Evaluation of an indigenous ELISA for diagnosis of Johne's disease and its comparison with commercial kits

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Abstract Country lacks sensitive and indigenous diagnostic kits for the screening of goats and sheep against Johne's disease. Therefore an indigenous ELISA kit was developed using protoplasmic antigen from native *Mycobacterium avium* subspecies *paratuberculosis* 'Bison Type' strain of goat origin (Kit 1). In the present study, kit 1 and two commercial kits (kit 2 and 3) were evaluated with respect to 'Gold Standard' fecal culture in 71 animals (55 goats and 16 sheep). Kit 1 using indigenous antigen (protoplasmic antigen) was sensitive at very low concentration (0.1 µgm / well) as compared to purified commercial protoplasmic antigen (4 µgm / well) used in kit 2, in the Type 1 reactors (strong positive as positive). Screening of 71 animals by fecal culture detected 38.0% animals (goats–40.0%, sheep–31.2%) as positive (clinical shedders of bacilli) from these farm animals. Of the farm animals located at Central Institute for Research on Goats, herds of goat were endemic whereas, sheep flocks were comparatively resistant to Johne's disease. The 29.5 and 61.9, 15.4 and 57.7 and 4.2 and 14.0% animals (goats and sheep) were in the category of sero-reactors type 1 and 2 of the ELISA kits 1, 2 and 3, respectively. In the type 1 sero-reactors, sensitivity and specificity of kit 1, 2 and 3 was 53.7 and 86.0, 17.8 and 86.0 and

3.5 and 94.7%, respectively. Indigenous ELISA test (kit 1) was significantly superior for the screening of goatherds and sheep flocks against JD as compared to commercial ELISA kits (Kit 2 and 3). In comparison to kit 2 and 3, kit 1 had highest sensitivity, comparable specificity and substantial to nearly perfect proportional agreement (Kappa Scores) with respect to 'Gold standard' fecal culture in goats and sheep. Disease being endemic in herds and flocks screened using ELISA kits, Type I sero-reactors had better correlation with fecal culture in comparison to Type II sero-reactors therefore, used for estimation of sero-prevalence. Newly developed Indigenous ELISA kit was simple, inexpensive, sensitive and reliable for screening of goats and sheep population against Johne's disease. The study reports high prevalence of Johne's disease in farm goatherds and sheep flocks, using sensitive tests (fecal culture and ELISA kit). Results of Type 1 reaction in kit 1 were optimally correlated with culture and were good for estimating the sero-prevalence. For controlling Johne's disease in endemic herds initial removal of the animals in strong positive category (Type 1 reactors), may help to remove heavy shedders.

KeyWords Johne's disease · *Mycobacterium avium* subsp. *paratuberculosis* · Protoplasmic antigen · Diagnosis · Fecal culture · ELISA kit

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Introduction

Johne's Disease (JD), a chronic infectious disease of ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and is a concern in developed countries¹. Information on prevalence of JD in India possessing 16.2% (120 million) goat population of the world is limited^{2,3,4}. In India, bio-burden of low producing goats and sheep is very

high and is taxing the scarce and fragile grazing resources. Populating of low and unproductive animals continue to increase in the country and is ecologically unsustainable. Earlier studies reported^{5,6,7} that JD is endemic in goatherds and sheep flocks in India (table 10), therefore culling of all positive goats in ELISA is not economical for controlling JD. Laboratory tests that can provide both diagnostic and prognostic information would be helpful to know infectivity status of each animal and adopt a suitable control strategy.

Goat and sheep farmers are very poor; consequently availability and cost of testing is major limiting factor in estimating prevalence of JD. However, most accurate and informative test results are often based on antibody detection using ELISA test. Commercial ELISA kits are popular for screening bovines⁸ against JD but the suitability of these kits with respect to sensitivity and specificity in small ruminants (goats and sheep) has not been investigated in India. In view of the lack of indigenous diagnostic kit against JD screening of country's huge population of large ruminants in general and small ruminants in particular was challenging. Therefore an indigenous ELISA kit was developed for screening goat and sheep. The first indigenous ELISA kit used protoplasmic antigen (PPA) from native MAP 'Bison Type' strain of goat origin. Since Isolation of MAP by fecal culture though time consuming was semi-quantitative and most reliable (detected as few as 10 cfu/g) for the diagnosis of JD in ruminants, therefore 'Gold standard' test⁹. Study aimed to compare a newly developed indigenous ELISA kit with two other commercial ELISA kits with respect to fecal culture (Gold standard) for the diagnosis of Johne's disease in goats and sheep.

Materials and methods

Fecal and serum samples were collected for screening against Johne's disease from 71 animals (55 goats and 16 sheep) belonging to farm animals located at Central Institute for Research on Goats (CIRG), Makhdoom, in Mathura district.

Fecal samples: The 71 fecal samples were processed for isolation of MAP by culture as per the method of Merkal¹⁰ with some modifications⁶. Culture method has been optimized using centrifugation, decontamination with hexadecyl pyridinium chloride at room temperature for 18–24 hours and Herrold's egg yolk medium (HEYM) with and without mycobactin J. Slants were examined for appearance of colonies at weekly interval after inoculation up to at least 120 days. Colonies were identified by slow growth, Mycobactin J dependence, acid fastness, colonial and bacterial morphology¹¹.

Serum samples: Each of the 71 serum samples was screened using 3 ELISA kits.

a. Indigenous ELISA kit – (Kit 1): Indigenous kit was developed for screening of goats and sheep using semi-purified soluble protoplasmic antigen (PPA) from native virulent and field isolates of MAP ('S-5') from a terminal case of JD in a goat (CIRG) and characterized as 'Bison type'^{12,13}. Antigen was prepared from this S 5 'Bison type' strain MAP at 16th passage level. Growth was inactivated (72°C for 2 hours) and pelleted at 10,000 g for 20 minutes at 4°C. Pellet was given 3 washing in 0.01 M PBS (pH 7.2). After 3rd washing pellet was suspended in sterilized normal saline solution in ratio of 0.2 g of wet cell weight / ml, in 30 ml and treated to ultrasonic disruption (sonication) at 100 watts (15 Hz) for 20 min. in ice slurry giving 20 cycle of 30 sec and 30 sec rest after each cycle. Sonicate was centrifuged (10,000 rpm for 30 min at 4 °C), dispensed and stored at –20 °C. Protein concentration was estimated by method of Lowry et al¹⁴.

Optimum concentration of antigen, serum and second antibody (conjugate) was determined by checkerboard analysis (Anti-goat conjugate, 1:8000; MAP 'Bison type' antigen (kit 1), 0.1 µg per well; Commercial 'bovine' antigen (kit 2), 4.0 µg per well, serum dilution, 1: 50; and incubation time 30 min. Positive and negative controls were selected from the goats, which were positive and negative respectively, both in culture and serum ELISA test in earlier studies, were used in kit 1 and 2. OD value of positive serum used as positive control was 0.176 and OD of negative serum used as negative control was 0.076 or the difference between OD values of positive and negative serum controls' should be at least 2–3 times.

Kit was standardized as per method of Milner¹⁵ with some modification¹⁶ (Singh et al., 2007). Briefly, 0.1 µg and 4.0 µg per plate of MAP 'Bison type' antigen (Kit 1), and commercial bovine antigen (Kit 2) respectively, were taken in 10 ml of carbonate-bi carbonate buffer, (pH 9.6) and coated in duplicate wells of flat bottom 96 well ELISA plate. Plate was incubated (4 °C over night), washed thrice with washing buffer (PBS + 0.05% Tween - 20), blocked (3% bovine serum albumin fraction V in PBS) and incubated (37 °C for 1 hrs). Plate was washed thrice (PBST), 100 µl of 1:50 diluted serum samples (dilution buffer: PBST + 1% bovine serum albumin) added in duplicate wells and incubated (2 hrs at 37 °C). Plate was washed (3 times with PBST for 5 min each), 100 µl of optimally diluted (1:8,000) Rabbit anti-goat horse radish peroxidase conjugate (Bangalore Genei) added to all wells and incubated (1 hour at 37 °C). Plate was washed (3 times with PBST for 5 min each), 200 µl of freshly prepared substrate, (OPD, 8 mg / plate in substrate buffer, pH - 5.0) was added to each well and was incubated (in dark for 30 min at room temperature. Absorbance was read at 450 nm in ELISA reader (Multiskan,

Finland). Positive, negative, substrate and conjugate controls were run with serum samples.

Calculation of S/P ratios from OD values:

Positive and negative controls had OD values of 0.176 and 0.076, respectively. OD values of test samples were transformed to S/P ratio¹⁷. Since JD is endemic in goatherds located at CIRG, therefore, goats in the strong positive group: (Type 1 sero-reactors) were treated as positive for JD. Standard procedure was used to calculate sensitivity and specificity of ELISA test¹⁸.

b. ELISA kit with commercial antigen (Kit 2): Kit was standardized using commercial purified protoplasmic antigen (PPA) of MAP 'Bovine' origin. Positive and negative serum controls were also procured along with antigen from Allied Monitor Inc. (USA). The antigen was diluted and used as per the instructions of manufacturer and finally per well protein concentration was 4.0 µg (Lowry et al 14).

c. Commercial ELISA Kit (Kit 3): Commercial ELISA kit recommended for cattle, sheep and goats was also based on purified commercial protoplasmic antigen (PPA) from MAP and was provided by Pourquier, France as gift. Kit was used as per the instruction of manufacturer. Positive and negative serum controls were supplied with kit.

Performance of 3 ELISA kits with respect to 'Gold Standard' fecal culture were compared by calculating the Kappa Scores (Proportional Agreement) as per method given by Landis and Koch¹⁹ in 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 – 1.00, almost perfect). Sensitivity and specificity of each kit was determined using standard method.

Results and Discussion

Johne's disease is highly prevalent (endemic) in the goat-herds located at CIRG and other places in India (table 1) therefore, using ELISA kits two types of sero-reactors were identified.

- a. Type 1 sero-reactors:** Animals in the strong positive category in ELISA S/P ratio were classified as positive and rest categories (positive, low positive, suspected and negative) as negatives. For the control of Johne's disease in endemic herds it was absolutely safe to recommend removal of strong positive reactor irrespective of their correlation with fecal culture test.
- b. Type II sero-reactors:** Animals in low positive, positive and strong positive categories were considered positives and rest categories (suspected and negatives) as negatives. To stamp out the disease,

these positive reactors can be recommended for removal or vaccinated depending on the administrative will and economic resources.

On the basis of fecal culture, prevalence of Johne's disease was 38.0% in animals (goats–40.0%, sheep–31.2%) located at CIRG, Mathura. The 29.5 and 61.9, 15.4 and 57.7 and 4.2 and 14.0% goats and sheep were sero-reactors in the Type 1 and II sero-reactor categories of the kits 1, 2 and 3, respectively. Using kit 1 (indigenous 'ELISA kit'), the 29.5, 21.1 and 11.2% animals were categories as strong positive, positive and low positives, respectively. However, in fecal culture 71.4, 20.0 and 37.5% animals from strong positive, positive and low positive categories were positive, respectively. The 16.9 and 21.1% animals were in doubtful and negative categories of S/P ratio (kit 1) and of these 8.3 and 33.3% were positive in fecal culture (Table 1). Therefore positive reactors in strong positive category have maximum correlation with in fecal culture, though culture detected animals in all the categories of S/P ratio. Using purified commercial PPA (Allied Monitor Inc., USA), 15.4, 33.8 and 8.4% animals were categorized as strong positive, positive and low positive in ELISA, respectively. In kit 2, 45.4, 33.3 and 33.3% animals from strong positive, positive and low positive categories were positive in fecal culture, respectively. The 9.8 and 32.3% animals were in doubtful and negative categories of S/P ratio (kit 2) and of these 28.5 and 43.4% were also positive in culture (Table 2). Here also animals in strong positive category (among positives) had maximum correlation with culture, however, large number of culture positives were missed by this kit. Using kit 3 (Pourquier kit) 18.3, 32.3 and 11.2%

Table 1 Kit 1 – Comparison of S/P ratios with culture status of goats and sheep.

S/P ratio	Disease status	Kit 1	Culture
0.00–0.09	Negative	15	5
0.10–0.24	Suspected	12	1
0.25–0.39	Low positive	08	3
0.40–0.99	Positive	15	3
1.0–10.0	Strong Positive	21	15

Table 2 Kit 2 – Comparison of S/P ratios with culture status of goats and sheep.

S/P ratio	Disease status	Kit 2	Culture
0.00–0.09	Negative	23	10
0.10–0.24	Suspected	07	2
0.25–0.39	Low positive	06	2
0.40–0.99	Positive	24	8
1.0–10.0	Strong Positive	11	5

Table 3 Kit 3 – Comparison of S/P ratio with culture status of goats and sheep.

S/P ratio	Disease status	Kit 3	Culture
0.00–0.09	Negative	55	23
0.10–0.24	Suspected	6	3
0.25–0.39	Low positive	1	0
0.40–0.99	Positive	6	0
1.0–10.0	Strong Positive	3	1

Table 4 Sensitivity and specificity of 3 ELISA kits for the diagnosis of Johne's disease in goats and sheep (Type I reactors).

ELISA kits	Sensitivity %	Specificity %
Kit 1	53.7	86.0
Kit 2	17.8	86.0
Kit 3	3.5	94.7

Table 5 Sensitivity and specificity of 3 ELISA kits for the diagnosis of Johne's disease in goats and sheep (Type II reactors).

ELISA kits	Sensitivity %	Specificity %
Kit 1	87.5	47.7
Kit 2	60.0	40.9
Kit 3	3.7	79.5

animals were categories as strong positive, positive and low positive. Of the strong positive, positive and low positive animals, 33.3, 0.0 and 0.0% were positive in fecal culture, respectively. The 8.4 and 77.4% animals were in doubtful and negative groups of S/P ratio (kit 3) and of these 50.0 and 41.8% were positive in culture (Table 3). In kit 3 also strong positives animals had maximum correlation with culture (among positives) and large number of culture positives were missed by this kit 3.

In the type 1 category of sero reactors, sensitivity and specificity of the kits 1, 2 and 3 was 53.7 and 86.0, 17.8 and 86.0 and 3.5 and 94.7%, respectively (Table 4). Whereas, in the type II category of sero reactors, sensitivity and speci-

ficity of the kits 1, 2 and 3 was 87.5 and 47.7, 60.0 and 40.9 and 3.7 and 79.5%, respectively (Table 5). The Kappa scores of Kit 1, 2 and 3 (Type I reactors) with respect to fecal culture were 0.74, 0.60 and 0.60 in goats and sheep, 0.69, 0.61 and 0.60 in goats and 0.93, 0.56 and 0.06 in sheep, respectively (table 6, 7 and 8). Species-wise sensitivity and specificity of the Kit 1, 2 and 3 were 83.3 and 90.0, 33.3 and 60.0 and nil and 90.0 in sheep and 45.4 and 70.0, 13.6 and 93.9 and 4.5 and 96.9 in goats, respectively (Table 9).

Growth in small ruminant sector in India is number driven and per animal productivity remains very low and chronic infections like Johne's disease are mainly responsible for this poor productivity. Poor productivity may be due to high prevalence of Johne's disease, as reported in this study. Lack of priority provided to JD may be due to lack of indigenous diagnostic kits and control measure (vaccine). This has led to increased incidence of Johne's disease in goat and sheep population of the country. Earlier studies showed that wherever investigated, JD has been reported to be endemic in the domestic ruminants, especially goats (table 10). To know the prevalence of JD in nearly 200 millions goats and sheep, there is no indigenous, cost effective and sensitive diagnostic test. Therefore, first time an indigenous ELISA kit was developed and compared with other 2 commercial ELISA kits with respect to the Gold standard culture test in fecal and serum samples from 71 animals (goats, 55 and 16 sheep). The indigenous kit 1 was standardized using 0.1 µgm / well of soluble semi-purified antigen (PPA) obtained from native homologous MAP 'Bison Type' isolate of goat origin^{12,13} as compared to 4.0 µgm / well of purified PPA from MAP of 'bovine origin' in kit 2. The kit 3 was also based on purified PPA from MAP 'bovine' and recommended for cattle, goats and sheep by manufacturers. Efficiency of indigenous PPA (kit 1) in very low concentrations, exhibited the value of using antigen of native MAP isolates. Results of screening of goats and sheep using ELISA test showed that sensitivity of Kit 1 was higher (53.7 and 87.5% sero-reactors in Type 1 and 2) as compared to kit 2 (17.8 and 60.0%) and kit 3 (3.5 and 3.7%). Similarly fecal culture test revealed high

Table 6 Comparative evaluation of 4 tests (3 ELISA kits and fecal culture) for diagnosis of Johne's disease in goats and sheep.

Tests	Number of samples	Combinations				Kappa Scores
		1	2	3	4	
1. Culture	71	+	-	+	-	
2. ELISA	71	+	-	-	+	
2 a. Kit 1	71	15	38	12	6	0.74
2 b. Kit 2	71	5	38	22	6	0.60
2 c. Kit 3	71	1	42	26	2	0.60

Table 7 Comparative evaluation of 4 tests (3 ELISA kits and fecal culture) for diagnosis of Johne's disease in goats.

Tests	Number of samples	Combinations				Kappa Scores
		1	2	3	4	
1. Culture	55	+	-	+	-	
2. ELISA	55	+	-	-	+	
2 a. Kit 1	55	10	28	12	5	0.69
2 b. Kit 2	55	3	31	19	2	0.61
2 c. Kit 3	55	1	32	21	1	0.60

Table 8 Comparative evaluation of 4 tests (3 ELISA kits and fecal culture) for diagnosis of Johne's disease in sheep.

Tests	Number of samples	Combinations				Kappa Scores
		1	2	3	4	
1. Culture	16	+	-	+	-	
2. ELISA	16	+	-	-	+	
2 a. Kit 1	16	5	10	0	1	0.93
2 b. Kit 2	16	2	7	3	4	0.56
2 c. Kit 3	16	0	10	5	1	0.06

(38.0%) prevalence of JD in farm animals. In the absence of diagnosis, these sub-clinical animals continue to shed *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in the environment. In the screening of animals using fecal culture, irregular or no shedding of MAP in early stages of infection, beside slow growth in artificial medium are major constrain. Since prevalence of JD was high in farm animals therefore, two-way strategy can be adopted for the control of JD, depending upon the scope of improvement in management practices and financial resources. Animals in strong positive category considered as positive in Type

1 sero-reactors category, warrant immediate removal from the herds / flocks. Once these super-shedders (strong positives) are removed from the herds and flocks, next group of positives and low positives can be either removed or vaccinated. 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, positive and low positive categories were considered positive (Type II sero-reactors), JD is highly endemic (61.9%) in farm animals and situation demands urgent measures to control it. In such situation vaccination is the only option to reduce the sero-incidence²⁰. JD has been reported endemic in goat and sheep population⁶. Similar, study in North India reported 29.0% sero-prevalence of JD by screening 1441 ruminants using indigenous ELISA kit²¹.

Comparative evaluation of kit 1 (Type 1 sero-reactors) showed high sensitivity (53.7%) and specificity (86.0%) and also showed better proportional agreement (PA) with 'Gold standard fecal culture (0.74) in goats and sheep (goats – 0.69, sheep – 0.93) as compared to kit 1 and 2. The kit 2 had lowered sensitivity (17.8%) and similar specificity (86.0%) and lowered PA value (0.60) with respect to fecal culture in goats and sheep (goats – 0.61, sheep – 0.56) as compared to kit 1 but was superior to kit 3. Whereas, kit 3 had very poor sensitivity (3.5%) but very good specificity (94.7%) and had low PA value (0.60), with respect to fecal culture in goats and sheep (goats – 0.60, sheep – 0.06), as compared to kit 1 and 2. As per Landis and Koch method, kit

Table 9 Species-wise sensitivity and specificity of ELISA Kits with respect to fecal culture.

ELISA kits	Sensitivity (%)	Specificity (%)
a. Sheep - Total screened - 16		
Kit 1	83.3	90.0
Kit 2	33.3	60.0
Kit 3	-	90.0
b. Goats - Total screened - 55		
Kit 1	45.4	70.0
Kit 2	13.6	93.9
Kit 3	4.5	96.9
c. Sheep and goats; Total screened - 71		
Kit 1	53.7	86.0
Kit 2	17.8	86.0
Kit 3	3.5	94.7

Table 10 Prevalence of Johne's disease (MAP infection) in goats in India.

Sn.	Test used	Prevalence (%)	Reference
1	ELISA	13.5	Goswami et al., ³
	CIE	8.5	
	AGID	20.0	
2	Tissues culture	3.5	Tripathi & Parihar ²³
3	Tissues culture	27.2	Kumar et al., ⁴
4	Milk culture (sediment)	0.5	Singh & Vihan, ²⁴
5	Milk culture	69.8	Kumar et al., ²⁵
6	Fecal culture	44.8	Vihan et al., ⁵
7	Fecal culture	23.0	Singh et al., ²⁶
8	Fecal culture	58.0	Kumar et al., ⁴
9	Serum ELISA (<2 months kids)	8.5	Singh et al., ¹⁶
10	Serum ELISA	33.3	Singh et al., ²¹
11	Milk ELISA	53.7	Kumar et al., ²⁵
12	Microscopic Examination	38.0	Kumar et al., ⁴
13	Microscopic Examination *	46.4	Gupta et al., ²⁶
14	Microscopic Examination	18.0	Singh & Vihan, ²⁸⁷
15	Microscopic Examination	4.1	Singh et al., ²⁸

* Goats and sheep

1 had substantial PA value as compared to moderate in kit 2 and 3 in goats and sheep. In goats, PA value was substantial in kit 1 and 2 and moderate in kit 3. However, in sheep, kit 1 had almost perfect PA value as compared to moderate and slight in kit 2 and 3, respectively. The study clearly showed the superiority of indigenous ELISA kit over imported commercial purified PPA (kit 2) and imported and commercial ELISA kit (kit 3). Though kit 1 was based on homologous host (MAP 'bison type' of goat origin), but it performed much better in sheep (table 4 and 9). This may be due to high sensitivity of present fecal culture test (standardized in our lab) in goats or higher susceptibility of goats to MAP 'Bison type' strain as compared to sheep. The specificity of kit 1 can be increased in goats by employing adsorption step (*M.phlei*), as used in commercial kits. The higher susceptibility of goats as compared to sheep located at CIRG, Mathura, has been reflected in the production and health data over the years. Sheep flock had significantly high growth rate and low morbidity and mortality as compared to goats herds located at CIRG, Mathura. Type 2 sero-reactors in general had poor correlation with culture in all the 3 kits and therefore, not suitable for Indian conditions. Salgado et al²² compared serum and milk ELISA kit (IDEXX laboratories Inc.) with fecal culture and reported 64.0 and 48.0% sensitivity, respectively and 100.0% specificity in both the cases, in goats. The 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. However, indigenous kit 1 had comparable sensitivity and specificity with IDEXX kit, without adsorption of serum with *M. phlei*. Positives and low positives animals can be marked as 'high and low risk groups' and will need repeated screenings. In S/P ratios of kit 1, 2 and 3 showed that animals in negative and suspected category were also shedding bacilli, however, it had low correlation with kit 1 as compared to kit 2 and 3. In the present study, of the 3 kits, kit 1 using indigenous PPA of native MAP 'Bison type' had shown better efficacy. Use of antigen at very low quantities (0.1 µgm / well), also reduced the chances of cross reactivity with other mycobacterial antigens. Individually though culture was superior test for diagnosis of JD, however, culture and kit 1 had comparable efficacy (Table 6, 7 and 8). Whereas in goats, after culture kit 1 using PPA of goat origin was superior to kit 2 and 3, for the screening of JD. Sensitivity of ELISA is dependent on the antigen and as found in this study, species-specific antigen has been recommended by many workers. MAP 'Bison Type' has been reported both from goats and sheep population¹¹, may be reason for better efficacy of kit 1 in diagnosing Johne's disease.

Conclusion

Newly developed indigenous ELISA test (kit 1) was significantly superior for the screening of goatherds and sheep

flocks against JD as compared to commercial ELISA kits (Kit 2 and 3). In comparison to kit 2 and 3, kit 1 had highest sensitivity, comparable specificity and substantial to nearly perfect proportional agreement (Kappa Scores) with respect to 'Gold standard', fecal culture in goats and sheep. Using sensitive fecal culture and ELISA kit, study showed high prevalence of Johne's disease in farm goatherds and sheep flocks. Since disease is endemic in herds and flocks, using ELISA kits, in comparison to Type II sero-reactors, Type 1 sero-reactors had better correlation with fecal culture and therefore, used for estimation of sero-prevalence. Study underlined the need for large-scale screening of domestic ruminants to estimate the prevalence of JD and subsequent control.

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