Seroprevalence and risk factors for bovine brucellosis in domestic yaks (*Bos grunniens*) in Tibet, China

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Abstract
A cross-sectional study was conducted in three counties (Damxung, Maizhokunggar and Yadong) in Tibet in April and May 2015 to investigate the prevalence and risk factors for brucellosis in yaks. A total of 1523 yaks owned by 181 herders were randomly selected and blood sampled. Sera were tested using the Rose Bengal Test (RBT) and a Competitive immune-enzymatic assay (C-ELISA) and test results interpreted in parallel. The individual yak prevalence was 2.8% (95%CI: 2.0-3.7) with a herd prevalence of 18.2% (95%CI: 12.9-24.6). On the individual level, a binary logistic regression model was constructed to evaluate the association between seropositivity and putative risk factors. Two predictor variables, age and production system were significantly associated with seropositivity. At the herd level, an association between infection with *Brucella* and a history of abortions in the herd was observed. The results of the survey indicate that bovine brucellosis is endemic among the yak population in the plateau region of China, and the risk factors identified in the study should be considered in the epidemiology of the disease and when developing control programs for the disease.

Keywords: *Brucellosis, Yak, Prevalence, Risk factors*

Introduction
Bovine brucellosis is principally caused by *B. abortus*, although *B. melitensis* and *B. suis* can occasionally cause disease if animals are grazed and/or managed with sheep and goats, or pigs, respectively (1). Although bovine brucellosis has been eradicated in some countries, such as Australia and New Zealand (2), it remains endemic in many countries, including China (3). Tibet is one of the largest pastoral regions in China and the grazing of yaks (*Bos grunniens*) plays an important role in the lives of the pastoralist herders through the production of meat, milk, fibre and hides (4).

Brucellosis was recognised in Tibet in the 1980s as a disease of both livestock and humans. Through the implementation of vaccination and slaughter of affected animals the disease was reported to have been controlled (5); however recently human infection in Tibet has re-emerged (6). As infection in humans always arises from contact with domestic or wild animal reservoirs and their products (7), in order to effectively control the disease China initiated a surveillance programme of livestock in 2009 (8). In this surveillance programme the prevalence of brucellosis in livestock including yaks, cattle, sheep and goats from three counties of Tibet was reported to be 1.25% (9). Currently there is little up-to-date information about the disease on the Tibetan plateau consequently the current study was designed to determine the seroprevalence, distribution and risk factors for brucellosis in yaks.

Materials and methods
Study areas
Damxung, Maizhokunggar and Yadong Counties.

Sample size
The sample size to estimate the prevalence using a design prevalence of 10% (10), with 95% confidence and 5% precision was calculated in Epitools (11).

Serological tests
Each serum sample was tested with a commercial RBT (Institute Pourquir, IDEXX Montpellier, France) and a C-ELISA (INGEZIM BRUCELLA Compac 2.0, SPAIN) using the protocol outlined by the manufacturer. These tests had not previously been validated in yaks. When tests were interpreted in parallel (an animal with a positive result on either test was classified as positive) the calculated sensitivity and specificity of the combined tests in *Bos Taurus* were 99.6 and 86.2%, respectively.

Statistical analyses
Data were entered in a spread-sheet (Microsoft Excel 2010, Redmond, USA). Seroprevalence together with 95% CI were calculated and further analysed using the statistical software R (12). A binary logistic regression model was constructed to explore the associations between factors and seropositivity. The model was evaluated through the Hosmer-Lemeshow goodness-of-fit test. Additionally, a Receiver Operating Characteristic (ROC) curve was generated to display the predictive accuracy of the model (13).

Results
A total of 1523 yaks belonging to 181 herders originating from 30 villages were sampled in the three selected counties.

Serological results
The seroprevalence on the RBT was 1.8% (95%CI: 1.2-2.6) which was similar to the C-ELISA result of 1.5% (95% CI 1.0-2.3) ($p=0.67$, $\chi^2=0.18$). When the tests were interpreted in

parallel, the seroprevalence of antibodies against brucellosis was 2.8% (95% CI: 2.0-3.7) (Table 1). 18.2% (95% CI:12.9-24.6) of herds contained one or more seropositive animals.

Table 1. Serological detection of antibodies against Brucella using RBT and C-ELISA

Serological results	Number of cases	Test Prevalence (%)	95% CI
RBT(+)	27	1.8	1.2, 2.6
C-ELISA(+)	23	1.5	1.0, 2.3
RBT(+) C-ELISA(+)	8	0.5	0.2, 1.0
RBT(+) C-ELISA(-)	19	1.3	0.8, 1.9
RBT(-) C-ELISA(+)	15	1	0.6, 1.6
RBT or C-ELISA(+)	42	2.8	2.0, 3.7

Analyses at the individual level

The four factors age, herd size, county and production system all had a $p<0.20$ on the univariable analyses and were selected for inclusion in the saturated multivariable logistic regression model. In the final multivariable logistic-regression model only the production system and age of animals were significantly associated with seropositivity in yaks at the animal level. Yaks in the agro-pastoral production system (OR=2.66, 95% CI: 1.44-5.0) and aged >2 years old (OR=2.90, 95% CI: 1.23-8.51) had significantly higher odds of seropositivity compared to yaks in the pastoral production system and aged ≤2 years old, respectively (Table 2). A Hosmer-Lemeshow goodness-of–fit value (p=0.86), indicated that the model was a good fit of the data. The area under the ROC curve was 0.65, indicating that the model had moderate to good predictive ability.

Table 2. Multivariable logistic regression analysis of risk factors associated with brucellosis seropositivity

Risk factors	Category levels	Estimate	SE	OR	95%CI	P-Value
Constant		-4.86	0.49			
Production system	Pastoral			1		0.002
	Agro-pastoral	0.98	0.32	2.66	1.44, 5.00	
Age	Young (≤2)			1		0.027
	Old (>2)	1.07	0.48	2.9	1.23, 8.51	

Estimate

Regression coefficient; SE: Standard error; CI: Confidence interval

Analyses at the herd level

Herds with a history of abortion in the preceding year to the survey were more likely to be seropositive (OR=3.62, 95%CI: 1.25, 10.54) than herds without abortions.

Discussion

Although there is an official bovine brucellosis surveillance strategy in Tibet, this study is one of the first epidemiological studies on the disease investigating the prevalence in yaks managed under different production systems in the region. The study showed that the seroprevalence in individual yaks was relatively low (2.8%) although the herd prevalence was higher (18.2%). A higher animal level prevalence in yaks has been reported in India (21.1%) and Nepal (22%). In China the prevalence reported in yaks has varied between locations being 6.5 to 13.4% in Qing-Tibet plateau (14), 5.2 to 12.8% in Xinjiang autonomous region to the northwest of Tibet (15). One or both of the tests in the current study were also applied in these other studies.

At the individual animal level, age and production system were significant in the logistic regression model. Others have similarly reported an increased seroprevalence with age (16). This is likely to be associated with an increased chance of exposure to the bacterium through environmental exposure to contaminated materials such as aborted foetuses, fluids and milk. The production system was identified as a risk factor for seropositivity in the current study as has similarly been reported by others (17). The lower population density associated with a nomadic system would decrease potential exposure to *Brucella,* in contrast the higher stocking density in an agro-pastoral system would provide a greater opportunity for non-infected animals to have contact with infected yaks (18).

In this study a history of abortions in the herd in the 12 months preceding the survey was strongly linked to seropositivity ($p<0.05$). This is consistent with other studies (19) and is not unexpected given the association between infection and abortion. The presence of abortion storms is characteristic of infection with brucellosis in a naïve herd.

In conclusion, this study demonstrated that the seroprevalence to brucellosis in domestic yaks (*Bos grunniens*) was low in Tibet. To minimise future spread of infection it is recommended that an annual vaccination program should be implemented as has previously been adopted for cattle (20). There is a need for educational material to be developed for yak herders to minimise infection of humans who are in close association with these animals or who consume unpasteurised milk and milk products.

Acknowledgements

We would like to thank the John Allwright Fellowship from ACIAR (Australian Centre for International Agricultural Research) and Murdoch University for sponsoring this study as part of a PhD studentship. The Tibet Livestock Research Institute and Veterinary stations in the three sampled counties are acknowledged for their field assistances during sample collection. The authors would like to thank for all pastoralists of participating the project. Also, we appreciated the staff of China Animal Health and Epidemiology Centre for laboratory assistances.

References

1. **OIE**. 2009. Available from: http://www.oie.int.
2. **Díaz.** *Rev Sci Tech.* 32(1), 43-51, 53-60, 2013
3. **Shang *et al.*** *Vet. Microbiol.* 90, 165-182, 2002
4. **Ji et al.** Nairobi, Kenya: International Livestock Research Institute (ILRI), 2002
5. **Anonymous.** *Tibetan Journal of Science and Technology* 4, 47-49, 1985

6. **Zhan Dui** *et al. Disease Surveillance* 23(5), 285-286, 2008
7. **Godfroid *et al.*** *Vet. Res.* 36(3), 313-326, 2005
8. **Bureau of Veterinary**. Beijing: China Agriculture Press, 2009
9. **Wu et al.** *Tibetan Journal of Animal Husbandry and Veterinary* 2, 15-17, 2011
10. **Gao *et al.*** *Cattle practice* 21, 109-111, 2013
11. **Sergeant.** 2016. Available from: http://epitools.ausvet.com.au.
12. **R Development Core Team.** 2013. Available from: http://www.r-project.org.
13. **Sing *et al.*** *Bioinformatics* 21(20), 3940-3941, 2005
14. **He *et al.*** *China Cattle Science* 41(4), 94-95, 2015
15. **Dilixiati *et al.*** *Xinjiangxumuye* 4, 35-37, 2001
16. **Kebede *et al.*** *Rev. Med. Vet.* 159(1), 3-9, 2008
17. **Jiwa *et al.*** *Prev. Vet. Med.* 26(3), 341-346, 1996
18. **Omer *et al.*** *Epidemiol. Infect.* 125(02), 447-453, 2000
19. **Muma *et al.*** *Rev. Sci. Tech.* 26(3), 721-730, 2007
20. **Olsen *et al.*** *Veterinary Clinics of North America: Food Animal Practice* 26(1), 15-27, 2010