



High seroprevalence of bovine herpesvirus-1 and *Brucella abortus* in yak populations of Arunachal Pradesh, India, correlates with abortion

A K BERA¹, M HUSSAIN², C MAJI³, S MAITI⁴, J DOLEY⁵, D BHATTACHARYA⁶, J BAM⁷,
S DEORI⁸, V PAUL⁹, T K BISWAS¹⁰ and S M DEB¹¹

ICAR-National Research Centre on Yak, Dirang, West Kameng, Arunachal Pradesh 790 101 India

Received: 21 January 2015; Accepted: 8 March 2015

Key words: Abortion, *Brucella*, IBR, India, Seroprevalence, Yak

Yak (*Poephagus grunniens* L.), a unique multipurpose bovid, is reared mostly by the poor and marginal tribal farmers of rural and remote hilly regions at an altitude of 3000-4500m above sea level. In the Peoples Republic of China, Mongolia, Bhutan, Nepal, Russia and India, yaks are farmed even at 5000m above sea level (Weiner *et al.* 2003, Bandyopadhyay *et al.* 2007). About 90% of yak populations of the world inhabit the Qinghai-Tibetan Plateau in China, where they are of high economic importance to native herdsman (Lie *et al.* 2008). Amongst infectious diseases brucellosis and infectious bovine rhinotracheitis (IBR) are known to cause reproductive problems in the form of abortions and subfertility in dairy animals worldwide. Brucellosis is a well-known zoonotic disease that causes huge economic losses to dairy farmers through abortion and other reproductive failure (Dhand *et al.* 2005). Infectious bovine rhinotracheitis (IBR) is an important emerging disease of livestock caused by bovine herpes virus- 1 (BHV-1). Infection by BHV-1 causes different clinical syndromes including vulvovaginitis, respiratory disease, pustular balanoposthitis, infertility, abortion (Radostits *et al.* 2000), keratoconjunctivitis (Bandyopadhyay *et al.* 2009a) in a range of domestic and wild cattle, yak, mithun, goat, sheep and camelid (Campos *et al.* 2009, Rahman *et al.* 2007, Rajkhowa *et al.* 2004). Previous studies have measured the sero-prevalence of brucellosis and BHV-1 in yak from India (Bandyopadhyay *et al.* 2007) and China (Xulong *et al.* 2011), however these studies did not correlate with the seroprevalence levels with cases of abortion in the different stages of pregnancy. In this study the levels of seroprevalence in yak for *Brucella* and BHV-1 were correlated to levels of abortion.

Farmed and free-ranging yaks from the West Kameng and Tawang districts of Arunachal Pradesh, India were selected for the study. Serum samples (328) from yak (134 male and 194 female) that were collected for detection of antibodies against *Brucella* and BHV-1. A detailed history on abortion in these yaks revealed that 102 of the yaks had aborted for a total of 132 times in the past. Blood samples were collected from the yaks by venipuncture and serum was separated using standard procedures. Serum samples were stored at -20°C. Testing for antibodies to *Brucella abortus* and BHV-1 were carried out using an AB-ELISA kit procured from the Project Directorate on Animal Disease Monitoring and Surveillance, Bengaluru, India. The AB-ELISA was performed following the methodology as described. Briefly, the test and control (strongly positive, moderately positive and negative) sera diluted in blocking buffer containing 1% gelatin and 0.1% Tween-20 in 0.01 M PBS were added to respective wells in duplicate and incubated at 37°C for 1 h with periodic shaking. The plates were washed and then 100 µL biotinylated anti-bovine IgG (1:30,000 diluted in blocking buffer) was added to each well and incubated for 1 h at 37°C. After washing, the plates were incubated with 100 µL of avidin-horseradish peroxidase conjugate (1:15,000 diluted in blocking buffer) followed by incubation for 30 min and 20 min for brucellosis and BHV-1 respectively at 37°C. The plates were washed again and treated with 100 µL of substrate chromogen complex (100 µL of O-phenylene diamine dihydrochloride with 5 µL of 30% H₂O₂) and were incubated for 10 min at room temperature. Finally, the reaction was stopped by 50 µL of 1 M H₂SO₄. Absorbance was taken at 492 nm and percentage positivity (PP) was calculated using the formula given below.

$$PP (\%) = \frac{OD \text{ of the test well} \times 100}{\text{Median OD of the strong positive sample}}$$

A value greater than or equal to 40 was considered positive.

Out of the 328 serum samples, 32.92% tested positive for *Brucella*, which comprised of 20.37% male and 79.63%

Present address: ¹Senior Scientist (Veterinary Medicine) (asitmed2000@yahoo.com), ²Senior Technical Officer (hussainmokhtar61@gmail.com), ³SRF (lordchinmoy@gmail.com), ^{4,5,7,8,10}Scientist (sanjit.ndri@gmail.com, juwarvet_dol@yahoo.co.uk, jode.vet@gmail.com, sourabhd1@rediffmail.com, drtapas007@rediffmail.com), ⁶Principal Scientist (debasis63@rediffmail.com), ⁹Senior Scientist (vpaul.nrcy@gmail.com), ¹¹Director (sm_deb@yahoo.com), ICAR-NRC on Yak.

Table 1. Data on seroprevalence screening against brucellosis and IBR in the studied yak population

Total animal tested	328	Male Female	134 194	Heifer Lactating	38 156
<i>Brucella</i> positive	108 (32.92%)	Male Female	22 (20.37%) 86 (79.63%)	Heifer Lactating	18 (20.93%) 68 (79.07%)
IBR positive	100 (30.49%)	Male Female	26 (26%) 74 (74%)	Heifer Lactating	8 (10.81%) 66 (89.19%)
Both <i>Brucella</i> and IBR positive	52 (15.85%)	Male Female	10 (19.23%) 42 (80.77%)	Heifer Lactating	4 (9.52%) 38 (90.48%)

Table 2. Occurrence of abortion in relation to different number of lactation in yak

Number of lactation	1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	8 th	9 th	Total number of abortion
Number of abortion	30 (22.73%)	18 (13.64%)	34 (25.76%)	16 (12.12%)	6 (4.55%)	14 (10.61%)	6 (4.55%)	4 (3.03%)	4 (3.03%)	132

female yaks. Out of brucellosis positive female yaks, 79.07% were in lactation. Similarly, 30.49% of the yaks tested positive for BHV-1 antibodies by ELISA of which 74% were female. Amongst the IBR positive female, 89.19% were lactating animals (Table 1).

This indicated that yak populations of West Kameng and Tawang districts of Arunachal Pradesh, India are affected with both IBR and brucellosis. Diagnosis of abortion often poses a challenge to the herd owner and the veterinarian, although the gradual increase in abortion rate in a herd over a period of many years is likely to be recognized.

It was observed that 15.85% of the yaks were positive for both *Brucella* and BHV-1 antibodies, out of which 80.77% were female. Brucellosis is highly contagious zoonotic and economically important bacterial disease of animal worldwide (OIE 2000). The economic losses through lower milk production in animals with brucellosis have been described in previous reports (McDermott and Arimi 2002). Besides, it is second most important zoonotic disease in the world after rabies (FAO 2003) which demands a large scales survey in the area to detect the carrier animals and their treatment or prevention following the standard protocol. IBR caused by BHV-1 is amongst the most important emerging diseases of cattle and buffalo in India (Jain *et al.* 2008). Serological evidence for IBR has been reported in different animals including cattle (Sontakke *et al.* 2008), mithun (Rajkhawa *et al.* 2004) and yak (Bandyopadhyay *et al.* 2010). IBR is responsible for a variety of manifestations including reproductive disorders (Jain *et al.* 2008). Amongst these female yaks that were antibody positive for both pathogens, 90.48% were lactating. The data further revealed that 102 of the yaks (65.38%) had a history of having either a single or multiple abortions. Yaks in their third lactation showed the highest incidence of abortion (25.76%) followed by first lactation

(22.73%), second lactation (13.64%) and fourth lactation (12.12%) (Table 2).

The zoonotic nature of brucellosis puts at risk to field workers, veterinarians, butchers and milkers (Shafee *et al.* 2011). Abortion, either by brucellosis or BHV-1, may cause a reduction in milk production indirectly by length of lactation. It is a global problem of wild and domestic animals especially cattle, sheep and goats causing decrease in reproductive efficiency and increase in abortion rate (Rijpens *et al.* 1996). Further, it was observed that abortion cases were highest in third lactation and third trimester which further aggravate the economic loss (Shafee *et al.* 2011).

The incidence of abortion was highest in the third trimester (39.39%) followed by first trimester (36.36%) and second trimesters (24.24%). BHV-1 antibodies have been reported in yak from Arunachal Pradesh, India (Bandyopadhyay *et al.* 2009a). They also opined that antibodies against BHV-1 can be found in bovines in all continents, and in many wild species. Prevalence varies greatly depending upon herd size and management practices. However, previous studies in yaks (Bandyopadhyay *et al.* 2009a, Bandyopadhyay *et al.* 2009b, Bandyopadhyay *et al.* 2010) did not correlate the seroprevalence data with the history of abortion and stage of pregnancy in which abortion occurred. It has been suggested that the mixing of domestic animals in pasture during grazing is a major contributor to the spread of transmissible diseases (Poester *et al.* 2002). Yaks are reared in a transhumance (free range) system of management, therefore there is a high possibility that yak, cattle, sheep and other wild animals will co-graze and mix, resulting in the spread of infectious diseases between these species. In this present investigation, it was observed that 90.48% of lactating yaks were positive for both IBR and brucellosis,

Table 3. Data on abortion history of yak and seroprevalence of brucellosis and IBR

Total number of animals with abortion history	Total number of animals tested for both <i>Brucella</i> and IBR	Number of animals with positive titre either or both <i>Brucella</i> and IBR	Number of animals positive for <i>Brucella</i>	Number of animals positive for IBR	Number of animals positive for <i>Brucella</i> and IBR also
102	62	44 (70.97%)	38 (61.29%)	26 (41.94%)	20 (32.26%)

resulting in potential high economic losses due to lower milk production.

Out of these 102 animals with a history of abortion, 62 were tested for antibodies to BHV-1 and *Brucella abortus*. The results revealed that 70.97% of these tested animals were positive for either or both *Brucella* and BHV-1 antibodies where as 61.29, 41.94 and 32.26% were positive for antibody against *Brucella*, BHV-1 and both pathogens, respectively (Table. 3). This high level of infection could lead to severe economic losses through lower reproductive and productive performance. Under field conditions, natural servicing is usually practiced in yak herds, which could result in the repeated use of IBR or *Brucella* infected yak bulls for natural insemination, which could transmit the infections and thus contribute to the high levels of seropositivity seen in the female yaks (Bandyopadhyay *et al.* 2009b). Seroprevalence surveys for brucellosis in yak and transhumant pastoralists have been carried out in Nepal (Jackson *et al.* 2014) which indicated that seropositivity for *Brucella* species in yak was wide spread and associated with reported human disease. Brucellosis has also been confirmed in yak in the erstwhile Soviet Union (Schley 1967), China (Xulong *et al.* 2011) and India (Bandyopadhyay *et al.* 2009b).

SUMMARY

Animals with history of abortion were positively correlated with IBR, brucellosis or both. Out of 328 animals tested, 32.92% were seropositive for *Brucella*, 30.49% were positive for BHV-1 and 15.85% were positive for both pathogens. Based on retrospective data, 102 yaks could be traced as having a history of aborting on at least one occasion in the past. Cases of abortion were highest (25.76%) in the third lactation as well as third trimester (39.39%). This has further proved the etiological factors of abortion in yak population are mainly infectious in origin and out of which brucellosis (61.29%) was the prominent factor followed by IBR (41.94%). Abortion in yak due to infectious etiological factor causes significant economic loss includes abortion, loss of milk production, low fertility rate and cost of replacement of animals. Well-kept records on clinical and serological findings will often be of benefit during the investigation of abortion problems.

ACKNOWLEDGEMENT

The authors are thankful to the Director, ICAR-National Research Centre on Yak for providing laboratory facilities

and scientific aid and Director of the Project Directorate on Animal Disease Monitoring and Surveillance, Bangalore, India for supplying AB-ELISA Kit without which this study could not have taken place.

REFERENCES

- Bandyopadhyay S, Saravanan B C, Ghosh M K, Sarkar M, Ramesha K P and Bhattacharya M. 2007. Common health ailments of yaks (*Poephagus grunniens* L.). *Technical bulletin*, NRC-Y publication, Dirang, AP, India.
- Bandyopadhyay S, Chakraborty D, Sarkar T, Pal B, Sarkar S, Sasmal D, Biswas T K, Ghosh M K and Sarkar M. 2009a. A serological survey of antibodies against bovine herpesvirus-1 in yak (*Poephagus grunniens*) in Arunachal Pradesh in India. *Revue scientifique et technique (International Office of Epizootics)* **28**: 1045–50.
- Bandyopadhyay S, Sasmal D, Dutta T K, Ghosh M K, Sarkar M, Sasmal N K and Bhattacharya M. 2009b. Seroprevalence of brucellosis in yak (*Poephagus grunniens*) in India and evaluation of protective immunity to S19 vaccine. *Tropical Animal Health Production* **41**: 587–92.
- Bandyopadhyay S, Das S, Baruah K K, Chakravarty P, Chakraborty D, Sarkar T, Pal, De S, Pan D, Bera A K, Bandyopadhyay S and Bhattacharya D. 2010. Detection of bovine herpes virus 1 sequences in yaks (*Bos grunions*) with keratoconjunctivitis, using a highly sensitive nested polymerase chain reaction. *Revue scientifique et technique (International Office of Epizootics)* **29**: 695–703.
- Campos F S, Franco A C, Hubner S O, Oliveira M T, Silva A D, Esteves P A, Roehe P M & Rijsewijk F A. 2009. High prevalence of co-infections with bovine herpes virus 1 and 5 found in cattle in southern Brazil. *Veterinary Microbiology* **139**: 67–73.
- Dhand N K, Gumber S, Singh B, Aradhana, Bal M S, Kumar H, Sharma D R, Singh J and Sandhu K S. 2005. A study on the epidemiology of brucellosis in Punjab (India) using Survey Toolbox. *Revue scientifique et technique (International Office of Epizootics)* **24**: 879–85.
- FAO. 2003. Progress in understanding brucellosis. *Veterinary Record* **641**.
- Jackson D S, Nydam D V and Altier C. 2014. Prevalence and risk factors for brucellosis in domestic yak (*Bos grunniens*) and their herders in a transhumant pastoralist system of Dolpo, Nepal. *Preventive Veterinary Medicine* **113** (1): 47–58.
- Jain L, Kanani A N, Patel T J, Purohit J H, Jhala M K, Chuahan H C and Chandel B S. 2008. Seroprevalence of bovine herpesvirus-1 (BHV-1) in Indian breeding bulls of Gujarat. *Buffalo Bulletin* **27**: 165–69.
- Lie J, Cai J Z, Zhang W, Liu Q, Chen D, Han J P and Liu Q R. 2008. Sero-epidemiology of *Neospora caninum* and *Toxoplasma gondii* infection in yaks (*Bos grunniens*) in Qinghai, China. *Veterinary Parasitology* **152**: 330–32.

- McDermott J J and Arimi S M. 2002. Brucellosis in sub-Saharan Africa: epidemiology, control and impact. *Veterinary Microbiology* **90**: 111–34.
- OIE. 2000. Bovine brucellosis. *Manual of Standards for Diagnostic Tests and Vaccines*. 4th edn. pp. 328–45. OIE, Paris, France.
- Poester F, Goncalves V S P and Lage A P. 2002. Brucellosis in Brazil. *Veterinary Microbiology* **90**: 55–62.
- Radostits O M, Gay C C, Blood D C and Hinchcliff K W. 2000. *Veterinary Medicine: A Textbook Of The Diseases Of Cattle, Sheep, Pigs, Goats and Horses*. 9th edn. pp. 1877. W.B. Saunders, London.
- Rahman H, Bhattacharya M, Rajkhowa J, Soud N, Nandankar U and Mukherjee S. 2007. Seroprevalence of infectious bovine rhinotracheitis in yaks (*Poephagus grunniens*) in India. *Indian Journal of Animal Sciences* **77**: 793–95.
- Rajkhowa S, Rajkhowa C, Rahman H and Bujarbaruah K M. 2004. Seroprevalence of infectious bovine rhinotracheitis in mithuns (*Bos frontalis*) in India. *Revue scientifique et technique (International Office of Epizootics)* **23**: 821–29.
- Rijpens N P, Jannes G, Van Asbroeck M, Rossau R and Herman L M F. 1996. Direct detection of brucella spp. In raw milk by PCR and reverse hybridization with 16S-23S rRNA spacer probes. *Applied and Environmental Microbiology* **62**: 1683–88.
- Schley P. 1967. Der Yak und seine Kreuzung mit dem Rind in der Sowjetunion. Giessener Abhandlungen zur Agar-und Wirtschaftsforschung des Europäischen Ostens. *Wiesbaden Germany* **44**: 132.
- Shafee M., Rabbani M., Sheikh A. A., Ahmad M. and Razzaq A. 2011. Prevalence of bovine brucellosis in organized dairy farms, using milk ELISA, in Quetta City, Balochistan, Pakistan. *Veterinary Medicine International*. Article ID 358950, pp–3.
- Sontakke S B, Deshmukh V V and Gujar M. B. 2008. Evaluation of humoral and cell mediated immune responses in cattle against experimental infectious bovine rhinotracheitis killed vaccine. *Indian Journal of Animal Sciences* **78**: 367–68.
- Weiner G, Jianlin H and Ruijun L. 2003. *The Yak*. 2nd edn. FAO Regional office for Asia and Pacific, Bangkok.
- Xulong L, Hailong Q, Zhaoyang B, Vanling Y, Chunhui S, Xiaoyan L, Jinshan C, Rullin M, Yijuan F and Xinglong W. 2011. Seroprevalence of *Brucella* infection in yaks (*Bos grunniens*) on the Qinghai-Tibet plateau of China. *Tropical Animal Health Production* **43**: 305–06.