been previously encountered in Sudan (32). Moreover, when bacteriology is performed caution should always prevail when identifying *Brucella* spp. to the species and biovar levels. Therefore, the identification and biotyping should always be confirmed in reference laboratories. The present study highlights that differentiating *B. melitensis* biovar 2 from *B. abortus* biovar 6 can only be done with certainty in such reference laboratories. Hence, previous studies mentioning the isolation of *B. melitensis* biovar 2 from small ruminants need to be re-examined.

The authors wish to emphasise that correctly identifying *Brucella* spp. to the species and biovar levels is not a trivial exercise. Indeed, only the isolation, identification and characterisation of *Brucella* spp. will allow the implementation of a sound brucellosis control programme. Such a programme should target interventions towards the reservoir species in order to stop the transmission within the reservoir species but also to prevent spillover to other livestock species, wildlife and humans (21).

Given the high transmission rate of *B. melitensis* in its preferential host (sheep) and the absence of a centrally organised brucellosis control programme in Sudan, the low true prevalence of the disease in the sheep in this study was difficult to understand. It pointed towards the possibility of a spillover of another *Brucella* species in the sheep population. This was confirmed by the isolation of *B. abortus* biovar 6.

The results of the present study suggest that the global brucellosis epidemiology needs to be carefully re-evaluated in Africa, where mixed herding is commonly practised and where diagnosis relies almost exclusively on serology. Isolation of *Brucella* spp. is critical in order to identify the reservoir of *Brucella* spp. and implement sound control measures. Therefore, the use of the Farrell's medium for the isolation of *Brucella* spp. should be recommended in sub-Saharan Africa by the OIE and the FAO and financial resources should be allocated for the isolation and characterisation of *Brucella* spp. in Africa.

Acknowledgements

The authors would like to thank the director and technical staff in the Veterinary Research Institute and Kassala Veterinary Research Laboratory.

■

Prévalence sérologique de la brucellose chez les ovins et isolement de *Brucella abortus* biovar 6 dans l'État de Kassala, Soudan oriental

M.M. Gumaa, H.M. Osman, M.M. Omer, E.M. El Sanousi, J. Godfroid & A.M. Ahmed

Résumé

La brucellose est l'une des maladies zoonotiques les plus graves affectant le bétail dans le monde. Les auteurs rapportent les résultats d'une étude visant à estimer la prévalence de la brucellose et à isoler les espèces de *Brucella* chez les ovins dans l'État de Kassala, Soudan oriental. Au total, 2005 échantillons de sérum ont été prélevés de manière aléatoire dans neuf localités différentes. Les échantillons sériques ont été soumis à l'épreuve au rose Bengale sur plaque (RBPT) et à l'épreuve RBPT modifiée (mRBPT). Les résultats positifs se sont répartis comme suit : 43 échantillons positifs au RBPT (2,15 %, intervalle de confiance [IC] à 95 % compris entre 1,6 et 3,0) et 68 échantillons positifs au mRBPT (3,4 %, IC à 95 % compris entre 2,6 et 4,2). Compte tenu du fait que le mRBPT a démontré posséder une sensibilité diagnostique de 86,6 % et une spécificité diagnostique de 97,6 %, la prévalence réelle a été estimée à 1,2 % (IC à 95 % compris entre 0,3 et 2,2). Différents tissus ont été prélevés à partir des 41 animaux ayant donné des résultats positifs au mRBPT. *Brucella abortus* biovar 6 a été isolée à partir de fluides utérins d'une brebis séropositive atteinte de pyomètre. Il est important de remarquer que les méthodes bactériologiques courantes ne permettent pas de distinguer *B. abortus* biovar 6 de *B. melitensis* biovar 2. Seule une lysotypie réalisée par un laboratoire de référence permet d'identifier la souche avec exactitude. Le fait que *B. abortus* biovar 6 ne nécessite pas de CO_2 pour sa croissance et qu'elle ait été isolée chez un petit ruminant lors de l'étude aurait facilement pu entraîner une erreur d'identification (en tant que *B. melitensis* biovar 2), aboutissant à des conclusions épidémiologiques erronées et à la mise en œuvre de mesures de contrôle inappropriées. Les résultats présentés ici semblent indiquer que les ovins sont des hôtes incidents, comme cela a déjà été constaté chez les chameaux, et que l'espèce bovine constitue le véritable réservoir de *B. abortus* biovar 6 dans l'État de Kassala au Soudan oriental. Cette étude souligne l'importance d'isoler et d'identifier les *Brucella* spp. dans différentes espèces d'animaux d'élevage afin d'élucider avec exactitude l'épidémiologie de la brucellose en Afrique subsaharienne.

Mots-clés

Afrique subsaharienne – Animal d'élevage – *Brucella abortus* biovar 6 – *Brucella melitensis* biovar 2 – Brucellose – Épidémiologie – Hôte incident – Hôte réservoir – Soudan oriental.

■

Seroprevalencia de la brucelosis en ovejas y aislamiento del biovar 6 de *Brucella abortus* en el estado de Kassala (Sudán oriental)

M.M. Gumaa, H.M. Osman, M.M. Omer, E.M. El Sanousi, J. Godfroid & A.M. Ahmed

Resumen

La brucelosis es una de las enfermedades zoonóticas de importancia que afectan al ganado. Los autores exponen un estudio encaminado a estimar la prevalencia de esta afección y aislar las brucelas presentes en ovejas del estado de Kassala, en la parte oriental del Sudán. Tras la obtención aleatoria de 2005 muestras séricas en nueve localidades distintas, todas ellas fueron sometidas a las pruebas de aglutinación en placa de rosa de Bengala (APRB) y APRB modificada (APRBm). De esas muestras, 43 (2,15%, intervalo de confianza [IC] 95%: 1,6-3,0) y 68 (3,4%, IC 95%: 2,6-4,2) resultaron positivas con las pruebas de APRB y APRBm, respectivamente. Atendiendo a los parámetros descritos para la prueba de APRBm, a saber, una sensibilidad de diagnóstico del 86,6% y una especificidad de diagnóstico del 97,6%, se calculó que la prevalencia real era del 1,2% (IC 95%: 0,3-2,2). Después se extrajeron diferentes muestras tisulares de los 41 animales positivos a la APRBm. En muestras de exudado piométrico de una oveja seropositiva se aisló el biovar 6 de *Brucella abortus*. Es importante señalar que las técnicas bacteriológicas habituales no permiten distinguir entre el biovar 6 de *B. abortus* y el biovar 2 de *B. melitensis*. El único método para identificar la cepa con exactitud es el de la tipificación por fagos que llevan a cabo los laboratorios de referencia. El hecho de que el biovar 6 de *B. abortus* no necesite CO_2 para crecer y de que en este estudio fuera aislado en un pequeño rumiante podría haber llevado fácilmente a identificarlo (erróneamente) como el biovar 2 de *B. melitensis*, y a partir de ahí a extraer conclusiones epidemiológicas equivocadas e instituir medidas de lucha inadecuadas. Los resultados aquí presentados dejan suponer que las ovejas son hospedadores no preferentes, función ya descrita en los camellos, y que en el estado de Kassala (Sudán oriental) el verdadero reservorio del biovar 6 de *B. abortus* es el ganado vacuno. Este estudio pone de manifiesto la importancia de aislar e identificar a las brucelas en distintas especies ganaderas para desentrañar con precisión la epidemiología de la brucelosis en el África subsahariana.

Palabras clave

África subsahariana – *Brucella abortus* biovar 6 – *Brucella melitensis* biovar 2 – Brucelosis – Epidemiología – Ganado vacuno – Hospedador no preferente – Hospedador reservorio – Sudán oriental.

■

References

1. Alton G.G., Jones L.M., Angus R.D. & Verger J.M. (1988). – Techniques for the brucellosis laboratory. Institut National de la Recherche Agronomique, Paris.

2. Corbel M.J. (1997). – Brucellosis: an overview. *Emerg. infect. Dis.*, **3** (2), 213–221.

3. Foster G., Osterman B.S., Godfroid J., Jacques I. & Cloeckaert A. (2007). – *Brucella ceti* sp. nov and *Brucella pinnipedialis* sp. nov. for *Brucella* strains with cetaceans and seals as their preferred hosts. *Int. J. syst. evolut. Microbiol.*, **57**, 2688–2693.

4. Scholz H.C., Hubalek Z., Sedlacek I., Vergnaud G., Tomaso H., Al Dahouk S., Melzer F., Kampfer P., Neubauer H., Cloeckaert A., Maquart M., Zygmunt M.S., Whatmore A.M., Falsen E., Bahn P., Gollner C., Pfeffer M., Huber B., Busse H.J. & Nockler K. (2008). – *Brucella microti* sp. nov., isolated from the common vole *Microtus arvalis*. *Int. J. syst. evolut. Microbiol.*, **58**, 375–382.

5. Scholz H.C., Nockler K., Gollner C., Bahn P., Vergnaud G., Tomaso H., Al Dahouk S., Kampfer P., Cloeckaert A., Maquart M., Zygmunt M.S., Whatmore A.M., Pfeffer M., Huber B., Busse H.J. & De B.K. (2010). – *Brucella inopinata* sp. nov., isolated from a breast implant infection. *Int. J. syst. evolut. Microbiol.*, **60** (4), 801–808.

6. World Organisation for Animal Health (OIE) (2012). – Chapter 2.7.2. Caprine and ovine brucellosis (excluding *Brucella ovis*). *In* Manual of diagnostic tests and vaccines for terrestrial animals, 7th Ed. OIE, Paris. Available at: www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.07.02_CAPRINE_OVINE_BRUC.pdf (accessed on 15 September 2014).

7. Blasco J.M. & Molina-Flores B. (2011). – Control and eradication of *Brucella melitensis* infection in sheep and goats. *Vet. Clin. N. Am. (Food Anim. Pract.)*, **27** (1), 95–104.

8. Godfroid J., Scholz H.C., Barbier T., Nicolas C., Wattiau P., Fretin D., Whatmore A.M., Cloeckaert A., Blasco J.M., Moriyón I., Saegerman C., Muma J.B., Al Dahouk S., Neubauer H. & Letesson J.J. (2011). – Brucellosis at the animal/ecosystem/human interface at the beginning of the 21st century. *Prev. vet. Med.*, **102** (2), 118–131.

9. Roth F., Zinsstag J., Orkhon D., Chimed-Ochir G., Hutton G., Cosivi O., Carrin G. & Otte J. (2003). – Human health benefits from livestock vaccination for brucellosis: case study. *Bull. WHO*, **81** (12), 867–876.

10. Zinsstag J., Schelling E., Roth F., Bonfoh B., de Savigny D. & Tanner M. (2007). – Human benefits of animal interventions for zoonosis control. *Emerg. infect. Dis.*, **13** (4), 527–531.

11. Musa M.T. & Jahans K.L. (1990). – The isolation of *Brucella melitensis* biovar-3 from a testicular hygroma of a ram in a nomadic flock of sheep and goats in Western Sudan. *J. comp. Pathol.*, **103** (4), 467–470.

12. Refai M. (2002). – Incidence and control of brucellosis in the Near East region. *Vet. Microbiol.*, **90** (1–4), 81–110.

13. El-Ansary E.H.H., Mohammed B.A., Hamad A.R.A. & Karom A.G.O. (2001). – Brucellosis among animals and human contacts in eastern Sudan. *Saudi med. J.*, **22** (7), 577–579.

14. Omer M.M., Abdelaziz A.A., Abusalab M.A. & Ahmed A.M. (2007). – Survey of brucellosis among sheep, goats, camels and cattle in Kassala area, eastern Sudan. *J. Anim. Vet. Adv.*, **6** (5), 635–637.

15. Food and Agriculture Organization of the United Nations (FAO) (2003). – Guidelines for coordinated human and animal brucellosis surveillance. FAO Animal Production and Health Paper No. 156. FAO, Rome.

16. World Organisation for Animal Health (OIE) (2012). – Chapter 2.4.3. Bovine brucellosis. *In* Manual of diagnostic tests and vaccines for terrestrial animals, 7th Ed. OIE, Paris. Available at: www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.04.03_BOVINE_BRUCELL.pdf (accessed on 15 September 2014).

17. Ferreira A.C., Cardoso R., Dias I.T., Mariano I., Belo A., Preto I.R., Manteigas A., Fonseca A.P. & De Sa M.I.C. (2003). – Evaluation of a modified Rose Bengal test and an indirect enzyme-linked immunosorbent assay for the diagnosis of *Brucella melitensis* infection in sheep. *Vet. Res.*, **34** (3), 297–305.

18. Minas A., Stournara A., Minas M., Stack J., Petridou E., Christodoulopoulos G. & Krikelis V. (2007). – Validation of a fluorescence polarization assay (FPA) performed in microplates and comparison with other tests used for diagnosing *B-melitensis* infection in sheep and goats. *J. immunol. Meth.*, **320** (1–2), 94-103.

19. Reiczigel J., Foldi J. & Ozsvari L. (2010). – Exact confidence limits for prevalence of a disease with an imperfect diagnostic test. *Epidemiol. Infect.*, **138** (11), 1674–1678.

20. Godfroid J., Cloeckaert A., Liautard J.P., Kohler S., Fretin D., Walravens K., Garin-Bastuji B. & Letesson J.J. (2005). – From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis. *Vet. Res.*, **36** (3), 313–326.

21. McDermott J.J. & Arimi S.M. (2002). – Brucellosis in sub-Saharan Africa: epidemiology, control and impact. *Vet. Microbiol.*, **90** (1–4), 111–134.

22. Godfroid J., Al Dahouk S., Pappas G., Roth F., Matope G., Muma J., Marcotty T., Pfeiffer D. & Skjerve E. (2013). – A One Health surveillance and control of brucellosis in developing countries: moving away from improvisation. *Comp. Immunol. Microbiol. infect. Dis.*, **36** (3), 241–248.

23. Nielsen K. (2002). – Diagnosis of brucellosis by serology. *Vet. Microbiol.*, **90** (1–4), 447–459.

24. Perrett L.L., McGiven J.A., Brew S.D. & Stack J.A. (2010). – Evaluation of competitive ELISA for detection of antibodies to *Brucella* infection in domestic animals. *Croatian Med. J.*, **51** (4), 314–319.

25. Emslie F.R. & Nel J.R. (2002). – An overview of the eradication of *Brucella melitensis* from Kwazulu-Natal. *Onderstepoort J. vet. Res.*, **69** (2), 123–127.

26. Muendo E., Mbatha P., Macharia J., Abdoel T., Janszen P., Pastoor R. & Smits H. (2012). – Infection of cattle in Kenya with *Brucella abortus* biovar 3 and *Brucella melitensis* biovar 1 genotypes. *Trop. anim. Hlth Prod.*, **44** (1), 17–20.

27. Musa M.T., Eisa M.Z.M., El Sanousi E.M., Abdel Wahab M.B. & Perrett L. (2008). – Brucellosis in camels (*Camelus dromedarius*) in Darfur, Western Sudan. *J. comp. Pathol.*, **138** (2–3), 151–155.

28. Holt H.R., Eltholth M.M., Hegazy Y.M., El-Tras W.F., Tayel A.A. & Guitian J. (2011). – *Brucella* spp. infection in large ruminants in an endemic area of Egypt: cross-sectional study investigating seroprevalence, risk factors and livestock owner's knowledge, attitudes and practices (KAPs). *BMC public Hlth*, **11**, 341–350.

29. Verger J.M., Garin-Bastuji B., Grayon M. & Mahe A.M. (1989). – *Brucella melitensis* infection in cattle in France. *Ann. Rech. vét.*, **20** (1), 93–102.

30. Álvarez J., Sáez J.L., García N., Serrat C., Pérez-Sancho M., González S., Ortega M.J., Gou J., Carbajo L., Garrido F., Goyache J. & Domínguez L. (2011). – Management of an outbreak of brucellosis due to *B. melitensis* in dairy cattle in Spain. *Res. vet. Sci.*, **90** (2), 208–211.

31. Ocholi R.A., Kwaga J.K.P., Ajogi I. & Bale J.O.O. (2004). – Phenotypic characterization of *Brucella* strains isolated from livestock in Nigeria. *Vet. Microbiol.*, **103** (1–2), 47–53.

32. Omer M., Musa M., Bakhiet M. & Perrett L. (2010). – Brucellosis in camels, cattle and humans: associations and evaluation of serological tests used for diagnosis of the disease in nomadic localities in eastern Sudan. *Rev. sci. tech. Off. int. Epiz.*, **29** (3), 663–669.

33. Menshawy A.M., Pérez-Sancho M., García-Seco T., Hosein H.I., García N., Martínez I., Sayour A.E., Goyache J., Azzam R.A., Domínguez L. & Álvarez J. (2014). – Assessment of genetic diversity of zoonotic *Brucella* spp. recovered from livestock in Egypt using multiple locus VNTR analysis. *Biomed. Res. Int.*. E-pub.: 6 January 2014. doi:10.1155/2014/353876.