Retrospective analysis of the prevalence of *Brucella* antibodies in sheep in the Karas Region of Namibia

Oscar Madzingira

Division of Veterinary Public Health, Directorate of Veterinary Services, P. O. Box 27, Gobabis, Namibia. email: omuzembe@gmail.com; Tel: +264813593072

Cheryl M McCrindle

SSHPH, Faculty of Health Sciences, University of Pretoria, P. Bag X20, Hatfield, Pretoria 0028, South Africa

Abstract

In this retrospective study, complete *Brucella* serology data from the annual national brucellosis testing program and disease investigation for the years 2008-2010 was collated and analyzed to estimate the prevalence of brucellosis in sheep in the Karas Region of Namibia. A total of 22994 serological results from 762 flocks screened using the Rose Bengal Test (RBT) and confirmed using the Complement Fixation Test (CFT) were analyzed. An overall prevalence of 0.14% was recorded over the three years. Yearly prevalence was 0.19% (2008), 0.05% (2009) and 0.18% (2010). At district level, brucellosis prevalence was estimated to be between 0% and 0.49%. On positive farms (n=32), prevalence was between 2.25% and 30%. True prevalence was zero at district level and in all the three study years. We concluded that the prevalence of *Brucella* antibodies in sheep was low taking into account that some farmers may have vaccinated against the disease. The low prevalence confirms the effectiveness of existing brucellosis control measures implemented by the official veterinary services.

Keywords *Brucella*, sheep, prevalence, Namibia

Introduction

Brucellosis is a world-wide zoonosis caused by Gram-negative bacteria of the genus *Brucella.* The agent is endemic in most African countries (Mangen et al. 2002). *Brucella melitensis* causes disease in adult sheep (SANCO 2001; Robinson 2003). Economic losses due to abortions, birth of weak and sickly newborns and reduced fertility in livestock have been widely reported (SANCO 2001). In humans, *B. melitensis* is the most pathogenic agent and causes a chronic debilitating disease (SANCO 2001). The disease is an occupational risk for slaughter men, butchers, farmers and veterinarians (FAO 2006). The incidence of brucellosis in humans is related to the prevalence of infection in livestock species. In Namibia, *B. melitensis* was first reported in the Karakul breed of sheep in 1953 (Godfroid et al. 2004). Over the years, serological prevalence of between 0.2% and 2.19% has been reported in sheep (Magwedere et al. 2009).

Sheep meat is one of the most affordable sources of protein in Namibia. About 750 000 sheep are slaughtered annually at local and export abattoirs. To meet the sanitary requirements of importing countries, the Directorate of Veterinary Services (DVS) embarked on an annual voluntary brucellosis testing of sheep farms to certify sheep flocks as free of the disease for meat export purposes. The approach currently used for controlling sheep brucellosis in Namibia is: when a sheep flock tests positive for brucellosis, the farm is placed under movement restriction and all sheep above six months of age are serologically tested for *Brucella* antibodies. All sheep that test positive on the Complement Fixation Test (CFT) are eliminated. Quarantine restrictions

on the remaining sheep are removed after two consecutive negative CFT serological results at least three months apart. In addition, farmers are advised to keep a closed flock and purchase replacement sheep from brucellosis-free flocks (DVS 2011).

An outbreak of brucellosis due to *B. melitensis* on a sheep farm in one of the major sheep producing regions of Namibia (Magwedere et al. 2009); the availability of brucellosis testing results data from both passive and active surveillance and the paucity of published information on the prevalence of brucellosis in sheep in Namibia necessitated this study.

The estimated prevalence will assist risk managers in planning the management of this disease and in assessing whether the sanitary conditions imposed by importing countries are justified. The objective of this study was to estimate the prevalence of *Brucella* antibodies in sheep in the Karas Region of Namibia – a region with the greatest sheep population, using testing results data from 2008 to 2010.

Materials and methods

Study area and study population

Data for the study was obtained from the Karas Region. The region is located at the extreme southern end of Namibia. The region is divided into four magisterial districts namely Keetmanshoop, Karasburg, Bethanie and Luderitz. The latter district has predominantly mining activities and was therefore not included in this study. The Karas Region is the major sheep producing region of the country with a total of 700 sheep

commercial farms rearing approximately one million sheep. The region has a hot and dry climate, with unpredictable average summer (October to March) rainfalls of between 142-152mm. In the hottest months, temperatures reach 40^{o}C, whilst in winter temperatures frequently drop below freezing point at night (KRC 2011). The Dorper is the predominant sheep breed in the study area. Other breeds such as the Damara, Karakul and Merino are also present but in smaller numbers. Sheep are managed extensively on natural pastures year wide. The study population comprised of sheep of unknown vaccination status.

Study design

Data for this study was obtained from brucellosis active and passive surveillance data for the years 2008, 2009 and 2010. These serological results were collated from the annual national brucellosis testing program and disease investigation testing in sheep. No distinction was made between passive and active surveillance data. A total of 762 farms and 22994 serological results were analyzed. The prevalence of brucellosis was estimated per farm, district and year, and comparisons made.

Collection of serum samples

Sample size determination, the collection of blood, storage, recovery of sera and transport was done in accordance with standard procedures described in the updated brucellosis protocol (DVS 2011) issued by the Directorate of Veterinary Services.

Ethical statement

All sheep in this study were handled by Animal Health Technicians of the Directorate of Veterinary Services who are trained in animal handling and welfare. The collection of

blood samples was according to standard procedures as described in the brucellosis sampling protocol (DVS 2011).

Serological testing

Serological testing was done by the Central Veterinary Laboratory. Testing for *Brucella* antibodies was done using the Rose Bengal Test (RBT) as a screening test and confirmation of all positive samples was done using the Complement Fixation Test (CFT) according to recommendations of the World Organization for Animal Health (OIE 2009).

Statistical analyses

Data from the study was stored and processed in Microsoft Excel® software. Brucellosis prevalence was calculated per farm, district and year. To account for the possible clustering effect of sampling on the farms, 95% confidence intervals around the mean prevalence were adjusted according to Reiczigel et al. (2010).

Results

Thirty-two sera and eight farms tested positive over the three year period. The serological prevalence of brucellosis over the three year period was 0.14% (95% CI: 0.1%-0.2%). Annual prevalence was 0.19% (95% CI: 0.11%-0.33%), 0.05% (95% CI: 0.02%-0.13%) and 0.18% (95% CI: 0.11%-0.30%) for 2008, 2009 and 2010 respectively. The prevalence of serologically positive farms was 1.05%. On these farms, brucellosis prevalence was 8.23% (95% CI: 4.47%-13.42%), 2.25% (95% CI: 0.77%-5.46%) and 30% (18.49%-43.90) in 2008, 2009 and 2010 respectively.

At district level, the prevalence of *Brucella* antibodies was between 0% and 0.49%. Results for each year are illustrated in Tables 1-3. A summary of the district and annual

Table 1 Results for sheep sera collected in the Karas Region in 2008

District	Number of farms tested	Total sera tested	Number of positive farms	Number of positive sera	% positive farms	% positive sera
Bethanie	8	241	0	0	0	0
Karasburg	100	3045	1	2	1 (0.05-5.14)	0.07 (0.02-0.24)
Keetmanshoop	112	3433	3	11	2.68 (0.73-7.29)	0.32 (0.18-0.57)
Total	220	6719	4	13	1.82 (0.62-4.58)	0.19 (0.11-0.33)

Values in brackets are 95% confidence limits (Reiczigel et al. 2010)

Table 2 Results for sheep sera collected in the Karas Region in 2009

District	Number of farms tested	Total sera tested	Number of farms positive	Number of sera positive	% positive farms	% positive sera
Bethanie	48	1445	1	3	2.08 (0.10-10.74)	0.21 (0.07-0.61)
Karasburg	44	1343	0	0	0	0
Keetmanshoop	174	5290	1	1	0.57 (0.02-2.95)	0.02 (0.00-0.11)
Totals	266	8078	2	4	0.75 (0.13-2.66)	0.05 (0.02-0.13)

Values in brackets are 95% confidence intervals (Reiczigel et al. 2010)

Table 3 Results for sheep sera collected in the Karas Region in 2010

District	Number of farms tested	Total sera tested	Number of farms positive	Number of sera positive	% positive farms	% positive sheep
Bethanie	40	1139	0	0	0	0
Karasburg	76	2672	1	13	1.32 (0.06-6.77)	0.49 (0.28-0.83)
Keetmanshoop	160	4386	1	2	1.25 (0.22-4.42)	0.05 (0.01-0.17)
Totals	276	8197	2	15	0.72 (0.12-2.56)	0.18 (0.11-0.30)

Values in brackets are 95% confidence limits (Reiczigel et al. 2010)

prevalence over the three years is shown in Table 4. At district level, Bethanie district had no positive reactors in 2008 and 2010.

True prevalence as calculated from apparent prevalence according to Reiczigel et al. (2010) using CFT test sensitivity and specificity of 81% and 98% (Bercovich 1998) respectively was zero in all districts as shown in Table 4.

Table 4 A summary of brucellosis prevalence (%) for 2008, 2009 and 2010

Year	Keetmanshoop	Karasburg	Bethanie	Overall Prevalence
2008	0.32 (0)	0.07 (0)	0 (0)	0.19 (0)
2009	0.02 (0)	0 (0)	0.21 (0)	0.05 (0)
2010	0.05 (0)	0.49 (0)	0 (0)	0.18 (0)

Values in brackets represent true prevalence calculated according to Reiczigel et al. (2010)

Discussion

An overall prevalence of 0.14% was determined for the Karas Region over the three year period. This figure is comparable to a prevalence of between 0.2% and 2.19% reported in sheep on commercial farms in Namibia (Magwedere et al. 2009). Depner (1993), reported a higher prevalence of between 0.9% and 20% in sheep on communal farms in Namibia. In South Africa, a prevalence of between 1.23% and 4.02% has been reported (Emslie and Nel 2002). In Southern Africa, sheep brucellosis prevalence has been reported to be between 5.6% and 14.5% (McDermott and Arimi 2002). Our results show that the prevalence recorded in this study is lower than reported elsewhere. The difference may be attributed to the different environments and sheep management systems prevailing in the different geographical areas and the serological tests employed. The study by Depner (1993) utilised a competitive enzyme immunoassay with a higher specificity and sensitivity than the RBT and CFT. In this study, the study area was characterized by very hot and dry climatic conditions which are well documented to be unfavorable for the survival and transmission of *Brucella* species bacteria (SANCO 2001). In addition, the extensive management of sheep on natural pastures and the fact that flocks from different farms did not mix may have reduced the infection rate (Hesterberg 2008). It is well documented that sheep flocks whose movement is restricted on farms as in our study, have lower brucellosis prevalence than mobile flocks (McDermott & Arimi 2002).

The prevalence of *Brucella* antibodies in 2009 (0.05%) was lower than the prevalence in 2008 (0.19%) and 2010 (0.18), although the number of sera and farms tested were greater than the other years. The control and preventive measures that were

implemented in the country in 2009 following an outbreak of brucellosis on one farm in a neighboring region may have played a part in reducing brucellosis prevalence.

The prevalence of positive farms was low (0.72% - 1.82%), but brucellosis prevalence on such farms was relatively high (2.25% to 30%). These findings indicate that *Brucella* positive sheep were concentrated on a few farms. Therefore, if control measures are focused on these farms, it should be possible to further reduce prevalence in the region. Follow up investigations on positive farms also revealed that these farms had a history of introducing sheep from other flocks. These movements may have been the source of infection because the introduction of new sheep without implementing biosecurity measures is a well documented risk factor for the introduction of brucellosis in clean flocks (SANCO 2001; McDermott and Arimi 2002).

True prevalence was zero in all districts over the three years. The low prevalence could therefore be due to false positive results or to cross reactions with other organisms such *Yersinia enterocolitica* O: 9 (Nielsen et al. 2006; OIE 2009). Vaccinations against *B. melitensis* using *B. melitensis* Rev. 1 vaccine may have confounded the prevalence recorded in our study.

The major limitation of the retrospective study was that the data was drawn mainly from a voluntary testing program. Interpretation of the results must therefore be made with caution. However, the number of serological results (n = 22994) and study farms (n = 762) were considerably large enough to provide a representation of the brucellosis situation in the Karas Region.

Our results show that the prevalence of *Brucella* antibodies in sheep in the Karas Region was low over the three years. These results correlate with the low number of clinical cases of sheep brucellosis reported in the Karas Region by official veterinary services and confirm that existing measures based on the test-and-slaughter principle are effective. According to Nicoletti (1993) and the FAO (2003), the test-and-slaughter approach is effective when brucellosis prevalence is less than 2% as recorded in our study. These findings may be used to negotiate for economically friendly brucellosis sanitary requirements for live sheep and sheep meat with importing countries.

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Conflict of interest

The author declares that he has no conflict of interest.

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