Haryana Vet. **46** (December, 2007), pp 69-71

Research Article

SERO-PREVALENCE OF JOHNE'S DISEASE IN BOVINE BREEDING BULLS USING INDIGENOUS ELISA KIT

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ABSTRACT

Indigenous ELISA kit developed using protoplasmic antigen from native *Mycobacterium avium* subsp. *paratuberculosis* 'Bison Type' strain of goat origin was adapted to screen bovine bulls. Serum samples were collected from 49 breeding bulls from dairy farms in Ludhiana (Punjab). The study showed moderate rate of sero-prevalence of Johne's disease in dairy farm bulls (14.2%). Using S/P ratio, bulls were grouped as negative, suspected, low positive, moderately positive and strong positive for Johne's disease in 12.2, 6.1, 8.1, 59.1 and 14.2 samples, respectively. The prevalence rate was high (73.4**%**) in Type II category of sero-reactors. Using ELISA kit, Type 1 sero-rectors had better correlation with fecal culture as compared to Type II sero-reactors, therefore, recommended for estimating sero-prevalence. As first step to control Johne's disease, removal of strong positives sero-reactors may help to reduce shedder bulls from herds. Bulls in suspected, low positive and moderately positive categories may be vaccinated against Johne's disease as second step of control measures.

Key words: Johne's disease, *Mycobacterium avium* subsp. *paratuberculosis*, fecal culture, ELISA kit

Johne's disease (JD) is chronic and incurable disease of ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Disease is a major concern in developed countries (Manning and Collins 2000) and is endemic in their high yielding dairy animals. However, role of MAP infection in reduced productivity of cattle has been seldom realized in India. The MAP has also been associated with inflammatory bowel disease (Crohn's disease) in human beings (Hermon -Taylor *et al*., 2000). Earlier studies have shown that in India wherever investigated, JD was found endemic in the domestic ruminants (Sharma *et al*., 2007, Singh *et al*., 2007a). However, in the absence of screening and removal of shedders, infected animals continue to shed MAP in feces and thereby contaminating the environment. Lack of attention has led to increased incidence of Johne's disease in bovine population of the country (Sharma *et al*., 2007, Yadav *et al*., 2007). Therefore, ELISA kit originally developed for testing goats (Singh *et al*., 2007b) and cattle (Sharma *et al*., 2007) was employed for screening of prospective bovine bulls.

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Johne's disease is in the list B of OIE, therefore, certification of breeding bulls becomes mandatory otherwise it invites trade restrictions. Transmission of MAP by breeding bulls through semen is well documented (Buergelt *et al*., 2004), however, information is limited in India (Sharma *et al*., 2007). Breeding bulls and their semen are being used for breeding of animals without certification. Therefore, this study aimed to estimate sero-prevalence of Johne's disease in bovine dairy bulls, using indigenous goat based ELISA kit.

MATERIALS AND METHODS

Animals: The 49 breeding bulls belonging to dairy farm of Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana (Punjab) were screened for JD using indigenous ELISA kit. The serum samples were collected aseptically in May 2007 and sent to Central Institute for Research on Goats for screening.

Indigenous ELISA kit: The kit using protoplasmic antigen (PPA) from native MAP 'Bison Type' strain of goat origin, initially developed for goats (Singh *et al*., 2007a) was

used for screening of breeding dairy bulls. Known positives and negatives sera with respective OD value of 0.584 and 0.384 were used as controls.
Calculation of S/P ratios: Absorbance was read at 450 nm in ELISA reader (Multiscan, Finland). The results of sero-screening of breeding bovine bulls were read on continuous scale, as per Collins (2002). The OD values were transformed to S/P ratio (Sample to positive ratio). On the basis of screening by goat based indigenous ELISA kit, Johne's disease status of dairy breeding bulls was grouped as negative, doubtful, low positive, moderately positive and strong positive with a S/P ratio in the range of 0.00-0.09, 0.10-0.24, 0.25-0.39, 0.40-0.99, 1.0-10.0 respectively. Based on two types of sero-reactors in S/P ratios, bulls were considered JD positive as given below.
a) Type 1 sero-reactors: Bulls classified as strong positive in S/P ratio were considered positive and rest (including moderately positive, low positive, suspected and negative) as negative.
b) Type II sero-reactors: Bulls in moderately positive and strong positive categories were considered as positive and rest including low positives, suspected and negatives as negative.

RESULTS AND DISCUSSION

On the basis of screening by indigenous goat based ELISA kit, the status of breeding bovine dairy bulls with respect to Johne's disease was classified as 6 negatives (12.2%), 3 doubtful (6.1%), 4 low positives (8.1%), 29 moderately positives (59.1%) and 7 strong positives (14.2%). Sero-prevalence of JD was 14.2% in bovine dairy bulls in Ludhiana (Punjab) on the basis of type I sero-reactors. However, on the basis of type II sero-reactors, the sero-prevalence was 73.3%.

Sero-studies in developed countries are based on commercial ELISA kits, using antigen from MAP 'Bovine' strain, irrespective of the origin of country (Collins *et al*.,1994). Indigenous ELISA kit using protoplasmic antigen (PPA) from MAP 'Bison type' of goat origin has been used to screen goats (Kumar *et al*., 2007) cattle (Sharma *et al*., 2007), and buffaloes (Yadav *et al*., 2007) in India. The ELISA using purified PPA from MAP 'Bovine' (Allied Monitor Inc, USA), none or few of the serum sample were found as positive (Yadav *et al*., 2007, Kumar *et al*., 2007). Salgado *et al.* (2005) compared serum and milk ELISA kit (IDEXX laboratories Inc.) with fecal culture and reported 64 and 48 % sensitivity, respectively and 100 % specificity in both the cases, in goats. High specificity of commercial kits may be due to adsorption of serum or milk with *M. phlei* cultures. Pourquier kit reports that in milk ELISA (bulk tank), only strong positive can be detected. Indigenous ELISA kit was simple and had comparable sensitivity and specificity with IDEXX kit (Singh *et al*., 2007b).

Using ELISA as screening test, targeted population can be sero-monitored for JD and animals can be partitioned into high and low risk groups. If animals in strong positive and moderately positive categories were considered positive (Type II sero-reactors), prevalence of MAP was high (73.3%) in dairy farm breeding bulls. Since JD is endemic in bovine population of country (Sharma *et al*., 2007), therefore, bulls in the strong positive category (Type I sero-reactors) were only considered as positive for JD. Using this criterion study showed moderate prevalence of Johne's disease in prospective breeding bovine bulls from dairy farms in Ludhiana (Punjab), using indigenous goat based ELISA kit. Similar, study in 38 randomly selected young prospective Murrah buffalo breeding males in their home tract showed low (10.5%) prevalence (Type I category). However, in Type II category, sero-prevalence was high (Singh *et al*., 2007b). Ocepek *et al*., (2002), estimated sero-prevalence of JD in bull's mother's herds, in Slovenia.

Johne's disease is endemic in cattle in developed countries and variable prevalence of MAP has been reported from 5 – 55.0% clinically (Taylor *et al*., 1981, Giese and Ahrens, 2000) and 2 – 12.0% sub-clinically (Sweeney *et al*., 1994, Streeter *et al*., 1995, Jakobsen *et al*., 2000) affected cows. In India though cattle are known for low productivity, yet there are few reports on the prevalence of JD. Sharma *et al*., (2007), reported high (84.0%) lacto-prevalence of MAP by milk culture and 32.1% by milk ELISA, in 6 dairy herds in Agra, Etah and Mathura districts. Sivakumar *et al*. (2005) reported 12.0 and 2.0% incidence of JD by fecal culture and 22.0 and 10.0% by absorbed ELISA of 50 each cattle and

buffaloes, respectively maintained at dairy farm of IVRI, Izatnagar, UP.

Bull is the nucleus in upgrading JD control programmes therefore, screening of bulls by a sensitive test should be mandatory at farm level. This goat based ELISA kit can be used to determine the prevalence of MAP in bovines in the country. Strong positive bull in Type 1 sero-reactors warrant their immediate removal from breeding stock. Study underlined the need for national level screening of domestic ruminants to estimate prevalence of Johne's disease and subsequent control.

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