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Revisited immune reactivity between native semi-purified protoplasmic (caprine) versus commercial purified protoplasmic (bovine) antigens for the screening of goat herds endemic for Johne's disease

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The present study compared the immune-reactivity of 3 antigens of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) sourced from different geographical regions and livestock species for the diagnosis of Johne's disease (JD) in goats. Screening of 360 faecal and serum samples of goats by microscopy, i-ELISA, b-ELISA and r-ELISA gave 56.9, 40.0, 34.4 and 5.2% positive samples, respectively. Considering all the 4 tests (microscopy, i-ELISA, b-ELISA & r-ELISA kits), 3.0 and 35.2% goats were found common positive and negative, respectively. Of 3 ELISA tests, i-ELISA had the highest sensitivity, followed by b-ELISA and r-ELISA kit. 'i-ELISA' based on 'indigenous antigen' recovered from native ('Indian Bison Type') MAP strain of goat origin had superior immune reactivity as compared to imported commercial PPAs (protoplasmic antigens) of bovine origin (b-ELISA from Allied Monitor Inc., USA) and commercial ELISA kit for ruminant species (ID Vet, France). Lower immune-reactivity of commercial antigens as compared to 'indigenous antigen' indicated that search for universally acceptable 'ELISA kit' is not practically possible.

Keywords: *Mycobacterium avium* subsp. *paratuberculosis* (MAP), AFBs, indigenous ELISA, Allied Monitor PPA ELISA, ID-VET ELISA

Introduction

Johne's disease (JD) is chronic granulomatous enteritis of ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection. Goat is the fastest growing livestock species despite 43.0% slaughter rate[1]. JD is endemic in goatherds[2] and reported from Canada[3], several European countries[4], Nepal[5], Pakistan[6] and India[7,8]. Clinical symptoms are non-specific (progressive weight loss, diarrhea, poor fertility, loss in productivity *etc.*). Emaciation, hide bound condition and eventual death occur at very late stage of the disease. Being incurable and terminal disease[9], it has negative impact on the growth of goat industry in the country. Lack of accurate cost-effective test is the major stumbling block in the control of the disease. 'Test and cull' method used for the control by developed countries and by Central Institute for Research on Goats (CIRG) in India failed to deliver any good[10]. Instead the disease continued to flourish both in intensity and spread to newer goats. Poor sensitivity and specificity of the ELISA in sub-clinical stages of the disease is key obstacles in the management of JD in goat herds. Search for 'universal diagnostic test' has been the subject of research globally[11] in order to reduce MAP infection in goat herds[7] and also in human beings[12], to whom it spreads through food chain[13].

Goat being 'poor man's cow', the 'in-put' cost of test is a crucial factor. The high cost of the imported kits is a major constrain in screening of goats against JD. ELISA being sensitive and cost effective test is the most widely used test for the screening of JD in goats[14]. The theme of present study is based on the hypothesis that 'imported kits' are made from MAP strains originating from different livestock species (sheep & cattle) from geographically different regions and, therefore, have poor immune-reactivity. Further, is it possible to have 'universal 'ELISA kit/s'[15]? JD has been endemic in goatherds located at the CIRG, Mathura[16] and non-availability of 'indigenous ELISA kits' (i-ELISA) was the major limiting factor for its control. Hence 'i-ELISA kit' using native species specific antigens was first time developed in 1998[16].

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The immune-reactivity of 'i-ELISA kit' using native semi-purified protoplasmic antigen (sPPA) from novel MAP bio-type ('Indian Bison Type') of goat origin (Strain 'S 5'), under serial passage since isolation in 1999, was re-validated *vis-à-vis* commercial purified protoplasmic antigens (cPPA) of bovine origin from Allied Monitor, USA and cPPA of bovine origin from ID Vet, France (supplied with a new ELISA kit for ruminants) after the gap of >10 years (first time 2005-06 and now again in 2015-16) against serum samples of goats[14,17], with known status of MAP. The present study was also part of the efforts to evaluate microscopy and three antigens in ELISA protocol developed for 'i-ELISA kit' for goats.

Materials and Methods

Samples

Both serum and faecal samples were collected from 360 goats at different farms [Barbari, Jamunapari, Jakhrana, Sirohi and Experimental Units, CIRG; and Jamunapari Unit, Etawah and private farms (Kalpataru Goat Farm, Mathura; United Goat Farm, Garh-Mukteshwar, Uttar Pradesh; and Bhavika goat farm, Gurgaon, Haryana)] in North India. At the time of sampling, 23 goats suffered from clinical JD (emaciated, diarrhoeic, unproductive), 189 had sub-clinical JD (weak, unthrifty) and 148 had no symptoms. Screened goats were of Barbari, Jamunapari, Sirohi, Jakhrana, Totapari and non-descript breeds of North India. Serum samples were stored in –20ºC till further use.

Shedding of MAP in Faeces

The sampled faeces (2 g) were homogenized finely in tap water and concentrated by centrifugation at 4500 rpm for 45 min at room temperature. Smears prepared from the middle layer were heat fixed, stained with Ziehl-Nielsen (ZN) method and examined under microscope for pink (acid fast) short rods indistinguishable to MAP[18].

Semi-purified Protoplasmic Antigen (sPPA)

sPPA prepared from native 'Indian Bison Type' strain (S 5) of MAP, isolated from a terminal case (goat) of JD[19], was initially developed as a goat based 'indigenous ELISA (i-ELISA) kit[14].

i-ELISA Test Protocol

i-ELISA kit developed by Singh *et al*[14] was used. Briefly, native sPPA was standardized (0.1 µg per well). Serum samples and anti-goat horseradish peroxidase conjugate (Sigma Aldirch, USA) were used in 1:50 and 1:5000 dilution, respectively. Serums of culture positive and negative goats were used as positive and negative controls, respectively. Optical densities (ODs) expressed as sample-to-positive (S/P) ratios as per Collins[20].

Analysis of OD (Absorbance) Values

The S/P ratio was calculated as follows:

S/P ratio value = [(Sample OD–Negative OD)/ (Positive OD–Negative OD)]

The value of S/P ratios and corresponding status of JD is given below.

Calculated value of S/P ratio	JD status in animal
0.00-0.09	Negative
0.10-0.24	Suspected or Borderline
0.25-0.39	Low Positive
0.4-0.99	Positive
1.0-10.0	Strong Positive

*Serum samples in positive (P) and strong positive (SP) were taken as positive.

Sensitivity and specificity of i-ELISA kit was 83.3 and 90.0%, respectively[14].

Purified Protoplasmic Antigen (PPA) of MAP of Bovine Origin (USA) (b-ELISA)

Commercial PPA was used as per specifications of the manufacturer 'Allied Monitor Inc., USA' at 0.4 µg per well (b-ELISA) and rest steps were like i-ELISA kit, including interpretation of test[20].

Purified Protoplasmic Antigen (PPA) of MAP of Bovine Origin (France) (r-ELISA)

r-ELISA was done as per manufacturer instructions on previously coated antigen of bovine origin supplied by ID-VET, France. Anti-ruminant conjugate was provided. Positive, negative and test serum were diluted with buffer 6 in 1:11 ratio. Samples were incubated after transferring 100 µL of previously diluted samples to respective wells plate. Plates were incubated and washed 3 times with 300 µL of the 1× wash solution. Anti-ruminants IgG peroxidase conjugate (10×) was prepared to 1× in dilution buffer 3 and transferred 100 µL of diluted conjugate to each well of the plate and incubated. After incubation, conjugate was removed and plate was washed 3 times with 300 µL of the 1× wash solution. Substrate solution (100 µL) was added and incubated for 15 min at 21ºC. Stop solution was added to each well of plate and OD recorded at 450 nm. Sensitivity and specificity was reported as 42.0 and 99.0%[21].

Positive and Negative Controls

Previously tested positive and negative control serum were used in each plate. Positive control had 3-4 times difference in OD values with respect to negative control.

Interpretation

Samples mean OD value was converted to S/P percentage using following formula:

S/P percentage = [(Sample OD–Negative OD)/ (Positive OD–Negative OD)] × 100

The S/P per cent and corresponding status of JD is given below.

S/P per cent	Status of JD
≤ 60%	Negative
≥ 60% to ≤70%	Doubtful
≥ 70%	Positive

Statistical Analysis

Mc Nemar's test and kappa agreement was applied for the measure of statistical significance between the results of two tests (GraphPad Software, USA).

Ethical Approval

The study was part of the project on JD vaccination, which was approved by Institute Animal Ethics Committee. Serum samples used in this study were collected in the vaccine project and no animal was bled separately for the present study.

Results

Table 1 exhibits clinical profile of goats with respect to the status of MAP infection when OD values were converted to S/P ratio as per Collins[20]. Cut off for MAP infected animals (positives) in i-ELISA and b-ELISA included animals in strong positive and positive categories. Low positives were not taken as positive since MAP is endemic in the domestic livestock of country. Whereas in r-ELISA, there was only three categories, positive, suspected and negative for MAP infection. Goats in positive category were taken as positives for MAP infection. Table 1 shows the number of goats positive for JD as per the 'standard operating protocol' of 3 ELISA kits. Screening of 360 serum samples of goats for MAP infection showed 40.0, 34.4 and 5.2% samples positive using i-ELISA (goat based), b-ELISA (bovine based) and r-ELISA (ruminant based), respectively. In Table 2, the results of 4 tests (3 types of ELISA & microscopy) are compared. Of the 360 fecal and serum samples screened by all the 4 tests (microscopy, i-ELISA, b-ELISA & r-ELISA kits, 3.0 and 35.2% goats were common positive and negative, respectively (Table 2). Four tests together, detected 64.7% goats infected with MAP and 23.3% goats were detected positive in independent tests (positive in any one of the 4 test) (Table 2). Highest percent of goats (20.8%) were positives alone by fecal microscopy (Fig. 1), followed by 1.1, 0.8, and 0.5%

Table 1 — Status of MAP infection with respect to the OD values conversion into S/P ratios as per Collins[20] in i-ELISA, b-ELISA and r-ELISA tests in 2015-16

Tests	Status *n* (%)				
	SP	P	LP	S	N
i- ELISA*	6	138	67	91	58
	P-144 (40.0)		(18.6)	N-149 (41.3)	
b-ELISA*	11	113	65	87	84
	P-124 (34.4)		(18.0)	N-171 (47.5)	
r-ELISA**	19		6	335	
	P-(5.2)		(1.6)	(93.0)	

*SP-Strong positive; P-Positive; LP-Low positive; S-Suspected; N-Negative
*SP+P = Positive in i-ELISA and b-ELISA
**Positive-P, Suspected-S, Negative-N in r-ELISA
Total positive in i-ELISA-144 (40.0%)
Total positive in b-ELISA-124 (34.4%)
Total positive in r-ELISA-19 (5.2%)

Table 2 — Comparison of fecal microscopy, i-ELISA, b-ELISA and r-ELISA for detection of MAP infection in goats in 2015-16

Diagnostic tests	Combinations														Total *n* (%)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Microscopy	+	-	+	-	-	-	+	+	+	-	-	+	+	+	205 (56.9)
i-ELISA	+	-	-	+	-	-	+	-	-	+	+	+	-	+	144 (40.0)
b-ELISA	+	-	-	-	+	-	-	+	-	+	+	+	+	-	124 (34.4)
r-ELISA	+	-	-	-	-	+	-	-	+	-	+	-	+	+	19 (5.2)
Total= 360	11	127	75	4	3	2	24	7	1	18	1	83	1	3	233*
Percent	3.0	35.2	20.8	1.1	0.8	0.5	6.6	1.9	0.2	5.0	0.2	23.0	0.2	0.8	64.7

Positive in single tests independently-84 (23.3%), *Total positives 233 (64.7%)
Positive in two test combinations:
Microscopy + i-ELISA- 228 (63.3%)
Microscopy + b-ELISA- 227 (63.0%)
Microscopy + r-ELISA- 208 (57.7%)

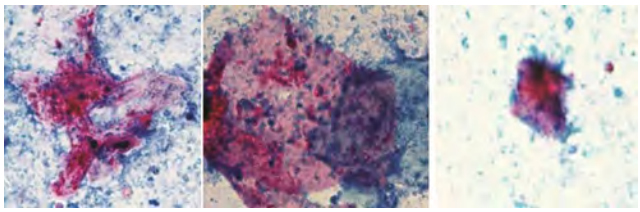

Fig. 1 — Fecal microscopy (ZN staining) of goats exhibiting heavy sheeding of MAP bacilli of clinical Johne's disease.

positives in i-ELISA, b-ELISA and r-ELISA, respectively. The 56.9, 40.0, 34.4 and 5.2% goats were detected positive in fecal microscopy, i-ELISA, b-ELISA and r-ELISA, respectively. However, of the 64.7% (233) samples detected by all the four tests, 12.0 (28), 38.2 (89), 46.7 (109) and 91.8% (214) goats were missed in microscopy, i-ELISA, b-ELISA and r-ELISA, respectively (Table 2). In two test combinations, microscopy with each of the ELISA kits; i-ELISA, b-ELISA and r-ELISA detected, 63.3, 63.0 and 57.7% goats as positives, respectively (Table 2). Table 3 compares 3 ELISA test combinations, wherein a total of 43.8% (158) goats were found positive by 3 ELISA tests (positive in one, two and three tests). Independently, i-ELISA, b-ELISA and r-ELISA tests alone detected, 7.7, 2.7 and 5.2% goats positive, respectively (Table 3).

Sensitivity and Specificity of Diagnostic Tests

Sensitivity of i-ELISA, b-ELISA and r-ELISA with respect to microscopy was 59.0, 49.7 and 7.8%, whereas specificity was 85.1, 52.1 and 98.0%, respectively (Table 4).

Statistical analysis in 4 tests by Mc-Nemar test and Kappa Agreement

P values calculated for 6 different comparative combinations of tests were found statistically significant (<0.0001). Kappa agreement between microscopy and b-ELISA was fair and was moderate between microscopy and i-ELISA, and i-ELISA and b-ELISA (Table 5).

Table 3 — Comparative evaluation of 3 ELISA tests (i-ELISA, b-ELISA, r-ELISA) for the diagnosis of JD in goats in 2015-16

ELISA tests	Combinations								Total *n* (%)
	1	2	3	4	5	6	7	8	
Goat based (i-ELISA)	+	-	+	-	-	+	+	-	144 (40.0)
Bovine based (b-ELISA)	+	-	-	+	-	-	+	+	124 (34.5)
Ruminant based (r-ELISA)	+	-	-	-	+	+	-	+	19 (5.2)
Total= 360	12	202	28	10	3	3	101	1	158*
Percent	3.3	56.1	7.7	2.7	0.8	0.8	28.0	0.2	43.8

* Total positives-158 (43.8%)
Positive in two test combinations:
i-ELISA + b-ELISA = 155 (43.0%)
b-ELISA + r-ELISA = 130 (36.1%)
r-ELISA + i-ELISA = 148 (41.1%)

Discussion

Paratuberculosis and other mycobacterial infection are the major threat to both animals and human population[22]. JD is major cause of low productivity in domestic livestock by reducing productive life span and animals go early culling or slaughter for harvesting meat. Culture used for the identification of pathogen and prevalence of disease has been costly and time-taking, hence it is replaced by microscopy. Microscopy for identification of disease has been standardized and used at CIRG for the past 32 years. In the present study, goat based 'i-ELISA kit' developed at CIRG was re-evaluated for immune-reactivity *vis-à-vis* cPPA (Allied Monitor Inc., USA) and new ELISA kit from ID Vet, France for ruminants after a gap of 10 years[14]. 'i-ELISA' kit had been under continuous validation with other 'International kits and antigens[14,17]. To estimate bio-incidence of JD[7] in goats was part of the study.

Of the total 360 goats screened, 64.7% were found positive using four tests (3 ELISA tests with microscopy combinations). Percent positivity was highest (56.9%) in microscopy, followed by i-ELISA (40.0%), b-ELISA (34.4%) and r-ELISA (5.2%). Per cent disagreement was low (1.1, 0.8 & 0.5%) in 4 tests regimen (microscopy added with ELISA tests), as compared to 7.7, 2.7 and 0.8% using 3 tests regimen (i-ELISA, b-ELISA & r-ELISA). Thus the results show the usefulness of multiple tests in detection of JD (Tables 2 & 3). Sensitivity and specificity of the tests increased as we increase the number of tests used in the screening. Similar to the findings of Singh *et al*[7], this study also reports high bio-incidence of MAP in domestic goats population and microscopy was found to be the most sensitive of the 4 tests used, hence microscopy was recommended as the preferred test[18]. In this study, microscopy detected highest number (56.9%) of goats as shedders as compared to 3 ELISA tests combined together (43.8%) (Tables 2 & 3). The chances of detecting saprophytes as MAP were low since the population of saprophytic Mycobacteria in the intestines of domestic livestock (in this study goats) has been replaced by more pathogenic and host dependent MAP in the last 10 years[23] (personal experience of

Table 4 — Permutation combinations of sensitivity and specificity in four diagnostic tests in goats in 2015-16

No.	Comparisons	TP	FP	TN	FN	Sensitivity & Specificity (%)
1	Microscopy – i-ELISA	121	84	132	23	84.0 & 61.1
	i-ELISA – Microscopy	121	23	132	84	59.0 & 85.1
2	Microscopy – b-ELISA	102	103	133	22	82.2 & 56.3
	b-PPA ELISA – Microscopy	102	22	133	103	49.7 & 52.1
3	Microscopy – r-ELISA	16	189	152	3	84.2 & 44.5
	r-ELISA – Microscopy	16	3	152	189	7.8 & 98.0
4	i-ELISA – b-ELISA	113	205	31	11	91.1 & 13.1
	b-ELISA – i-ELISA	113	11	31	205	35.5 & 73.8
5	i-ELISA – r-ELISA	15	129	212	4	78.9 &62.1
	r-ELISA – i-ELISA	15	4	212	129	10.4 & 98.1
6	b-ELISA – r-ELISA	13	111	230	6	68.4 & 67.4
	r-ELISA – b-ELISA	13	6	230	111	10.4 & 97.4

TP-True positives (positive in both tests), TN-True negatives (Negatives in both tests)
False positive (Positive in one test and negative in another), False negative (Negative in one test and positive in another)

Table 5 — Statistical analysis between different test by Mc-Nemar test and kappa agreement in goats in 2015-16

No.	Comparison	P value		Kappa	Strength of agreement	95% confidence interval
		Status	Value			
1	Microscopy *vs* i-ELISA	Significantly different	<0.0001	0.422	Moderate	0.334-0.509
2	Microscopy *vs* b-ELISA	Significantly different	<0.0001	0.33	Fair	0.248-0.420
3	Microscopy *vs* r-ELISA	Significantly different	<0.0001	0.051	Poor	0.013-0.089
4	i-ELISA *vs* b-ELISA	Significantly different	<0.0001	0.050	Poor	0.003-0.096
5	g- ELISA *vs* r-ELISA	Significantly different	<0.0001	0.100	Poor	0.038-0.162
6	b-ELISA *vs* r-ELISA	Quite significantly different	0.0896	0.751	Good	0.680-0.821

Kappa value (0.0-0.20, Poor; 0.21-0.40, fair; 0.41-0.60, moderate; 0.61-0.80, substantial; & 0.81-100, perfect)

Table 6 — Comparative evaluation of native sPPA prepared from native MAP strain (S 5), 'Indian Bison type' with commercial PPA (Allied Monitor, Inc., USA) in ELISA for the diagnosis of MAP infection in 2006-07

Species	Serum samples	Percent positives		Reference
	n	sPPA* from MAP 'Indian Bison type'	cPPA** from MAP 'Bovine'	
Goat kids^	47	8.5	0.0	Singh *et al*[14]
Goat kids^	179	58.6	2.7	Kumar *et al*[34]
Goats+	55	27.2	9.0	Singh *et al*[14]
Sheep+	16	37.5	37.5	Singh *et al*[14]
Buffaloes^	167	46.7	0.0	Yadav *et al*[36]
Humans	76	42.1	40.8	Singh *et al*[35]

*Semi-purified protoplasmic antigen (sPPA) from native MAP 'Bison type' of goat origin
**Purified commercial PPA (cPPA) from MAP 'Bovine'
+Farm animals (CIRG, Makhdoom), ^Farmers' animals

senior author). Hence, present study underlines the utility of microscopy as 'screening test', which is quick, repeatable, simple, cost effective and can be adopted in field laboratory for the screening of goats[18] belonging to poor farmers.

In the previous (2005-06) evaluation of cPPA of bovine origin (Allied Monitor Inc., USA) *vis-à-vis* native sPPA of goat origin, it showed poor immune-reactivity with serum of animals (goats, sheep, cattle & buffaloes)[7,14,17] (Tables 6 & 7), except human samples[24]. However, in this study, sensitivity of b-ELISA (34.4%) was comparable and detected almost similar percentage of goats positive as in i-ELISA (40.0%) and in microscopy (56.9%), after a gap of 10 years (2015-16). Change in immune-reactivity of cPPA of Allied Monitor Inc., USA, procured in the present study may be either due to change in strain used for making commercial antigen (cPPA) or change in microflora in the intestines of domestic livestock population (personal experience of senior author). Though the concentration of cPPA used per well was 4 times as compared to i-ELISA.

Table 7—Comparative sensitivity and specificity of five ELISA kits for the diagnosis of JD in goats and sheep in 2006-07

ELISA kits	Sensitivity (%)	Specificity (%)
i-ELISA kit (sPPA)	53.7	86.0
b-ELISA (cPPA)*	17.8	86.0
Pourquier ELISA kit (France)*	03.7	94.7
r-ELISA(cPPA)*	18.7	93.7
Adsorbed i-ELISA (sPPA)	42.1	95.1

Reference: Singh *et al*[14,17] and study based on 74 serum and fecal samples of goats tested by 'i-ELISA kit *vis a vis* other diagnostic kits
*Commercial diagnostic ELISA kits and antigen

Another major difference may be that we used semi-purified antigen (sPPA) in i-ELISA (40.0%) as compared to purified commercial PPA of bovine origin (Allied Monitor Inc., USA). In present study also per well concentration of cPPA was much higher as compared to 'i-ELISA test'.

Screening of goats by r-ELISA exhibited poor immune-reactivity and detected only 5.2% (19) goats as positive. The r-ELISA kit has been developed by ID Vet, France for screening of all livestock species, likewise i-ELISA is also for all species of domestic livestock including human beings. The r-ELISA kit was sent to our laboratory for validation. This may be due to use of MAP strain (of small ruminant origin or sheep type), which has not been reported so far in India[25]. Sensitivity and specificity, P-values and kappa values calculated in the present study had little significance as there was no 'Gold standard test' like culture. Earlier studies[14,17] also exhibited poor immune reactivity with commercial ELISA kits (Pourquier & ID-Vet, France) as showed by r-ELISA kit in the present study (Tables 1-3 & 7).

Different studies compared sensitivity and specificity of ELISA in MAP infection. Whittington developed ELISA using bovine strains of MAP to screen wild and domestic animals[26]. Ethanol vortex ELISA (EV ELISA) was developed using ethanol extract of MAP particularly for JD[27,28]. EV ELISA was reported significantly better to the Biocor ELISA, particularly in screening of low and middle level MAP shedders[27]. Similar study was carried out to measure the performance of EV ELISA for screening of serum samples collected from different herds of domestic and wild animals (red deer & cattle) including other experimentally infected animals with MAP[29]. Ferreira[30] has developed the PPA-ELISA and compared the sensitivity in relation to Herd Check, which was 47.5%, while specificity was 86.8%. Speer *et al*[31] have evaluated SELISA with commercial ELISA where sensitivity and specificity of SELISA appears superior to the commercial ELISAs for routine diagnosis of JD[31]. Shin *et al*[32] have reported that JTC-ELISA, which worked effectively for the detection of cattle infected with subclinical MAP infections, providing a cost-effective diagnostic that hold up the paratuberculosis control programmes in cattle herds. A goat origin antigen based 'i-ELISA kit' developed in India from 'Indian Bison type' strain ('S5') of MAP showed enhanced sensitivity and specificity than commercially available ELISA kits[14]. i-ELISA was optimally correlated with culture and was good for estimating the sero-prevalence[14]. Kumar *et al*[33] reported that sensitivity of fecal microscopy with culture and i-ELISA kit was 90.0 and 65.0%, respectively and revealed substantial agreement between two tests. In the absence of sensitive and specific field based screening tests, Singh *et al*[18] reported microscopy would be useful in screening of the domestic ruminants. Another study also reported that i-ELISA kit improved the detection rate of MAP in test samples. However, i-ELISA developed using native antigen from local strain of MAP was superior than AGPT in cattle[7,14]. Similar comparative studies were carried out where the sensitivity and specificity of i-ELISA kit was 66.6 and 75.0% and 68.1 and 66.6% with tissue culture and PCR, respectively and i-ELISA had superior diagnostic potential having high specificity with commercial ELISA kit[17,34].

The present study underlines the utility of multiple tests for the diagnosis of chronic infection like JD. It was prudent to use atleast two tests (microscopy may be preferred test in Indian conditions), where cost is big constrain in screening of goats. Microscopy and i-ELISA were sensitive and had superior accuracy as compared to commercial ELISA tests and were superior in detecting both early and late stages of MAP infections. Goat based 'i-ELISA' was highly superior in detecting all stages of MAP infection and performance of native sPPA from strain 'S 5' of MAP maintained its immune-reactivity despite serial passage in the last 10 years.

Conclusions

The present study demonstrated significant differences in the immune reactivity of commercial ELISA kit *vis-à-vis* 'indigenous ELISA' kit for the screening of goat population against JD. Thus instead of using 3 antigens from geographically distinct

regions of the world, it was prudent to use native antigen prepared from local strain of MAP. Faecal microscopy may be recommended as another 'screening test' to estimate shedders. Sensitivity of i-ELISA was highest in the 3 ELISA tests and is recommended for screening of goat herds. Low and poor immune-reactivity of b-ELISA and r-ELISA, respectively proved that there cannot be 'universal ELISA kit' and use of native and semi-purified antigens in ELISA is the best option.

Conflict of Interest

The authors declare that they have no conflict of Interest.

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