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Short Communication

SERO-PREVALENCE OF MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS IN BARBARI AND SIROHI BREEDS OF GOATS IN SEMI-ARID REGIONS OF NORTH INDIA

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SUMMARY

Information on prevalence of *Mycobacterium avium* subsp. *paratuberculosis* infection (MAP) cause of Johne's disease in farmer's herds is limited. Sixty six fecal and serum samples from 29 Barbari (South UP) and 37 Sirohi (Western Rajasthan) goats were screened through indigenous ELISA kit, developed at Central Institute for Research on Goats, and microscopic examination, respectively. Comparative evaluation showed that indigenous ELISA kit was significantly superior to microscopic examination. Study showed high prevalence (19.6-31.8%) of MAP in farmer's goat herds. Prevalence of MAP was higher in Sirohi (29.7-40.5%) breed of goats as compared to Barbari (6.8-20.6%). In ELISA kit, type I sero-reactors had substantial to nearly perfect proportional agreement with microscopic examination, therefore, can be used for large scale screening of goat herds against JD. Study underlined need for large scale screening of goats to estimate prevalence.

Key Words: Johne's disease, *Mycobacterium avium* subsp. *paratuberculosis*, protoplasmic antigen, ELISA kit

Johne's Disease (JD), a chronic infectious disease of ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is of major concern in developed countries. Earlier studies reported that JD is endemic in farm goat herds of India (Vihan *et al*., 1989, Singh *et al* 1996). However, information is limited on the prevalence of JD in farmer's herds. Singh *et al*., (1998) reported higher prevalence of JD in herds located in semi-arid region as compared to arid region. India lacks diagnostic kits to screen the goat population. Simple, rapid, and sensitive laboratory tests are needed to provide accurate diagnosis of infective status of each goat. Most accurate and informative test results are often based on antibody detection using ELISA test. First indigenous ELISA kit using protoplasmic antigen (PPA) from native MAP 'Bison Type' strain of goat origin was developed by Singh *et al*., (2007). Barbari and Sirohi are two important and popular breeds of goats in North India. These goats are taken from their native tracts to different parts of the country for starting new commercial goat farms. The present study aimed to determine prevalence of MAP in Barbari and Sirohi breeds of farmer's herds in their respective home tracts in two different geographical regions using indigenously developed ELISA and microscopic examination

Sirohi a large sized, sturdy and robust breed is native of semi-arid regions of the country in the districts of Sirohi, Ajmer and Nagaur in western Rajasthan. Farmer's maintain these goats on extensive grazing management system. Forty goats between 1-2 years of age and non-pregnant (4 goats primiparous) were purchased from 40 farmers each keeping 15–50 goats. Thirty-seven goats (33 females and 4 males) were purchased in March, 2007. Barbari is medium sized breed of goats and is very popular with commercial farmers. Twenty-nine females of Barbari goats in age group of 1-2 years were purchased in March, 2007 from Makhdoom, Gadaya, Sadabad, Tajganj and Khandauli villages of Mathura and Agra districts of South UP. Each farmer was maintaining 1-5 goats unit on extensive management in semi-arid region of South UP. After purchase these goats were transferred to Central Institute for Research on Goats and serum and fecal samples were collected.

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For microscopic examination feces were concentrated and smears were stained by Ziehl Neelsen stain to observe the presence of pink coloured cocco-bacillary rods of MAP. The sixty-six serum samples were screened using indigenous ELISA kit as per Singh *et al*. (2007). Serum absorbance was read at 450 nm in ELISA reader (Multiscan, Finland). Positive and negative controls had OD values of 0.584 and 0.384, respectively. The OD values of test samples were transformed to S/P ratio (Collins, 2002). Standard procedure was used to calculate sensitivity and specificity of ELISA test (Arizmendi and Grimes., 1995). Johne's disease is highly prevalent in the goat herds located at CIRG (endemic), therefore, using ELISA kits two types of sero-reactors were identified. Animals classified in the strong positive category (Type I category) in ELISA S/P ratio were considered as positive and rest categories as negatives. Animals in the positive and strong positive categories (Type II category) were considered as positive and rest categories as negatives. Performance of ELISA kit was compared with microscopic examination by calculating Kappa Scores (Proportional Agreement) as per Landis and Koch, 1977 (0 < poor, 0.0 – 0.20 slight, 0.21- 0.40 fair, 0.41- 0.60 moderate, 0.61 - 0.80 substantial and 0.81 - 100 almost perfect).

On traditional microscopic examination prevalence of MAP was 31.8% in two important breeds of goats belonging to farmer's herds located in arid and semi-arid regions of Rajasthan and Uttar Pradesh in North India, respectively. Independently, prevalence was 40.5 and 20.6% in Sirohi and Barbari breeds using microscopic examination. Using indigenous ELISA kit, sero-prevalence of MAP was 19.6% in two breeds of goats (Sirohi - 29.7% , Barbari - 6.8%), in Type 1 reactors. However, considering strong positive and moderately positive as positive (Type II reactors), sero-prevalence of MAP was 75.8% including 83.8% in Sirohi and 65.5% in Barbari goat breeds (Tables 1, 2, 3). The S/P ratios showed that in both the breeds, goats in strong positive, moderately positive, weak positive and suspected categories were also positive for shedding of MAP. However, correlation was high between shedders and strong positive and moderately positives categories. On comparison between ELISA and microscopic examination in Type I sero-reactors, proportional agreement (PA) was 40.0 and 55.0% in Sirohi and Barbari goat breeds, respectively (Tables 1, 2, 3). In Type II sero-reactors, PA value was 0.62 and 0.86, in Sirohi and Barbari goat breeds, respectively (Tables 1, 2, 3). Shedding of MAP in strong positive, moderately positive, weak positive and suspected categories exhibited that in chronic infections like JD, ELISA results were better read on continuous scale (Collins, 2002) as compared to single cut off in acute infections.

Gupta *et al*. (1996), Singh *et al*. (1998) and Kumar (2004) employed microscopic examination to screen fecal, milk and tissue samples from goats and centrifugation has improved sensitivity of this test. Using this test, study reports high prevalence of JD in Barbari and Sirohi goats. In present survey Sirohi goats from semi-arid regions of Rajasthan had significantly higher prevalence of MAP as compared to Barbari goats, native of South UP. High prevalence in Sirohi goats may be correlated with the practice of maintaining large sized goat herds (15-50) by farmers in Rajasthan as compared to small sized herds in UP (1-5). Climate in the two geographical regions was comparatively similar.

Table 1
Comparison of ELISA kit (S/P ratios) with respect to microscopic examination for the diagnosis of Johne's disease in goats

S/P ratio	Disease status	Sirohi goats		Barbari goats	
		ELISA	Microscopic examination	ELISA	Microscopic examination
0.00-0.09	Negative	0	0	1	0
0.10-0.24	Suspected	2	1	3	0
0.25-0.39	Low positive	4	2	6	0
0.40-0.99	Positive	20	6	17	4
1.0-10.0	Strong Positive	11	6	2	2

Table 2
Comparative evaluation of ELISA kit and microscopic examination for the diagnosis of Johne's disease (Type I sero-reactors)

Test	Combinations			
ELISA kit	+	-	+	-
Microscopic exam.	+	-	-	+
Sirohi goats – 37 *	6	17	5	9
Barbari goats – 29 **	2	23	0	4

* Proportional agreement – 0.62
** Proportional agreement – 0.86

Table 3
Comparative evaluation of ELISA kit and microscopic examination for the diagnosis of Johne's disease in Sirohi goats (Type II sero-reactors)

Test	Combinations			
ELISA kit	+	-	+	-
Microscopic exam.	+	-	-	+
Sirohi goats – 37 *	12	3	19	3
Barbari goats – 29 **	6	10	13	0

* Proportional agreement – 0.40
** Proportional agreement – 0.55

The ELISA kit developed has been frequently employed for the screening of goats (Singh *et al*., 2007). Using ELISA, target population can be sero-monitored for JD and goats can be partitioned into high and low risk groups. In ELISA, two types of sero-reactors (Type I and Type II), may be considered positive. Comparison of S/P ratios with microscopic examination showed substantial to almost perfect proportional agreement in Type I reactors, as compared to Type II sero-reactors (fair to moderate). Two screening tests showed high endemicity of JD in Barbari and Sirohi goats, in their respective home tracts. The JD has been reported endemic in goat population (Singh *et al*., 1996). Similar study in North India reported 29.0% sero-prevalence of JD by screening 1441 ruminants using indigenous ELISA kit (Singh *et al.*, 2008). Singh *et al.* (2007) reported 7.6% sero-prevalence in Sirohi kids below 2 months of age from Rajasthan. Hajra (2003) reported 8.3% and 36.3% incidence on the basis of fecal examination and tissue culture, respectively, in young Barbari kids (below 6 months age), from South-west Uttar Pradesh. Singh (1998) recorded 15.6% sero-positivity in serum samples obtained from slaughter house, Agra and usually belonging to Barbari type goats. Kumar *et al.* (2007) reported 35.4% and 58.6% sero-prevalence in kids belonging to farmer's and organized herds, respectively. Of 67 milk samples from farmer's herds (Barbari goats), 39 (58.2%) were positive in milk ELISA.

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