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Original Article

Seroprevalence to common infectious abortifacient and infertility causing agents in the dairy herds of India

Naveena, T.[1]; Sarangi, L. N.[2]; Rana, S. K.[3*]; Prasad, A.[4]; Prabha, T. S.[5]; Jhansi, D.[6]; Ponnanna, N. M.[2] and Sharma, G. K.[7]

[1]MSc in Microbiology, Group of Animal Health, National Dairy Development Board Research and Development Laboratory, IIL Campus, Gachibowli, Hyderabad 500032, Telangana, India; [2]Group of Animal Health, National Dairy Development Board Research and Development Laboratory, IIL Campus, Gachibowli, Hyderabad 500032, Telangana, India; [3]Group of Animal Health, National Dairy Development Board, Anand 388001, Gujarat, India; [4]MVSc in Veterinary Microbiology, Group of Animal Health, National Dairy Development Board Research and Development Laboratory, IIL Campus, Gachibowli, Hyderabad 500032, Telangana, India; [5]MSc in Biotechnology, Group of Animal Health, National Dairy Development Board Research and Development Laboratory, IIL Campus, Gachibowli, Hyderabad 500032, Telangana, India; [6]MTech in Biotechnology, Group of Animal Health, National Dairy Development Board Research and Development Laboratory, IIL Campus, Gachibowli, Hyderabad 500032, Telangana, India; [7]MVSc in Veterinary Microbiology, Group of Animal Health, National Dairy Development Board, Anand 388001, Gujarat, India

[*]***Correspondence:*** *S. K. Rana, Group of Animal Health, National Dairy Development Board, Anand 388001, Gujarat, India. E-mail: skrana@nddb.coop*

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Abstract

Background: Information on the prevalence of infectious agents in dairy farms forms the basis for formulating a suitable control strategy; especially in endemic situations. **Aims:** A cross-sectional study was undertaken to determine the prevalence of six economically important bovine diseases, causing reproductive disorders including bovine abortion in organized dairy herds in India. **Methods:** A total of 1,075 animals (cattle and buffaloes) from 09 dairy farms were screened by ELISA tests. **Results:** Bovine viral diarrhoea (BVD) was the most prevalent (56.5%) disease followed by infectious bovine rhinotracheitis (IBR) (45.4%). Prevalence of Q-fever (5.4%) and neosporosis (6.1%) were less on the farms. Although 16.3% of the samples turned positive for brucellosis, the contribution of calf-hood vaccination (*B. abortus* S19 vaccine) to the prevalence of antibodies cannot be ruled out. The overall prevalence of bovine anaplasmosis, known to cause sporadic abortions in dairy herds, was 34.1% in the 9 farms with a prevalence of less than 20% in 5 farms. Infection of multiple abortifacient (seroprevalence to more than two pathogens) was recorded in 56.8% of animals. A very strong association was observed between BVD and brucellosis (Odds ratio 14.2; P<0.001). Further, a positive association was also seen between seroprevalence of IBR and anaplasmosis, and neosporosis and Q fever (P<0.05). **Conclusion:** Viral diseases were found to be more common in the dairy herds than bacterial and protozoan diseases. Increased susceptibility of IBR seropositive cows to other bacterial and viral infections was observed.

Key words: Abortion, Bovine viral diarrhoea, Brucellosis, Infectious bovine rhinotracheitis, Neosporosis

Introduction

Infectious bovine abortions and infertility are serious economic concerns of dairy farms. Production loss, increased breeding interval, decreased output to nutritional inputs, and increase in treatment and maintenance costs associated with abortions, and infertility impact dairy farm economics (Njiro *et al*., 2011; Sahlu, 2015). The estimated cost of abortions in the USA averages between $ 500-$ 900, approximately $ 165 in Argentina, and around $ 89 per animal in India (Campero *et al*., 2003; De Vries, 2006; Deka *et al*., 2018).

Bovine diseases, including infectious bovine rhinotracheitis (IBR) caused by bovine alphaherpesvirus-1 (BoHV-1), bovine viral diarrhoea (BVD) by the BVD virus (BVDV), bovine brucellosis by *Brucella* spp., Q fever (coxiellosis) by *Coxiella* spp., leptospirosis, listeriosis, and neosporosis by *Leptospira*, *Listeria* and *Neospora canninum*, respectively constitute the major causes of infectious abortions and infertilities in dairy herds (Anderson, 2007; Barkallah *et al*., 2014; Dubey *et al*., 2015; Derdour *et al*., 2017; Noaman and Nabinejad, 2020; Gelalcha *et al*., 2021; Sarangi *et al*., 2021). Bovine anaplasmosis, one of the most prevalent tick-borne disease of the tropics and sub-tropics, is also known to cause sporadic abortions in dairy herds (Aubry and Geale, 2011).

While a number of studies have dwelled on the epidemiology, prevalence, and impact of each of the

diseases (Shome *et al*., 2019; Sarangi *et al*., 2021), reports are scarce on the association of multiple diseases in a herd. It has been reported that infection with one agent makes the animal susceptible or resistant to other infectious agents by modulating the host immune response (Candela *et al*., 2009). In tropics and sub-tropics such as India where most of the infectious diseases are endemic, the association of the diseases may significantly impact infertility and abortion in dairy herds. This study estimates the burden of major infectious agents known to cause abortion and infertility in some of the large to medium dairy farms located in various parts of India, and attempts to determine the likelihood of association between the pathogens.

Materials and Methods

Ethical considerations

In this study, serum samples submitted as part of routine brucellosis screening program and stored in the repository of NDDB R&D laboratory, Hyderabad were used. These serum samples were collected by the organized herds as per the standard protocol. The present study does not come under the category of experimental research on animals; therefore, formal ethical approval is not required.

Study design

A cross-sectional serosurvey was conducted on 09 intensive dairy farms located in different parts of India. The demographic detail of the farms is provided in Table 1. The dairy farms practiced modern farming procedures such as barn feeding, machine milking, artificial insemination, authorized entry, and exit procedures. Serum samples from the respective dairy herds were collected from June 2019 to December 2019 and submitted to the laboratory for the routine screening of bovine brucellosis. After the screening tests, the left-over serum samples were maintained in the laboratory at -20°C. For the current study, each farm was considered as an epidemiological unit and the sample size for a farm was determined with the expected prevalence of disease set at 50%, precision-5%, and confidence level-95%. Calves aged below one year were excluded from the study to exclude the influence of maternal antibodies in the study results. Samples from each farm were selected at random. The calculation of sample size and randomized selection was performed using the online tool - Epitools Epidemiological Calculators (Sergeant, 2007). A total of 1075 serum samples were screened in the study. The farm-wise description *viz*., location of the farm, various breeds maintained in the farm, herd size, and the number of samples screened, is given in Table 1.

Sample analysis

Commercial ELISA kits for the detection of antibodies to BoHV-1, BVDV, *Brucella* spp., *Coxiella burnetii*, *Neospora caninum*, and *Anaplasma marginale* were used for the seroprevalence determination of IBR, BVD, brucellosis, Q fever, and anaplsamosis, respectively. IBR seroprevalence in Farm 7 was determined using an additional companion IBR-gE ELISA kit for differentiating vaccinated and infected animals as the farm had been vaccinated with an inactivated marker (gE deleted) vaccine. The test performance and result interpretations were done as per the instructions of the kit manufacturer. The list of kits, the criterion for result interpretation (cut-off), reported sensitivity, and specificity are provided in Table 2.

Statistical analysis

Statistical analysis of data was performed using the online tool (Epitools Epidemiological Calculators: https://epitools.ausvet.com.au/). The true prevalence of the disease was determined based on the sensitivity and specificity of the test kit as reported previously (Greiner and Gardner, 2000). The confidence limit of true prevalence was determined using Blaker's interval as described previously (Reiczigel *et al*., 2010).

The likelihood of association between the seroprevalence of two diseases was calculated as the Odds-ratio using the online MedCalc® statistical software. Samples whose results were inconclusive in ELISA tests were considered negative for the calculation of Odds-ratio.

Results

The majority of the 1075 animals (85.6%) showed antibodies in sera to one or more of the pathogens causing the respective diseases. Only 155 (14.4%) animals were negative for all the diseases investigated in

Table 1: Demographic details of the farms included in the study

Farm No.	Location (State)	Species	Breeds	Herd size	Sample size
Farm 1	Maharashtra	Cattle	Gir, Sahiwal	72	59
Farm 2	Maharashtra	Cattle and buffalo	Jersey and Pandharpuri	116	91
Farm 3	Maharashtra	Cattle	HF, HF cross breed, Jersey cross-breeds	180	89
Farm 4	Gujarat	Cattle	Gir	100	74
Farm 5	Gujarat	Cattle	Gir, Kankrej, Red Sindhi, Sahiwal, HF, HF cross breeds, Jersey, Jersey cross breeds	345	178
Farm 6	Andhra Pradesh	Cattle	HF cross-breed	427	206
Farm 7	Telangana	Cattle	HF cross-breed	817	212
Farm 8	Madhya Pradesh	Cattle	Gir, Sahiwal, Malvi, HF cross-breeds	163	73
Farm 9	Punjab	Cattle	HF and Jersey	124	93

HF: Holstein Friesen

Table 2: Details of the bovine ELISA kits used in this study and the cut-off values used for determination of the sample status

Disease	Test method	Test kit	Target antigen	Cut-off value	Sensitivity (%)	Specificity (%)	Reference
Infectious bovine rhinotracheitis	Blocking ELISA	IBR gB antibody test kit (IDEXX)	gB protein of BoHV-1	Blocking % <45% = Negative, ≥45 to <55% = Suspect, ≥55% = Positive	97.4	99.8	Validation report IDEXX
	Blocking ELISA	IBR gE antibody test kit (IDEXX)	gE protein of BoHV-1	Sample/Negative (S/N) >0.70 = Negative, ≤0.70 to >60 = Suspect, ≤0.6 = Positive	97	99.8	Validation report IDEXX
Bovine viral diarrhoea	Competitive ELISA	Bovine BVDV Ab kit (Prionics)	P80	Percentage inhibition <50% = Negative, ≥50% = Positive	97.9	99.2	Kramps *et al*. (1999)
Brucellosis	Indirect ELISA	Brucella abortus antibody test kit (IDEXX)	Inactivated antigen	Sample/Positive (S/P) % <80 = Negative, >80 = Positive	64.5	97.3	Arif *et al*. (2018)
Q-fever	Indirect ELISA	Q-fever antibody test kit (IDEXX)	Phase I & II	Sample/Positive (S/P) <30 = Negative, ≥30% and <40% = Suspect, ≥40% = Positive	98.6	97.1	Horigan *et al*. (2016)
Anaplasmosis	Competitive ELISA	Anaplasma Antibody test kit (VMRD)	MSP5 protein	Inhibition % <30% = Negative, ≥30% = Positive	96	95.2	Torioni de Echaide *et al*. (1998)
Neosporosis	Indirect ELISA	Neospora caninum antibody test kit (IDEXX)	Sonicate lysate of tachyzoites	Sample/Positive (S/P) <30 = Negative, ≥30 and <40 = Suspect, ≥40 = Positive	100	93.3	Alvarez-García *et al*. (2013)

Table 3: Seroprevalence of the diseases across farms, species and cattle breed-types

Variable	Description	No of samples	True prevalence (95% confidence interval)					
			Infectious bovine rhinotracheitis	Bovine viral diarrhea	Brucellosis	Q fever	Anaplasmosis	Neosporosis
Farm	Farm 1	59	83.5 (71.4-91.6)	76.0 (63.2-85.6)	0 (0-10.2)	0 (0-6.37)	95.1 (84.2-100)	0 (0-2.2)
	Farm 2	91	72.2 (61.8-80.8)	17.3 (10.6-26.6)	18.8 (9.5-32.7)	0 (0-4.98)	1.8 (0-9.5)	77.6 (67.4-85.2)
	Farm 3	89	0 (0-4.1)	54.7 (44.1-65.0)	0 (0-8.3)	0 (0-3.34)	100 (95.8-100)	2.1 (0-10.5)
	Farm 4	74	98.5 (91.1-100)	2.0 (0-8.8)	0 (0-3.6)	6.8 (1.8-16.0)	100 (99.0-100)	0 (0-2.5)
	Farm 5	178	43.1 (35.9-50.7)	57.0 (49.5-64.3)	0 (0-0)	7.0 (3.3-12.4)	0.7 (0-5.5)	6.4 (1.9-12.5)
	Farm 6	206	30.3 (24.3-37.0)	31.2 (25.1-38.0)	0 (0-0)	7.1 (3.6-12.1)	18.5 (12.9-25.3)	0 (0-0)
	Farm 7	212	49.3 (42.5-56.2)	100 (97.3-100)	81.9 (71.0-92.6)	9.8 (5.9-15.1)	16.3 (11.0-22.7)	0 (0-2.8)
	Farm 8	73	73.1 (61.5-82.5)	34.5 (24.3-46.2)	0 (0-7.5)	7.0 (1.9-16.3)	74.3 (62.0-84.1)	4.3 (0-14.2)
	Farm 9	93	4.2 (1.5-10.6)	100 (94.4-100)	6.1 (0.5-17.3)	3.7 (0-10.9)	13.4 (6.5-23.2)	0 (0-2.2)
Species	Buffalo	71	89.6 (79.7-95.7)	3.5 (0.7-11.2)	0 (0-7.9)	0 (0-7.1)	3.8 (0-13.5)	78.8 (67.3-87.0)
	Cattle	1004	42.3 (39.2-45.5)	60.2 (57.0-63.3)	17.5 (14.3-21.2)	5.7 (4.1-7.7)	36.2 (33.0-39.5)	0.9 (0-2.9)
Cattle breed-type	Crossbred	615	36.9 (33.1-40.9)	63.0 (59.0-66.8)	25.6 (21.0-30.9)	6.3 (4.2-8.9)	26.7 (22.9-30.8)	0.2 (0-2.7)
	Exotic	145	7.6 (4.2-13.2)	88.7 (82.0-93.4)	18.0 (10.4-28.6)	1.3 (0-6.1)	20.3 (13.5-28.5)	8.1 (2.9-15.2)
	Indigenous	244	76.5 (70.6-81.7)	36.3 (30.4-42.7)	0 (0-0.4)	7.2 (4.0-11.8)	69.7 (63.0-75.8)	0 (0-2.6)
Grand Total		1075	45.4 (42.4-48.5)	56.5 (53.4-59.5)	16.3 (13.2-19.7)	5.4 (3.9-7.3)	34.1 (31.0-37.7)	6.1 (4.1-8.4)

Table 4: The likelihood of association (Odds ratio) between the diseases

Disease combination		Test results combinations				Odds ratio (95% CI)	P-value
		Positive-Positive	Positive-Negative	Negative-Positive	Negative-Negative		
IBR	Brucellosis	69	408	68	530	1.3 (0.9-1.9)	0.131
IBR	BVD	274	203	324	274	1.1 (0.9-1.4)	0.285
IBR	Anaplasmosis	207	270	180	418	1.8 (1.4-2.3)	<0.001
IBR	Q fever	54	423	33	565	2.2 (1.4-3.4)	0.0007
IBR	Neosporosis	83	394	53	545	2.2 (1.5-3.1)	<0.001
BVD	Brucellosis	128	470	9	468	14.2 (7.1-28.2)	<0.001
BVD	Anaplasmosis	192	406	195	282	0.7 (0.5-0.9)	0.003
BVD	Q fever	58	540	29	448	1.7 (1.0-2.6)	0.032
BVD	Neosporosis	55	543	81	396	0.5 (0.3-0.7)	0.0002
Brucellosis	Anaplasmosis	32	105	355	583	0.5 (0.3-0.8)	0.0011
Brucellosis	Q fever	14	123	73	865	1.3 (0.7-2.5)	0.330
Brucellosis	Neosporosis	14	123	122	816	0.8 (0.4-1.4)	0.36
Q fever	Neosporosis	10	77	126	862	0.9 (0.4-1.8)	0.735
Q fever	Anaplasmosis	27	60	360	628	0.8 (0.4-1.3)	0.315
Neosporosis	Anaplasmosis	30	106	357	582	0.5 (0.3-0.7)	0.0004

this study. Seropositivity to more than two diseases was recorded in 611 animals (56.8%). None of the animals were positive for all the six pathogens. Six animals showed seropositivity to five pathogens, while 42 animals to 4 pathogens. The results indicated the high prevalence of multiple infections in the dairy herds. The study recorded 65 different combinations of results (Supplementary Table 1 (ST1)).

The overall prevalence observed for Q fever, neosporosis, brucellosis, anaplasmosis, IBR, and BVD in this study was 5.4%, 6.1%, 16.3%, 34.1%, 45.4%, and 56.5%, respectively (Table 3). There was wide variation in the prevalence of the diseases among the different farms (Table 3).

As detailed in Table 4, the Odds ratio (OR) reveals a positive association between BVD and brucellosis (OR=14.2), IBR and Q fever (OR=2.2), IBR and neosporosis (OR=2.2), IBR and anaplasmosis (OR=1.8), brucellosis and Q fever (OR=1.3), IBR and brucellosis (OR=1.3), IBR and BVD (OR=1.1), and BVD and Q fever (OR=1.7).

Discussion

Brucellosis, leptospirosis, Q fever, bovine genital campylobacteriosis, anaplasmosis, listeriosis, IBR, BVD, neosporosis, and trichomonosis are the common infectious diseases causing bovine abortions (Morris *et al*., 2018). In addition to abortion, most of these diseases are also linked to other reproductive complications in dairy herds *viz*., infertility, retention of foetal membrane, repeat breeding, anoestrus, endometritis, and pyometra (Sahlu, 2015). The present study estimated the prevalence of major abortion and infertility causing agents in the modern dairy herds located in different parts of India. The study was limited to the major diseases for which serological assays are routinely used for diagnosis. The study also investigated the concomitant seroprevalence of IBR, BVD, Brucellosis, Q fever, anaplasmosis, and neosporosis in the dairy herds.

In the present study, BVD was the most prevalent disease with an overall prevalence of 56.5%. BVD is endemic in India and has been reported in various parts of the country (Sood *et al*., 2007; Kumar *et al*., 2018) with prevalence varying widely among the herds (2 to 100%). The mean of BVD seroprevalence is reported at 49.2% with a wide variation among herds in the world (Scharnböck *et al*., 2018). The variation is attributed to the differences in management practices, and known risk factors influencing prevalence *viz*., artificial insemination, sex, herd demographic structure, herd size, frequency of purchase, and trading activities (Scharnböck *et al*., 2018). Control of BVD infection in dairy farms is important to prevent direct losses due to the disease, as well as reduce the use of antibiotics and other reactive measures for resolving other infections resulting from the immunosuppressive effect of the virus (Yarnall and Thrusfield, 2017). While vaccination for the prophylaxis of BVD is not available in India, the effectiveness of the vaccines currently available elsewhere in the world for the control of HoBi-like pestivirus (HoBiPev) infection is questionable (Bauermann *et al*., 2013). Studies to determine the evaluation of vaccine effectiveness and also the development of novel, efficacious vaccines will herald the prevention of this economically important bovine infectious disease. Adoption of strict screening of new-born calves and newly introduced animals for the persistent infection of BVDV and their prompt removal from the herd has been proven as an effective strategy in mitigating disease transmission and incidence.

Seroprevalence of IBR has been reported from almost all parts of India albeit at a varied rate (Renukaradhya *et al*., 1996; Trangadia *et al*., 2012; Das *et al*., 2014). Cattle with a history of abortion, metritis, repeat breeding, and retention of the placenta have been shown to have higher seropositivity (Patil *et al*., 2017; Sibhat *et al*., 2018). In our study, IBR is the second most prevalent disease (45.4%). Only one of the 9 farms studied was found to be free from IBR. Among the seropositive herds, the prevalence showed wide variation (from 4.2% to 98.5%). Only one of the farms (Farm 2) housed both cattle and buffaloes, and the prevalence of IBR in buffaloes was very high (89.6%) on this farm than in cattle (10%). This difference was statistically significant ($P<0.001$), and we reckon, warrants further investigation. A pilot study on the effectiveness of the inactivated IBR marker (gE deleted) vaccine recently reported a significant reduction in disease incidence, and abortion rates (Sarangi *et al*., 2020a). In light of the high prevalence of IBR in the dairy herds, it may be prudent to adopt preventive vaccination of the respective herds routinely with the IBR marker vaccine to bring down the disease burden over time.

Bovine brucellosis is a major cause of contagious abortion in cattle. The disease is endemic in India and a nationwide survey has reported a seroprevalence of 8.4% in individual animals (Shome *et al*., 2019). A drastic increase in the prevalence rates has been reported in endemic herds (Lucchese *et al*., 2016; Sarangi *et al*., 2021). The overall prevalence of the disease in the current study is 16.3%. Six out of the nine farms in the study were free from brucellosis. High prevalence was recorded on two farms. However, both farms practiced calf-hood vaccination against brucellosis with live attenuated S19 vaccine. Although antibody response elicited by the *B. abortus* S19 vaccines usually wane below detection levels by one-year post-vaccination, the persistence of antibodies for a longer period extending up to 4.5 years has also been recorded (Simpson *et al*., 2018). Therefore, the possibility of vaccine-induced antibodies in *Brucella* seropositive animals could not be ruled out. Effective vaccination-based prophylaxis of brucellosis, therefore, necessitates the development of marker vaccines and companion diagnostic tests for differentiating infected and vaccinated animals (DIVA). Routine screening of the farms at periodic interval need to be undertaken and good managemental practices should be adopted in addition to the calf-hood vaccination to prevent the introduction of the disease to the farms as well as to reduce the incidence of the disease in the infected farms.

Q fever or coxiellosis is a zoonotic disease of public health concern caused by *Coxiella burnetii*. The disease is endemic in more than 51 countries and has been reported in India (Vaidya *et al*., 2010; Porter *et al*., 2011; Pradeep *et al*., 2017). In this study, the individual animal level true prevalence was 5.4% which is lower than

reported previously in India and elsewhere (Anastacio *et al*., 2016; Kesavamurthy *et al*., 2020).

The overall prevalence of anaplasmosis was 34.1% which is also lower than previous reports from India (Sarangi *et al*., 2020b; and references therein). Although all the 9 farms showed prevalence, the extent varied greatly from 0.7 to 100%. While four farms showed a high prevalence (74-100%), the rest showed a prevalence of less than 20% (Table 3). This wide variation could be due to managemental practices adopted by the farm especially the use of acaricides. Enzootic stability is an epidemiological concept proposed for vector-borne diseases which suggest that in highly endemic farms the possibility of severe clinical disease is rare (Oliveira *et al*., 2011). The Majority of the farms in this study, however, show low prevalence or enzootic instability (less than 20%), and hence adult cattle may be prone to severe clinical disease and mortality. Intensive tick control measures may be adopted on these farms to prevent disease outbreaks.

Seroprevalence to bovine neosporosis caused by the protozoan, *Neospora caninum* was 6.1%; lower than the previous reports from India and elsewhere (Mainar Jamie *et al*., 1996; Meenakshi *et al*., 2007; Nasir *et al*., 2011; Sengupta *et al*., 2013). Bovine neosporosis is mostly transmitted by feed and pasture contaminated with dog faeces (Haddad *et al*., 2005). Therefore, the prevalence of this disease is higher in conventional farms and smallholder cows where open grazing is the norm (Sengupta *et al*., 2013). Expectedly, the disease was absent in five of the studied farms, while the prevalence was low in the others (2.1-6.3%). Further reduction in the disease prevalence would be possible by screening at regular intervals and prompt removal of the repeat breeders. Surprisingly, the prevalence of neosporosis in farm 2 was high (77.6%). Both cattle and buffaloes of the farm showed high prevalence. The reasons for high prevalence while not obvious require further investigation.

Out of the 1075 animals, 611 (56.8%) were serologically positive for two or more pathogens. Multiple infections of cattle have been reported previously (Moshkelani *et al*., 2011; Yang *et al*., 2012; Lucchese *et al*., 2016; Sarangi *et al*., 2021). IBR and BVD are known to cause immunosuppression in the host and render increased susceptibility to other bacterial and viral infections (Hutching *et al*., 1990; Wellenberg *et al*., 2002). Epidemiological studies have also reported a positive association between these two viruses (Nikbakht *et al*., 2015; Noaman and Nabinejad, 2020). In this study, a very strong association was observed between the seropositivity of BVD with brucellosis. The Odds of BVD seropositive cows for brucellosis was 14.2 with a statistically significant ($P<0.001$) likelihood of association (Table 4). The Odds ratios of more than one were also recorded for IBR and other diseases *viz*., Q fever, neosporosis, brucellosis, BVD, and anaplasmosis (Table 4). The results suggest increased susceptibility of IBR seropositive cows to other bacterial and viral infections. BoHV-1 is known to impair host immune responses *viz*., the phagocytic function of macrophages and monocytes, reduced antibody-dependent cellular cytotoxicity function, poor T cell stimulation (Biswas *et al*., 2013; Jones, 2019), and hence rendering the IBR seropositive animals prone to secondary infections.

In the present study, viral diseases were more common in the dairy herds compared to bacterial and protozoan diseases with BVD, the most prevalent pathogen followed by IBR. Multiple infections were observed in a significant proportion of the animals in the dairy herds. While a strong association was observed between the seroprevalence of BVD and brucellosis, a positive association was observed between the seropositivity of IBR and other diseases. Preventive vaccination, stringent biosecurity measures, and animal health management practices should be adopted to reduce the incidence of the diseases, thereby improving productivity.

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Conflict of interest

The authors declare that there are no conflicts of interest in this article.

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