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PREVALENCE OF ANTIBODIES AGAINST PERSISTENT PRODUCTION-LIMITING INFECTIONS IN RUMINANTS IN INDIA

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ABSTRACT

The present study was aimed to screen ruminants for the prevalence of antibodies against four major persistent production-limiting infections in different agro-climatic zones of West Bengal (India). Two-hundred and twenty (n = 220) serum samples from cattle (n = 74), goats (n = 109) and sheep (n = 37) were collected from four agro-climatic zones of West Bengal. In present study, IBR antibodies was detected in 11 sera samples (11/74, 14.8%) collected from cattle only. Antibodies against BVD was detected in sera samples collected from three sheep (3/37, 8.1%) and one cattle (1/74, 1.3%). The MAP antibodies were detected in sera samples collected from three goats (3/109, 2.7%), two sheep (2/37, 5.4%) and one cattle (1/74, 1.3%). The antibodies against *Chlamydophila abortus* was confirmed in sera samples collected from two cattle (2/74, 2.6%). The study revealed lower seroprevalence of four major persistent and production limiting microbial infection in ruminants in Bengal.

Key words: BVD, *Chlamydophila*, ELISA, India, MAP, West Bengal

INTRODUCTION

The persistent microbial infections in ruminants is a major production limitation due to decrease in milk production and mortality, inferior reproductive performances, premature voluntary culling and reduced market opportunity. Most of these infections are associated with *Mycobacterium avium* subspecies *paratuberculosis* (MAP), infectious bovine rhinotracheitis (IBR) virus, bovine viral diarrhoea (BVD) virus and *Chlamydophila abortus* (CA) in cattle, sheep and goats (Bachofen *et al.*, 2008; Biswas *et al.*, 2013). The persistent MAP infections reportedly cause annual estimated economic loss of US $ 1196 per 100 cow with seroprevalence rate of 3.1% in cattle (Tiwari *et al.*, 2008). Similarly, infection with BVD was reported to cause economic loss of US $ 57 million per million calvings (Houe, 1999). In India, annual economic losses up to US $ 26,606.4 due to MAP in dairy farm has been reported (Rawat *et al.*, 2014).

M. avium subspecies *paratuberculosis* causes chronic inflammatory stomach infection in ruminants known as paratuberculosis or Johne's disease (Samanta, 2013). The animals with persistent infection can shed the bacteria in their faeces, milk and colostrum (Sorge *et al.*, 2012). IBR is caused by bovine herpesvirus-1 (BHV-1) which falls under genus *Varicellovirus*, subfamily *Alphaherpesvirinae* and family *Herpesviridae*. BHV-1 has a unique feature of latency establishment mostly in sensory neurons within trigeminal ganglia of host animals (Biswas *et al.*, 2013). BVD is caused by virus BVD-1 and BVD-2 which fall under genus *Pestivirus* and family *Flaviviridae* (Mishra *et al.*, 2009). Persistent infection of BVD develops in foetus by non-cytopathic strains of etiological viruses and the newborn calves act as major source of infection (Nettleton *et al.*, 1998).

Further, the non-cytopathic strains may mutate to cytopathogenic varieties of BVD (Mansour *et al.*, 2015). *Chlamydophila abortus* is an obligate intracellular bacterium under the family *Chlamydiaceae* and order *Chlamydiales* (Everett *et al.*, 1999). It causes abortion, polyarthritis, keratoconjunctivitis and pneumonia in ruminants (Reinhold *et al.*, 2011). *Chlamydophila* has a tendency to produce persistent infection in ruminants which is further augmented with antimicrobial treatment (Ouellette *et al.*, 2006).

Detection of these persistent carriers is required to ascertain the extent of infection as subsequent economic losses may rise with time. Although the identification of carrier is challenging due to lack of visible clinical signs (VanLeeuwen *et al.*, 2001) but detection of specific antibodies against the infection primarily identifies the exposure of infection and it can also affirm the active infection (Houe, 1999). Among the serological tests available for detection of persistent infection in ruminants, ELISA is considered as highly sensitive, less expensive, less time consuming (Park *et al.*, 2006). The present study was aimed to screen ruminants for the prevalence of antibodies against four major persistent production limiting infections in different agro-climatic zones of West Bengal, an eastern Indian state, which was earlier not included in such kind of seroprevalence studies.

MATERIALS AND METHODS

Serum (n = 220) samples from cattle (n = 74), goat (n = 109) and sheep (n = 37) were randomly collected from four agro-climatic zones (old alluvial, new alluvial, red laterite and coastal) of West Bengal (India) based on the willingness of owners and approachable communication of location. The collection area included Kolkata, Burdwan, Hooghly districts (old alluvial zone, n = 77), Nadia district (new alluvial zone, n = 52), West Medinipur district (red laterite zone, n = 61) and South 24-Parganas district (coastal zone, n = 30). The average minimum-maximum temperature and rainfall were 25-30ºC and 6 mm in old alluvial, 27-34ºC and 5 mm in red laterite, 26-33ºC and 7 mm in new alluvial and coastal zones, respectively, during the sample collection period (Sept.-Feb., 2014). The cattle belonged to both indigenous and cross breed whereas goats and sheep were Black Bengal, Garole and Sahabadi breed. All the animals were of either sex and divided into three age groups. For cattle, group 1 was $\leq$ 25 months age, group 2: 25 - 96 months and group 3: $\geq$ 96 months while for sheep and goat, group 1 $\leq$ 12 months ag, group 2: 12 - 60 months and group 3: $\geq$ 60 months age. The sampled cattle were kept in a herd of 2-3 animals in a shed and sheep and goat were kept in a separate shed.

The antibodies against MAP, IBR virus, BVD virus and *C. abortus* (CA) in animal were detected by ID Screen indirect kits (PARAS-5P, IBRS-5P, BVDC-5P and CHLMS-MS-2P) for paratuberculosis, IBR, BVD and CA as per the manufacturer's protocol (ID-VET, Montpellier, France). The optical density was read at 450 nm in an ELISA reader (ECI, India) and S/P percentage calculated as per below given formula. Positive S/P value was detected as indicated by the manufacturer.

$$S/P\ (\%) = \frac{OD_{sample} - OD_{negative\ control}}{OD_{positive\ control} - OD_{negative\ control}} \times 100$$

Logistic model was used to compare the seroprevalence rate between age, sex, breed and agro-climatic zones using SAAS (version 9.3) system for Windows (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

In present study, IBR antibodies was detected in 11 sera samples (11/74, 14.8%) collected from cattle only. Antibodies against BVD was detected in sera samples collected from 3 sheep (3/37,

Table 1: Species-, zone-, age- and sex-wise seroprevalence in animals

Agro climatic zone	Species	Age (months)	Sex	Breed	IBR	BVD	*Mycobacterium avium* subspecies *paratuberculosis* (MAP)	*Chlamydophila abortus*
	Cattle	72	F	*Deshi*	1	0	0	0
	Cattle	72	F	CB	1	0	0	0
	Cattle	36	M	*Deshi*	1	0	0	0
	Cattle	32	M	CB	0	0	0	1
	Cattle	50	F	CB	0	0	0	1
Old	Goats	12	F	Black Bengal	ND	ND	1	0
Alluvial	Sheep	24-36	F	Garole	ND	1	0	0
	Sheep	24-36	M	Garole	ND	2	1	0
New	Sheep	8	F	Garole	ND	0	1	0
Alluvial								
	Cattle	18	F	CB	2	0	0	0
	Cattle	24, 72	F	*Deshi*	2	0	0	0
	Cattle	4	M	CB	1	0	0	0
Red	Cattle	8	M	*Deshi*	1	0	0	0
Laterite	Goats	26	F	Black Bengal	ND	ND	1	0
	Goats	9	M	Black Bengal	ND	ND	1	0
Coastal	Cattle	8. 60	F	CB	2	1	1	0

CB = Cross breed; ND = Not detected; F = Female; M = Male;
IBR = Infectious bovine rhinotracheitis; BVD = bovine viral diarrhoea

8.1%) and 1 cattle (1/74, 1.3%). Whereas, MAP antibodies were detected in sera samples collected from 3 goats (3/109, 2.7%), 2 sheep (2/37, 5.4%) and 1 cattle (1/74, 1.3%). The antibodies against *Chlamydophila abortus* was confirmed in sera samples collected from two cattle (2/74, 2.6%) (Table 1). No statistically significant difference in seroprevalence rate was observed between age, sex, breed and agro-climatic zones in all the studied infections except IBR. In IBR seroprevalence was significantly more in cross breed cattle than *Deshi* (indigenous) cattle ($p < 0.05$).

The present study detected lower seroprevalence of IBR (14.8%) in West Bengal (India). In the studied population in West Bengal the cattle were reared in small household units with less stocking density and mixing of the animals which might be the reason for less possibility of viral exposure and the low seroprevalence (Solis-Calderon *et al.*, 2003). Similar lower seroprevalence was observed in livestock reared in Andaman-Nicobar islands (17.7%) and Garhwal region (10.4%) of India (Jai *et al.*, 2005; Jain *et al.*, 2006). Further the seroprevalence of IBR in cattle detected in the present study is within the range between 14.7-68.9% as detected in other states of India (Sharma *et al.*, 2006; Lata *et al.*, 2008; Nandi *et al.*, 2011). The finding of significantly higher occurrence of IBR in cross bred cattle was associated with exotic germplasm, which renders them more susceptible to diseases and environmental stress factors (Deka *et al.*, 2005). Although the age, sex and agro-climatic zone were not significantly associated with the occurrence of IBR, most of the cattle having antibodies were more than 2 years old which was also noted earlier (McDermott *et al.*, 1997).

Antibodies against BVD was detected in sera samples collected from three sheep (3/37, 8.1%) and one cattle (1/74, 1.3%). In different countries throughout the world the seroprevalence of *Pestivirus* antibodies varies between 0 - 50% (Nettleton *et al.*, 1998). In India, earlier studies reported the seroprevalence of BVD antibodies in sheep between 4.1-90.9% in different states, although the study did not include the samples collected from West Bengal. The flocks showing higher seroprevalence had a frequent contact with cattle which could be responsible for *Pestivirus* seropositivity (Mishra *et al.*, 2009). The sheep population in present study was kept in separate shed without any direct contact with cattle which may explain the lower seroprevalence of BVD. Further, a very low seroprevalence of BVD in cattle (1/74, 1.3%) was detected which might be comparable with 0% seroprevalence in cattle in some other states of India such as North-Eastern states, Madhya

Pradesh and Jammu & Kashmir (Sudharshana *et al.,* 1999). Detection of BVD antibodies in a herd is difficult because in persistent infection the virus is seroconverted into a heterologous strain which produced the specific antibody at below detectable level (Brownlie, 1985).

Mycobacterium avium subspecies *paratuberculosis* (MAP) antibody was detected in six sera samples from three goats (3/109, 2.7%), two sheep (2/37, 5.4%) and one cattle (1/74, 1.3%). The low seroprevalence of MAP in sheep and goats could be due to low sensitivity of ELISA in healthy and young small ruminants without symptom of Johne's disease as observed earlier (Kumthekar *et al.,* 2013). Similarly, lower seroprevalence of MAP in cattle was reported in UK and Korea with a commercial ELISA kit (Pence *et al.,* 2003; Park *et al.,* 2006). In India, higher seroprevalence of MAP with an indigenous ELISA kit has been detected in cattle in Uttar Pradesh and Punjab (Singh *et al.,* 2008). The commercial kit used in present study was prepared from an exotic strain of MAP which might be the reason behind the lower seroprevalence in cattle.

Chlamydophila abortus (CA) antibody was detected in two cattle samples (2/74, 2.6%) in the present study. Similarly, low seroprevalence (0.4%) or absence of CA antibody was detected in cattle in Sweden (Godin *et al.,* 2008; Karlsson *et al.,* 2010). The lower seroprevalence of CA antibody in cattle could be due to small herd size and lack of contact with sheep and goats considered as major reservoir (Reinhold *et al.,* 2011). The sheep and goat samples screened in the present study also revealed the absence of CA antibodies. Further studies with larger sample size in sheep and goat are required to explore their carrier status of CA. Earlier study in Indian yaks (*Poephagus grunniens*) reported higher prevalence of CA antibodies due to their close contact with sheep and goats during grazing (Bandyopadhyay *et al.,* 2009). Th present study revealed lower sero-prevalence of four major persistent and production limiting microbial infection in ruminants in West Bengal, India.

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