Bhutan Journal of Animal Science (BJAS)
Volume 6, Issue 1, Page 52-55, March 2022

SEROPREVALENCE OF BRUCELLOSIS IN GOATS IN SELECTED HIGH-RISK AREAS OF BHUTAN

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ABSTRACT: Brucellosis in goats is caused by *Brucella melitensis* and *Brucella ovis*. *Brucella melitensis* is also known to cause Brucellosis in human. It causes abortion, retention of placenta, and orchitis in male animals and losses is mainly accounted due to abortions in goats. Goat population is concentrated in the southern districts; free mixing up of animals with those of across the international border occurs in these areas leading to high risk of incursion of disease. Brucellosis in cattle has been reported in the country however, it's prevalence in goats have not been established before hence, study on seroprevalence in goats were conducted in two of the high-risk districts. Retrospective screening of 949 goat sera samples for Brucella antibody were conducted. The samples were collected during March 2021 from 16 villages of 3 sub-districts under Chukha district and 25 villages of 8 subdistricts under Sarpang district. Sera samples were tested using Rose Bengal Test and Enzyme Linked-immunosorbent Assay. All the samples, tested negative to Brucella antibodies. However, screening of more samples from other districts needs to be conducted.

Keywords: Brucella; ELISA; food safety; goats; health safety; RBT; screening; zoonoses

1. INTRODUCTION

Animal Brucellosis is caused by *Brucella abortus* (cattle), *Brucella melitensis* (goat), *Brucella suis* (pig), *Brucella canis* (dog) and *Brucella ovis* (sheep). *Brucella melitensis* and *Brucella ovis* are the common cause of brucellosis in goats and sheep, respectively (Tekle et al. 2019). It is an infectious zoonotic disease with significant economic impact on the livestock farming and also public health. In human, *Brucella melitensis* is the most virulent species (Corbel 2006).

Brucellosis in goats is reported to cause abortion in the late stage of pregnancy, retention of placenta, and orchitis in male animals and losses in goat production is mainly accounted through abortions. Approximately 90% of goats are being reared in the developing world, where they are considered one of the most important sources of protein for humans (Klopp et al 2014). Goat farming is not very popular in Bhutan especially on commercial scale. However, they are reared in the backyards commonly in the southern parts of the country where they are meant mainly for meat purpose and also as a source of income and common breeds include local, Black Bengal, Beetal, Sirohi, Babari and Jamnapari (Sherpa 2014). Since the goat population is concentrated in the southern districts and also free mixing up of animals with those from across the international border takes place, there is high risk of incursion and spread of Brucellosis in these areas. The animals in the country are not vaccinated against Brucellosis.

The disease has been reported in imported cattle in Bhutan recently and an investigation of abortion at National Jersey Breeding Centre, Samtse and monitoring for five different time points for a period of 2 years revealed and average prevalence of 32.80%, 40.11%, 22.39% and 37.17% through Rose Bengal Test, ELISA, culture & PCR and Complement Fixation Test respectively (Gurung et al. 2018). However, to date the study has not been conducted in the goats. Hence, a retrospective seroprevalence study was conducted in goats in the two of the southern districts (Chhukha and Sarpang) from high-risk areas in the southern part of the country for the regional baseline information.

2. MATERIALS AND METHODS

Retrospective study was conducted by screening the goat sera samples for Brucella antibodies. Purposive random sampling was done from Chhukha and Sarpang. A total of 949 sera samples these two districts (11 sub-districts, 41 villages) were collected (Table 1.)

2.1 Sample pool

All 949 samples were used to get 92 pools. A pool comprised of around 10 samples from each village thus, sample per village ≤ 5 were neither pooled nor screened.

2.2 Rose Bengal Test (RBT)

Rose Bengal Antigen (ID Vet, France) was used to perform RBT and screen pooled sample for antibody to Brucella species with a sensitivity of 92.1% and specificity of 99.6% (Blasco 1994). The test serum (0.06 ml) was mixed with 0.03 ml of RBT antigen on a glass slide to produce a zone of approximately 3 cm in diameter. The mixture was agitated gently for 4 min at ambient temperature and then observed for agglutination. Tests were considered positive when any visible reaction or agglutination were observed.

2.3 Enzyme Linked-immunosorbent Assay

Pooled sera samples were also subjected to Brucella antibodies detection using, Goat and Sheep Brucellosis antibody ELISA Kit (Elab Science, US, Catalog No: E-AD-E026). The ELISA Microtiter plate pre-coated with Brucellosis antigen was used to perform ELISA. Horseradish peroxidase (HRP) conjugate and other auxiliary reagents were used to detect Brucellosis antibody of goat and sheep in sera samples. The test was performed as per the protocol provided by the manufacturer. Serum samples (100 µl) were dispensed to each well of an ELISA plate pre-coated with antigen, mixed with gentle tapping on the sides and incubated for 45 min at room temperature. The plates were hand-washed three times with wash buffer, and a peroxidase-labelled monoclonal anti-ruminant IgG conjugate (100 µl) diluted in dilution buffer (1:100) was added to each well and incubated for 30 min at room temperature. The plates were washed as above and 100 µl of 3', 3', 5', 5' - tetra-methyl-benzidine (TMB) substrate was added and incubated for 10 min in dark. The chromogenic reaction was stopped by adding 100 µl of stop solution, and the optical density (OD) values were read at 450 nm using a plate reader (Heales MB-530).

The ELISA results were interpreted as the signal of the test sample as a proportion of the positive control. Samples with an OD_{450} value ≥ 0.38 was considered positive and sample with an OD_{450} value < 0.38 was considered negative.

3. RESULTS AND DISCUSSION

All the sera samples screened against Brucella antibodies were found negative by both RBT and ELISA. It indicated that the animals were not exposed to Brucella antigens; infective agents or vaccines. However, study in other districts also needs to be conducted. The seroprevalence in the goats were 2.4% in Bangladesh (Munsi et al. 2021), 2.6 % to 3.3% in Nepal (Pandeya et al. 2013; Gompo et al. 2021), 19.2% in Indonesia (Primatika et al. 2016) to as high as 28.17% in Ethiopia (Teshome, 2022) and 33.4% in Libya (Al-Griw et al. 2017). On the contrary, the seroprevalence study conducted in selected areas of high-risk areas of Bhutan did not reveal seropositivity to Brucella antibody. However, study in other districts of the high-risk areas is required to conclude the status of seroprevalence of Brucellosis in goats.

Brucellosis is a highly infectious zoonotic disease and humans get infection often by drinking raw milk from infected animals and also meat. Animal health workers, farmers, and abattoir workers are vulnerable to infection as they handle infected animals and aborted foetuses or placentae. Brucellosis has been an occupational risk for veterinarians, farmers and employees in the meat industries (Young 1995). Hence, human brucellosis can be best prevented by controlling the infection in animals. Brucellosis in cattle (*B. abortus*), sheep and goats (*B. melitensis*) and in swine (*B. suis*) are diseases listed in the World Organization for Animal Health (OIE) Terrestrial Animal Health Code and must be reported to the OIE.

Table 1: Sample area and sample size

District	Sub-district	Village	Breed	No of sample	Pool
Chukha	Lokchina	Amalay	Local	92	9
	Sampheling	Kortiline	Local	33	3
		Khaibatar	Local	10	1
		Gurungdara	Local	9	1
	Phuntsholing	Toribari	Local	48	6
		Nayabasti	Local	17	2
		Khairayni	Local	21	2
		Balaytar	Local	9	1
		Chamkuna	Local	15	1
		Kami Dara	Local	5	0
		Sadumadu	Local	15	1
		Kamaydara	Local	45	4
		Sukigaon	Local	25	2
		Lowar Malbashey	Local	23	2
		Limbukha	Local	22	2
		Katarey	Local	10	1
			Total	**399**	**38**
Sarpang	Chuzagang	Chaskhar	Local	72	7
		Yuling	Local	32	3
	Umling	Dangling	Local	19	2
		Lingar	Local	1	0
		Rijook	Local	25	2
		Thongjazor	Local	10	1
	Taraythang	Tashicholing	Local	6	1
		Yoezergang	Local	30	3
	Singye	Yarphuling	Local	3	0
		Sangaythang	Local	17	2
		Labtsakha	Local	26	3
		Bunakha	Local	5	0
	Samtenling	Samtenling	Local	74	7
	Gelephu	Pelrithang	Local	9	1
		Namkhaling	Local	29	3
		Rabdeyling	Local	10	1
		Jampayling	Local	24	2
	Dekiling	Yangchenphu	Local	31	3
		Trashiling	Local	10	1
		Dekiling	Local	11	1
		Mendrelgang	Local	7	1
		Dolomgang	Local	15	1
	Gakiling	Kawaipani	Local	10	1
		Omchuna	Local	27	3
		Gakiling	Local	47	5
			Total	**550**	**54**
Grand total				**949**	**92**

4. CONCLUSIONS & RECOMMENDATION

Absence of Brucellosis in goats as revealed by this study conducted in two of the goats' rearing districts of Bhutan is an assurance of food safety and occupational health safety. Goats are reared by marginal farmers where these animals are slaughtered in conventional method for home consumption or sale purpose. Slaughter process that involves significant carcass handling in conventional method unlike in machine operated slaughterhouse present higher risk of exposure to workers. Brucellosis is very important disease for livestock industry as well as to humans due to its high zoonotic potential. Hence, regular screening of the disease in animals is very essential. Screening of animals both at the entry point at the quarantine stations and also the animals within the country including small ruminants should be mandatory. Actual seroprevalence status in the goats in the high-risk districts will be known only after screening the representative population in other high-risk districts. Early detection of Brucellosis in animals and timely control helps prevent further spread in animals and also prevention of the disease in humans.

Acknowledgements

The authors are thankful to all the colleagues from regional offices and District Livestock Sector for supporting in sample collection from Sarpang and Chhukha districts. The authors are also thankful to the laboratory technicians who helped in testing samples.

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