



An investigation on infectious etiologies of bovine abortions in Northern Western Himalayan region of Himachal Pradesh

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Cattle husbandry is the core of livestock economics making an important contribution to the sustenance of small and marginal livestock keeping in India. Dairying is crucial for the rural economy as a whole and especially in the mountainous and semi-mountainous regions. Reproductive losses linked to infectious etiologies in bovines are major economic concerns worldwide. Reproductive disorders like abortions, metritis, stillbirths and infertility incur losses to the dairy sector. Abortions (loss between days 42 and 260 of the fetal stage) significantly impact the reproductive performance and production of dairy cattle (Hopper 2015). Bovine abortions can result from any of the nutritional, physiological, endocrine, genetic, toxicological and infectious factors. It is estimated that in 90% of the cases, abortions are of infectious origin (Sarangi *et al.* 2021). Infectious diseases causing abortions in bovines like brucellosis, chlamydiosis, toxoplasmosis, Q-fever, etc. are not only of veterinary importance but are also zoonotic and pose a significant threat to human health. These are established occupational hazards for veterinarians, farmers and dairy workers (Vidal *et al.* 2017, Sarangi *et al.* 2021).

Keeping in view the adverse impact of abortions on the dairy sector, it is important to investigate their incidence and etiologies. Infectious etiologies causing abortions in bovine has been reported in several states in India (Kumar *et al.* 2020). Isolation of etiological agents is considered the gold standard in the diagnosis of infectious disease. However, it is not always possible to isolate the etiological agent due to the fastidious growth requirements of infectious agents, lengthy isolation procedures and lack of proper laboratory infrastructure and trained manpower. Serological diagnostic techniques can detect circulating antibodies and are convenient methods for detecting present or past infections.

The present study was undertaken to ascertain infectious etiologies of bovine abortions in Northern Western Himalayan region of Himachal Pradesh through serological tests with the objective to design the control and preventive measures against the abortifacient infectious agents.

A total of 161 serum samples were collected from cattle with a history of abortions presented during the clinical camps (n=204) organized in different regions of Himachal Pradesh. Animals were reared under discrete climate, nutrition, and management systems. Blood samples were collected through jugular venipuncture in 10 mL vacutainer tubes without anticoagulant. Blood was allowed to clot at room temperature for 15 to 30 min and serum was collected after centrifugation at 3500 rpm for 10 min. Serum samples were apportioned into aliquots, labeled, and stored at –20°C until analyzed.

The stored serum samples were analyzed with enzyme-linked immunosorbent assay (ELISA), Rose Bengal plate test (RBPT) and agar gel precipitation test (AGPT) for the detection antibodies against of viral (bovine herpes virus, BHV), bacterial (*Brucella abortus* and *Leptospira*), protozoan (*Toxoplasma*), chlamydial, rickettsial (*Coxiella burnetii*) and fungal (*Aspergillus fumigatus* and *Candida albicans*) etiologies of abortion.

IgG antibody ELISA kit of IMMUNOLAB (Immunoconcept India Pvt. Ltd.) was used to detect BHV-1, *Aspergillus fumigatus*, *Candida albicans* and *Brucella abortus* infections. IgG antibody DRG ELISA kits (DRG diagnostics GmbH) were used to detect anti-*Toxoplasma* and anti-*Leptospira* antibodies. The stored serum samples as well as the ELISA kits were brought to room temperature and assays were performed using TECAN SUNRISE Microplate Absorbance Reader (TECAN Austria GmbH, Austria) as per the manufacturer's recommendations. Optical density (OD) values were calculated and results were interpreted as positive or negative as per the manufacturer's instructions.

RBPT was performed as per Morgan *et al.* (1969) to detect *Brucella*-specific agglutinins using antigen procured from Indian Veterinary Research Institute (IVRI),

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Izatnagar. AGPT was performed as described earlier (Barron *et al.* 1972) for the detection of the *Chlamydia* antibodies with some modifications using 1% agar in a normal saline solution. 4-5 mL of molten agar was poured onto a clean microscope slide. Wells of 4.0 mm diameter were cut aseptically into the solid agar at a 3-4 mm distance. The slides were incubated in moist chambers for 24-48 h. The antigen-antibody complex in the form of a clear precipitation line was observed in chlamydia-positive cases.

Overall, 16.77% (27/161) of the serum samples were detected positive for antibodies to at least one organism, while no antibodies were detected in 138 samples for the targeted etiological agents (Table 1). The highest prevalence was recorded for BHV-1 (8.07%, 13/161) followed by *Chlamydia* (3.11%, 5/161), *B. abortus* (2.48%, 4/161), *Toxoplasma* (1.86%, 3/161), *Leptospira* (0.62%, 1/161), and *C. albicans* (0.62%, 1/161). None of the tested samples was positive for *C. burnetii* and *A. fumigatus*.

BHV-1 and BHV-4 are common viral infections linked to bovine abortions. BHV-1 prevalence is higher than BHV-4, which is considered as an opportunistic viral pathogen (Holler 2012). In this study, 8.07% of the analyzed serum samples were detected sero-positive for BHV-1 by ELISA (Table 1). Lucchese *et al.* (2016) reported 9.52% BHV seroprevalence in cattle with or without a history of abortion. Sarangi *et al.* (2021) reported 87.5% sero-positivity for BHV-I by ELISA in cattle from an organized farm with a high abortion rate in Southern India. Irrespective of the history of abortions, Katoch *et al.* (2017) reported a higher disease prevalence of 24.24% in bovine of Himachal Pradesh.

Brucellosis is one of the five major notifiable bacterial diseases of zoonotic importance in the world (Franc *et al.* 2018). Bovine brucellosis is endemic in India (Deka *et al.* 2019). We detected 2.48% (4/161) serum samples positive for *Brucella* using the RBPT and ELISA (Table 1). One serum showed a weak reaction (+/++) in RBPT; whereas three sera showed strong agglutination (+++). RBPT is a sensitive, cheap, and rapid method but has low specificity (Lolli *et al.* 2016). RBPT in association with ELISA in a parallel interpretation scheme reduces the misclassification and improves the testing sensitivity (Musallam *et al.* 2015).

The overall serological prevalence of *Brucella* recorded in this study was much lower compared to 25.0%, previously reported in the bovines of Himachal Pradesh with a history of abortions (Chahota *et al.* 2003). Dhand *et al.* (2005) also reported a high prevalence (33.87%) of anti-*Brucella* antibodies in the bovines of Punjab with a history of abortions. In Southern India, 95.3% of the aborted cattle in an organized farm were detected as *Brucella* reactors (Sarangi *et al.* 2021).

Leptospira spp. infection has a chronic presentation in bovines and can cause severe reproductive problems such as abortions, stillbirths and low fertility. Bovines are considered as maintenance hosts of the serovars *Hardjoprajitno* and *Hardjobovis* and are transmitted directly among cattle. In bovines, leptospirosis is transmitted through direct contact with the urine of infected reservoirs, consumption of contaminated feed/fodder and water as well as by sexual mode (Loureiro and Lilenbaum 2020).

The results of the present study showed that 1 out of 161 investigated samples (0.62%) was found positive for *Leptospira* using ELISA (Table 1). Vidal *et al.* (2017) reported anti-*Leptospira* spp. antibodies in 21.4% of bovine abortion cases in Switzerland, with 11.5% of sera being positive for at least two serovars by ELISA. In a recent study from Southern India, *Leptospira* was detected in 54.69% of the aborted cattle (Sarangi *et al.* 2021).

Chlamydia in cattle has been reported sporadically all over the world and is implicated in respiratory and reproductive tract diseases (Chahota *et al.* 2015). In the present study, 3.11% of sera were found positive for *Chlamydia* infection using AGPT (Table 1). These estimates are in agreement with Chahota *et al.* (2015) who reported an overall 4.65% and 0.93% sero-prevalence in cattle and buffaloes, respectively. In Switzerland, chlamydial antibodies were detected in 38.5% of cases of bovine abortions, and an additional 12.6% of sera were suspected to be positive (Vidal *et al.* 2017). In Argentina, Chlamydiaceae DNA was detected in 4.78% of aborted fetuses, 83.33% of aborted animals, and 16.66% of stillborn. *C. abortus* was detected in 1.99% of the analyzed samples with significantly high detection rates (41.67%) in Chlamydiaceae-positive samples (Rojas *et al.* 2018). In Arizona, USA, enzootic chlamydiosis due to *C. pecorum*

Table 1. Seroprevalence of infectious etiologies of bovine abortions

Infectious agent (no. of serum samples tested)	Detection of antibodies		Gestation period		
	Number positive (%)	I st trimester (%)	II nd trimester (%)	III rd trimester (%)	
Bovine herpes virus-1(161)	13 (8.07)	0 (0.0)	07 (53.85)	06(0.0)	
<i>Brucella</i> (161)	04 (2.48)	0 (0.0)	0 (0.0)	04 (100)	
<i>Leptospira</i> (161)	01 (0.62)	0 (0.0)	01 (100)	0 (0.0)	
<i>Toxoplasma</i> (161)	03 (1.86)	01 (33.33)	02 (66.67)	0 (0.0)	
<i>Chlamydia</i> (161)	05 (3.10)	0 (0.0)	03 (60.0)	02 (40.0)	
<i>Candida albicans</i> (161)	01 (0.62)	0 (0.0)	01 (100)	0 (0.0)	
<i>Coxiella burnetii</i> (161)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
<i>Aspergillus</i> (161)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Total (161)	27 (16.77)	01(3.70)	14 (51.85)	12 (44.44)	

was reported as the cause of third-trimester abortions and vasculitis and meningoencephalitis in fetuses, calves, and cows (Struthers *et al.* 2021).

Toxoplasma gondii is a zoonotic protozoan that can cause reproductive losses in ruminants. Various studies have demonstrated the presence of *T. gondii* in aborted fetuses and stillbirths of cattle (Nayeri *et al.* 2021). In contrast to small ruminants, few reports are available on cattle on natural and viable *T. gondii* infections (Stelzer *et al.* 2019). The results of our study showed that 1.86% of tested serum samples were found positive for *Toxoplasma* infection (Table 1). A study from Iran detected antibodies to *T. gondii* in 13.0% of pregnant cattle from a farm with a history of abortions. Cattle sero-positivity for *T. gondii* was reported to be significantly associated with the history of abortions (Gharekhani and Yakhchali 2020).

The global prevalence of fungal abortions varies from 1 to 25% (Pal *et al.* 1985). *Candida spp.* are natural inhabitants of the gastrointestinal or genital mucosa and tissue invasion may occur secondarily to immune suppression (Talapko *et al.* 2021). Different *Candida spp.* had been isolated from bovine abortions (Vilander *et al.* 2016). The results of our study showed that 1 out of 161 investigated samples (0.62%) was found positive for *C. albicans* infection (Table 1). This is in agreement with the results of two previous studies having identified *Candida spp.* in 0.14% and 0.3% of bovine abortions (Foley and Schlafer 1987, Knudtson and Kirkbride 1992).

Mycotic infection due to *Aspergillus spp.* causing placentitis is an important cause of abortion in cattle, which generally occurs as an uncomplicated infection in the last trimester of pregnancy. However, early abortion, i.e. in the second trimester is also possible. Concomitant abortions may be detected in cattle due to massive environmental contamination by *Aspergillus* conidia (Knudtson and Kirkbride 1992). *Aspergillus spp.* had been reported as the most common cause of mycotic abortions in cattle (Foley and Schlafer 1987, Knudtson and Kirkbride 1992). None of the tested serum samples in this study was detected for anti-*Aspergillus* antibodies (Table 1).

C. burnetii infection in animals is generally subclinical with non-specific clinical signs. Ruminants are the main reservoirs of infection, may present with late abortion, stillbirths, metritis and infertility (Vidal *et al.* 2017). *C. burnetii* was absent in all bovine samples taken from the 161 aborted cows using ELISA (Table 1). These results are in agreement with Barkallah *et al.* (2014), reported no *C. burnetii* infection in 150 aborted and 64 normal cows by the real-time PCR. 15.9% of the aborted cattle had anti-*C. burnetii* antibodies in Switzerland (Vidal *et al.* 2017). Sarangi *et al.* (2021) reported 9.4% *C. burnetii* sero-positivity in aborted cattle.

We recorded highest incidence of abortion (51.85%, 14/27) during the second trimester of the pregnancy followed by the third (44.44%, 12/27) and first trimester (3.70%, 1/27) of pregnancy (Table 1). These findings are in agreement with Clothier and Anderson (2012)

and Wolf-Jackel *et al.* (2020) who reported 90 to 100% bovine abortions in the second and third trimesters of the pregnancy.

Antibodies to BHV-1 were detected in animals with a history of abortions in the second (53.85%, 7/13) and third trimesters (46.15%, 6/13) of pregnancy (Table 1). Anti-*Brucella* antibodies were detected exclusively (4/4, 100%) in animals with a history of abortions in the third trimester of pregnancy. 60% (3/5) of the *Chlamydia* detection was in the second trimester of pregnancy whereas 40% (2/5) was in the third trimester. *Toxoplasma* sero-positive animals were from the first (33.33%, 1/3) and second trimesters (66.67%, 2/3) of gestation. Anti-*Leptospira* (n=1) and anti-*Candida* (n=1) antibodies were detected in the second trimester of pregnancy. Studies conducted previously had also reported abortions due to these infectious agents in the same gestation intervals as observed in this study (Mahajan *et al.* 2013, Lucchese *et al.* 2016).

SUMMARY

This study was conducted to ascertain infectious etiologies of bovine abortions in Northern Western Himalayan region of Himachal Pradesh. Overall, 16.77% (27/161) serum samples were positive for antibodies to at least one bovine abortion-causing pathogen. The overall prevalence was highest for BHV-1 (8.07%) followed by *Chlamydia* (3.11%), *B. abortus* (2.48%), *T. gondii* (1.86%), *Leptospira* (0.62%), and *C. albicans* (0.62%). None of the tested samples was positive for *C. burnetii* and *Aspergillus*. The occurrence of bovine abortions was higher (51.85%) in the second trimester of gestation followed by the third (44.44%) and first trimester (3.70%). In comparison to previous studies, we recorded a lower prevalence of infectious etiologies of bovine abortions. Hence, it is important to investigate the role of the non-infectious etiologies of bovine abortions such as hormonal (progesterone insufficiency), nutritional (negative energy balance, feedstuff containing anti-nutritional factors) and miscellaneous causes including poor animal management, toxin infestation, and twin pregnancies.

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