Mukartal et al., *Cogent Food & Agriculture* (2017), 3: 1298391
http://dx.doi.org/10.1080/23311932.2017.1298391

cogent food & agriculture

ANIMAL HUSBANDRY & VETERINARY SCIENCE | RESEARCH ARTICLE

Assessment of Ovine Johne's disease in the Mandya sheep breed in South India using multiple diagnostic tests and bio-typing of *Mycobacterium avium* subspecies *paratuberculosis* infection

Shivalingappa Yamanappa Mukartal[1], Doddamane Rathnamma[1], Hogalagere Doddappaiah Narayanaswamy[2], Saurabh Gupta[3], Kundan Kumar Chaubey[3], Manju Singh[3], Zahra Hemati[4], Chikkahonnaiah Nishanth[5], Anjali Pachoori[3], Kuldeep Dhama[6] and ShoorVir Singh[3]*

Received: 30 June 2016
Accepted: 01 January 2017
First Published: 15 March 2017

*Corresponding author: ShoorVir Singh, Microbiology Laboratory, Animal Health Division, Central Institute for Research on Goats, Makhdoom, PO-Farah, Mathura 281122, Uttar Pradesh, India
E-mail: shoorvir.singh@gmail.com

Reviewing editor:
Pedro González-Redondo, University of Seville, Spain

Additional information is available at the end of the article

Abstract: Johne's disease is major infectious disease of domestic livestock in India. Despite low per animal productivity, the country is yet to initiate plan for the survey and control of disease at National scale. A total of 81 clinical samples from Mandya breed of sheep were collected from an organised farm (Livestock Research and Information Centre, Nagmangala) and farmer's flocks suspected for Johne's disease

ABOUT THE AUTHORS

Shivalingappa Yamanappa Mukartal, Assistant Professor, Department of Veterinary Microbiology, KVAFSU, Bengaluru, India.

Doddamane Rathnamma, Professor and Head, Department of Veterinary Microbiology, KVAFSU, Bengaluru, India.

Hogalagere Doddappaiah Narayanaswamy, Professor, Department of Veterinary Patahlogy, KVAFSU, Bengaluru, India.

Saurabh Gupta, RA, Microbiology Laboratory, Central Institute for Research on Goats, Makhdoom, Mathura, Uttar Pradesh, India.

Kundan Kumar Chaubey, RA, Microbiology Laboratory, Central Institute for Research on Goats, Makhdoom, Mathura, Uttar Pradesh, India.

Manju Singh, JRF, Microbiology Laboratory, Central Institute for Research on Goats, Makhdoom, Mathura, Uttar Pradesh, India.

Zahra Hemati, PhD Scholar, Department of Pathobiology, Shiraz University, Shiraz, Iran.

Chikkahonnaiah Nishanth, Assistant Professor, Department of Veterinary Public Health & Epidemiology, KVAFSU, Bengaluru, India.

Anjali Pachoori, Microbiology Laboratory, Animal Health Division, Central Institute for Research on Goats, Makhdoom, Mathura, Uttar Pradesh, India.

Kuldeep Dhama, Principal Scientist, Department of Pathology, Indian Veterinary Research Insitute, Izatnagar, Bareilly, Uttar Pradesh, India.

ShoorVir Singh, Principal Scientist & Head, Microbiology Laboratory, Animal Health Division, Central Institute for Research on Goats, Makhdoom, Mathura, Uttar Pradesh, India.

PUBLIC INTEREST STATEMENT

The present study give the information about the high incidence of Ovine Johne's disease in the Mandya sheep breed in South India. The prime interests of the authors were to sensitize the farmers and policy makers regarding there is immediate need for a Nationwide program for the control of Ovine Johne's disease in the country in order to reduce the production losses and frequent passage of MAP in the domestic livestock species (inter-species transmission) and to human population through food chain, specially milk and milk products. As conclusion the authors suggested screening of all the animals before introducing in to the flock with multiple tests should be mandatory. Utility of multiple diagnostic tests is suggested for confirmatory detection and epidemiological diseases investigations of Johne's disease in animals.

on the basis of clinical symptoms (weakness, emaciation, diarrhea and alopecia). Only 10.0% samples from two farms were screened for bio-load of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) using multiple tests (microscopy, indigenous ELISA, IS*900* PCR and culture). Results showed that 100.0% samples were positive for MAP infection both in microscopy and "Indigenous ELISA". IS*1311* PCR-REA bio-typed 60.0% (feces) and 100.0% (blood and tissue) isolates as "Indian Bison Type". Typing investigated for the first time that biotype was of Indian origin and present in sheep flocks of South India having wide geographical distribution and broad host range. Study reported high bio-load of MAP in Mandya breed of sheep in Nagamangala, Mandya district and Bilagi taluk of Bagalkot district in South India. Being Johne's disease a spectral disease, multiple tests were useful for the screening of MAP infection in sheep farms. Study emphasised the need for initiation of short and long term control programs to reduce production losses and sharing of MAP strains by other domestic livestock species and prevent contamination of human food chain and reduce risk to human population.

Subjects: Microbiology; General Science; Epidemiology

Keywords: *Mycobacterium avium* subspecies *paratuberculosis*; Ovine Johne's disease; Mandya breed; Indigenous ELISA; IS*900* PCR; culture

1. Introduction

Ovine Johne's disease is a chronic incurable inflammation of intestines caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Though considered primarily a disease of domestic ruminants, MAP infection has been reported from wide range of host species such as wild ruminants, primates and human (Singh, Kumar et al., 2014; Singh, Singh, Singh, et al., 2013; Singh, Singh, Singh, & Sohal, 2010; Singh, Singh, et al., 2014). Disease leads to significant economic losses to the dairy, meat and wool industry world-wide (Bauman, Jones, Menzies, Jansen, & Kelton, 2014; Chiodini, VanKruiningen, & Merkal, 1984; Norton, Johnson, Jones, & Heuer, 2010; Stau, Seelig, Walter, Schroeder, & Ganter, 2012) including India (Rawat, et al., 2014; Sonawane & Tripathi, 2013; VinodhKumar, Gunaseelan, Ronald, & Sakthivelan, 2013).

Variable prevalence (15.1–36.0%) of Ovine Johne's disease has been reported using traditional tests for diagnosis of Johne's disease (Gupta, Rani, Agrawal, & Kumar Gupta, 2012; Singh et al., 2009; VinodhKumar et al., 2013). However, Collins et al. (2005) suggested that no single diagnostic test has been recommended as a confirmatory test for the screening of herds. Based on IS*1311* PCR-REA, MAP strains have been genotyped as "Cattle type", "Sheep type" and "Bison type" (Sevilla, et al., 2005; Whittington, Marsh, & Whitlock, 2001). There is only one report of "Bison type" strain isolated from bison in Montana, USA (Whitlock, Sweeney, Fyock, & Smith, 2005). From India a new biotype, "Indian Bison type" has been reported by Sevilla et al. (2005) from goats and sheep. Later this new bio-type has been found to be highly pathogenic to native livestock population and has widest host range (Singh, Singh, Singh, et al., 2010; Singh, Singh et al., 2014). It has been shown that virulence of MAP strains was 'bio-type dependent' (Gollnick et al., 2007; Hajra, Singh, Srivastava, Chakraborty, & Dhama, 2005).

Sharing of MAP bio-types between species (inter-species transmission) has been frequently reported (Sohal et al., 2008). Earlier studies in India, invariably reported endemicity of Johne's disease in the livestock population and in established sheep farms primarily located in North India (Singh, Singh, Singh, et al., 2013; Singh, Singh et al., 2014).

cogent • food & agriculture

Data presented in this study is not based on random sampling of the domestic livestock species; therefore, the term "Prevalence" was not used. Since the information is based on screening of animals suspected of suffering from JD in naturally infected research farm and farmer's flocks, therefore, to represent the probable level of disease in animals, the term "Bio-load" was used. The present study was undertaken to estimate bio-load of Johne's disease in Mandya breed of sheep at Livestock Research and Information Centre (LRIC), Karnataka Veterinary and Fisheries Science University (KVAFSU) Nagmangala farm and local breed of sheep in Bagalkot region of Karnataka state in South India. This is first comprehensive study to screen the clinical samples (tissue, blood, faeces, serum and milk) from suspected population of Mandya breed of sheep of Mandya district, Karnataka in South India, for the diagnosis of MAP infection using standard diagnostic tests (Microscopy, culture, PCR and "Indigenous ELISA kit").

2. Material and methods

2.1. Collection of samples

During April 2015 a total of 81 clinical samples (10% samples of total farms) including feces (25), blood (2), serum (52), milk (1) and tissue samples (1) were randomly collected from suspected animals showing clinical symptoms of Johne's disease (weakness, emaciation, diarrhea and alopecia) from two farms, an research farm organised farm (LRIC, KVAFSU, Nagmangala) and farmer's sheep flocks (Bilagi taluk, Bagalkot) in India. Samples were sent to Microbiology laboratory at CIRG, Makhdoom and stored at −20°C till further processing.

2.2. Culture

Herrold's egg yolk medium (HEYM) with mycobactin J was used for the culture of MAP from tissues as per Whipple, Callihan, and Jarnagin (1991), with some modifications (Singh, 1998). Approximately 2 g of fecal and tissue sample was finely grounded in sterilized pestle and mortar in 12 ml normal saline solution (NSS). Grounded material (12 ml) was centrifuged at 4,500 rpm for 45 min at room temperature. Supernatant was discarded and middle layer was decontaminated in 40 ml of 0.9% hexadecyl pyridinium chloride (HPC) for 18–24 h at room temperature. After decontamination and sedimentation, supernatant was removed slowly and the sediment was inoculated on the slants of HEYM with mycobactin J slants were incubated at 37°C and screened for the appearance of colonies every 2 weeks.

2.3. Fecal microscopy

Shedding of MAP in fecal samples was monitored by microscopy as per Singh, Singh, Gupta et al. (2013). Shedding of MAP was measured as quantitative (+1 as low shedders, +2, +3 as moderate shedders and +4 as heavy shedders) and any animal that was found positive (+1 to +4 scale) was taken as positive for MAP infection.

2.4. Molecular characterization and genotyping

2.4.1. IS900 PCR

DNA was isolated from the feces, blood, tissue and milk samples and subjected to specific IS*900* PCR as per Singh, Singh, Kumar, Sohal, and Singh (2010) using primers used by Vary, Andersen, Green, Hermon-Taylor, and McFadden (1990). Presence and yield of the specific PCR product (229 bp) was considered as positive for MAP infection.

2.4.2. IS1311 PCR_REA

IS*1311* PCR was carried out using M56 and M119 primers as per Singh, Singh, Kumar, et al. (2010). Restriction digestion (IS*1311* PCR_REA) reaction was carried out using endonucleases *HinfI* and *MseI* as per Sevilla et al. (2005). Genotype profiles were interpreted as per Whittington et al. (2001).

cogent food & agriculture

Table 1. Values of sample to positive (S/P) ratios and corresponding status of Johne's disease

S. No	Calculated value of S/P ratio	Johne's disease status in animal
1	0.00–0.09	Negative
2	0.10–0.24	Suspected or borderline
3	0.25–0.39	Low positive
4	0.4–0.99	Positive
5	1.0–10.0	Strong positive

Note: Serum samples in positive and strong positive categories were taken as positive.

2.5. Indigenous ELISA

Serum samples collected during the study were screened by using "Indigenous ELISA kit", developed for screening of goats and sheep against Johne's disease infection. Indigenous ELISA kit uses semi-purified protoplasmic antigen (PPA) from the highly virulent native isolate of MAP "Indian Bison type" biotype of goat origin. The test was originally developed and standardized in goats and sheep (Singh, Singh, Singh, Sohal, & Singh, 2007) and was also standardized in bovines (Singh, Singh, et al., 2015). Optical density values were transformed to Sample to Positive (S/P) ratio and animals in positive and strong positive categories in S/P ratio were considered as positive for MAP infection or sero-converts (Collins, 2002).

2.5.1. Analysis of OD (absorbance) values

Sample to Positive (S/P) ratio was calculated as follows (Table 1):

$$\text{S/P ratio value} = [(\text{Sample optical density} - \text{Negative optical density}) / (\text{Positive optical density Negative optical density})]$$

3. Results

Out of the 52 serum samples screened, all (100.0%) sheep were positive for MAP antibodies using goat based "Indigenous ELISA kit". In Indigenous ELISA kit, 41 (78.8%) and 11 (21.2%) sheep were in positive and strong positive categories, respectively (Table 2). Screening of fecal samples showed that all (100.0%) of the sheep were shedding MAP bacilli as revealed by microscopy (Table 3). Fecal, blood, milk and tissue samples were subjected to screening by IS*900* PCR and 100.0% were positive for MAP infection (Table 3). Bio-typing of positive isolates by IS*1311* PCR-REA categorized 60.0% (fecal samples) and 100.0% (tissue and blood samples) as "Indian Bison type", bio-types (Table 3). In three tests combinations; 16 (59.2%) sheep were positive in all the three tests (IS*900* PCR, Microscopy and IS*1311* PCR-REA), 11 (40.7%) were positive in IS*900* PCR and Microscopy (Table 4). Typical MAP colonies (multi-bacillary) started appearing from 60 days onwards and maximum colonies appeared around 90 days post inoculation on HEYM (Figure 1). Initially, colonies were very small pinpoint size and were white, rounded in appearance (Figure 1).

Table 2. Bio-load of Ovine Johne's disease in Mandya breed of sheep from Livestock Research Information Centre, Karnataka Veterinary Animal Fisheries Sciences University, Nagmangala farm and Bagalkot region of District Bengaluru (India) using goat based indigenous-ELISA (*n* = 52)

ELISA test	Test results	
Categories	**Positive (P)**	**Strong Positive (SP)**
Sub-total	41 (78.8)	11 (21.2)
Total	52 (100%)	

Note: Serum samples in positive (P) and strong positive (SP) categories were taken as positive.

Table 3. Screening of clinical and necropsy samples for the diagnosis of *Mycobacterium avium* subspecies *paratuberculosis* infection in the Mandya breed of sheep from Livestock Research Information Centre, Karnataka Veterinary Animal Fisheries Sciences University, Nagmangala farm and Bagalkot region (India)

Samples	Positives *n* (%)		
	Microscopy	IS*900* PCR	IS*1311* PCR-REA
Faeces (*n* = 25)	25 (100.0)	25 (100.0)	15 (60.0)
Tissue (*n* = 1)	1 (100.0)	1 (100.0)	1 (100.0)
Milk (*n* = 1)	1 (100.0)	1 (100.0)	0 (0.0)
Blood (*n* = 2)	ND	2 (100.0)	2 (100.0)
Total (*n* = 29)	27 (100.0)	29 (100.0)	18 (62.0)

Note: 27/27= 100.0%, because two blood microscopy samples were not done

Table 4. Comparative study of different tests for the detection of *Mycobacterium avium* subspecies *paratuberculosis* in 27 samples (faeces, milk and tissue) of sheep from Livestock Research Information Centre, Karnataka Veterinary Animal Fisheries Sciences University, Nagmangala farm and Bagalkot region (India)

Diagnostic tests	Test combinations							
	1	2	3	4	5	6	7	8
Microscopy	+	–	+	–	+	+	–	–
IS*900* PCR	+	–	+	+	–	–	+	–
IS*1311* PCR-REA	+	–	–	+	+	–	–	+
Total (*n* = 27)	16 (59.2)	0 (0.0)	11 (40.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Notes: Figures in parenthesis are percentages. + = positive in test, – = Negative in test.
1 to 8 are maximum permutation and combinations possible in 3 test regimen.

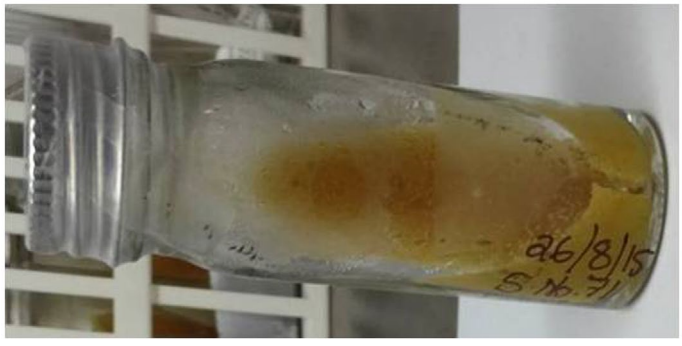

Figure 1. *Mycobacterium avium* subspecies *paratuberculosis* colonies on HEY medium.

4. Discussion

Like many other developing and un-developed countries, in India also, sampling of desired animals based on random table number is one of the biggest constrains. Therefore, information on the National prevalence of Ovine Johne's disease and losses caused by disease, as available in developed countries, is extremely limited. In these conditions it is not easy to present easy picture of diagnosis especially in chronic infections like Johne's disease, where clinical symptoms are also not specific; therefore it is prudent to use multiple screening tests.

In the present study, fecal microscopy and "Indigenous ELISA kit" were used as screening tests. IS*900* PCR and IS*1311* PCR were used as confirmatory tests. Limited numbers of samples were bio-typed (IS*1311* PCR_RE) to know MAP bio-type infecting Mandya sheep. This study reports high bio-load of MAP infection in the suspected population of Mandya breed of sheep belonging to Nagmangala KVAFSU farm and Bagalkot region of South India. Higher bio-load of MAP directly correlated with low

per animal productivity and increased risk of human exposure (VinodhKumar et al., 2013). Present study reported 100.0% bio-load of MAP infection in suspected sheep population (farm and farmer's flocks) by microscopy, "Indigenous ELISA kit" and IS*900* PCR. High incidence of MAP as reported in the present study may be due to high endemicity of disease and screening of limited number of suspected sheep. In bio-typing using IS*1311*-REA, "Indian Bison Type" strain of MAP was found the dominant biotype in the flocks studied. Singh, Singh et al. (2014) also reported high (32.7%) bio-load of MAP in sheep population in the last 28 years (1985 to 2013) of survey in India. "Indian Bison Type" was also reported earlier as the dominant biotype, irrespective of domestic livestock species screened and the geographical region surveyed (Singh, Chauhan et al., 2015). Recent study by Sonawane and Tripathi (2013) reported 100.0% sensitivity for detection of MAP in sheep with the help of quantitative real-time PCR (qPCR) assay employing IS*900* gene specific primers of MAP. Earlier, in sheep, prevalence of MAP was found to be moderate in sheep from Tamil Nadu as compared to Rajasthan (Singh, Singh, Gupta et al., 2013). Unlike holy cows, buffaloes, goats and sheep undergo high rate of slaughter in order to meet ever growing demand for meat. This high rate of slaughter indirectly result in "natural selection" in these three livestock species by regular removal of low productive animals (personal experience of corresponding author). Moreover, similar findings related to higher incidence of MAP have been reported in the four4 domestic livestock species in different regions of the country using multiple screening tests (Barad et al., 2013; Gupta et al., 2012; Shroff et al., 2013; Singh, Singh, Singh, et al., 2013; Singh, et al., 2008). In the four diagnostic tests used for the screening of sheep against MAP infection, microscopy was found to be more sensitive because shedding was often seen prior to peripheral immune response (Lybeck, Storset, Djønne, Valheim, & Olsen, 2011). However, multiple tests were found to be useful for the screening of MAP infection in domestic livestock of the country. High bio-load of MAP in livestock was directly associated with low productivity of domestic livestock in India, which in turn increased the chances of spread to human population (Singh, Singh, Gupta et al., 2013). Hence, there is immediate need for a Nationwide program for the control of Ovine Johne's disease in the country in order to reduce the production losses and frequent passage of MAP in the domestic livestock species (inter-species transmission) and to human population through food chain, specially milk and milk products. This is the first exploratory study reporting high bio-load of MAP infection in the Mandya breed of sheep in South India. Similar to the findings in North India by Singh, Singh et al. (2014) "Indian Bison Type" bio-type was the dominant bio-type infecting Mandya breed of sheep flocks in South India.

Acknowledgement
Authors are thankful to Indian Council of Agricultural Research (ICAR) and Karnataka Veterinary Animal and Fisheries Sciences University (KVAFSU), for provision of funding and facilities through ICAR-NAE project "Animal Disease Registry and Tissue Bank", Department of Veterinary Pathology, Veterinary College, Hebbal, Bengaluru.

Funding
This work was supported by Indian Council of Agricultural Research, New Delhi.

Competing Interests
The authors declare no competing interest.

Author details
Shivalingappa Yamanappa Mukartal[1]
E-mail: mukartal@rediffmail.com
ORCID ID: http://orcid.org/0000-0002-2094-280X
Doddamane Rathnamma[1]
E-mail: doddamanerathnamma@yahoo.co.in
Hogalagere Doddappaiah Narayanaswamy[2]
E-mail: drhdnswamy9@gmail.com
Saurabh Gupta[3]
E-mail: saurabhbiotech12@gmail.com
Kundan Kumar Chaubey[3]
E-mail: kundan2006chaubey@gmail.com
ORCID ID: http://orcid.org/0000-0001-7469-4752
Manju Singh[3]
E-mail: manjuchaudhary080191@gmail.com
Zahra Hemati[4]
E-mail: z.hemati28@gmail.com
Chikkahonnaiah Nishanth[5]
E-mail: biotechnology.saurabh@gmail.com
Anjali Pachoori[3]
E-mail: pachoori.anjali81191@gmail.com
Kuldeep Dhama[6]
E-mail: kdhama@rediffmail.com
ORCID ID: http://orcid.org/0000-0001-7469-4752
ShoorVir Singh[3]
E-mail: shoorvir.singh@gmail.com

[1] Department of Veterinary Microbiology, Veterinary College, KVAFSU, Hebbal, Bengaluru, India.
[2] Department of Veterinary Pathology, Veterinary College, KVAFSU, Hebbal, Bengaluru, India.
[3] Microbiology Laboratory, Animal Health Division, Central Institute for Research on Goats, Makhdoom, PO-Farah, Mathura 281122, Uttar Pradesh, India.
[4] Department of Pathobiology, Shiraz University, Shiraz, Iran.
[5] Department of Veterinary Public Health & Epidemiology, Veterinary College, KVAFSU, Hebbal, Bengaluru, India.
[6] Department of Pathology, Indian Veterinary Research Insitute, Izatnagar, Bareilly, Uttar Pradesh, India.

Citation information
Cite this article as: Assessment of Ovine Johne's disease in the Mandya sheep breed in South India using multiple diagnostic tests and bio-typing of *Mycobacterium avium* subspecies *paratuberculosis* infection, Shivalingappa Yamanappa Mukartal, Doddamane Rathnamma, Hogalagere Doddappaiah Narayanaswamy, Saurabh Gupta, Kundan Kumar Chaubey, Manju Singh, Zahra Hemati, Chikkahonnaiah Nishanth, Anjali Pachoori, Kuldeep Dhama & ShoorVir Singh, *Cogent Food & Agriculture* (2017), 3: 1298391.

References

Barad, D. S., Chandel, B. S., Dadawala, A. I., Chauhan, H. C., Kher, H. S., Shroff, S., ... Chaubey, K. K. (2013). Comparative potential of traditional versus modern diagnostic tests in estimating incidence of Caprine Johne's disease. *Advances in Animal and Veterinary Sciences, 1*, 35–40.

Bauman, C., Jones, B. A., Menzies, P., Jansen, J., & Kelton, D. (2014). Evaluation of seven paratuberculosis diagnostic tests in the dairy goat and dairy sheep industries of Ontario, Canada. *Proceedings of the 12th International Colloquium on Paratuberculosis, 57*, 169–175.

Chiodini, R. J., VanKruiningen, H. J., & Merkal, R. S. (1984). Ruminant paratuberculosis (Johne's disease): The current status and future prospects. *Cornell Veterinarian, 74*, 218–262.

Collins, M. T. (2002). Interpretation of a commercial bovine paratuberculosis enzyme-linked immunosorbent assay by using likelihood ratios. *Clinical and Diagnostic Laboratory Immunology, 9*, 1367–1371. http://dx.doi.org/10.1128/CDLI.9.6.1367-1371.2002

Collins, M. T., Wells, S. J., Petrini, K. R., Collins, J. E., Schultz, R. D., & Whitlock, R. H. (2005). Evaluation of five antibody detection tests for diagnosis of bovine paratuberculosis. *Clinical and Diagnostic Laboratory Immunology, 12*, 685–692.

Gollnick, N. S., Mitchell, R. M., Baumgart, M., Janagama, H. K., Sreevatsan, S., & Schukken, Y. H. (2007). Survival of *Mycobacterium avium subsp. paratuberculosis* in bovine monocyte-derived macrophages is not affected by host infection status but depends on the infecting bacterial genotype. *Veterinary Immunology and Immunopathology, 120*, 93–105. http://dx.doi.org/10.1016/j.vetimm.2007.07.017

Gupta, A., Rani, S. M., Agrawal, P., & Gupta, P. K. (2012). Sero-prevalence of paratuberculosis (Johne's Disease) in cattle population of South-Western Bangalore Using ELISA Kit. *Open Journal of Veterinary Medicine, 2*, 196–200. http://dx.doi.org/10.4236/ojvm.2012.24031

Hajra, S., Singh, S. V., Srivastava, A. K., Chakraborty, S., & Dhama, K. (2005). Pathobiology of spontaneous and experimental paratuberculosis (S-5 strain) in goats with special reference to early lesions. *Asian Journal of Animal and Veterinary Advances, 9*, 467–475.

Lybeck, K. R., Storset, A. K., Djønne, B., Valheim, M., & Olsen, I. (2011). Faecal shedding detected earlier than immune responses in goats naturally infected with *Mycobacterium avium* subsp. *paratuberculosis*. *Research in Veterinary Science, 91*, 32–39. http://dx.doi.org/10.1016/j.rvsc.2010.08.012

Norton, S., Johnson, W. O., Jones, G., & Heuer, C. (2010). Evaluation of diagnostic tests for Johne's Disease (*Mycobacterium Avium* subspecies *paratuberculosis*) in New Zealand dairy cows. *Journal of Veterinary Diagnostic Investigation, 22*, 341–351.

Rawat, K. D., Chaudhary, S., Kumar, N., Gupta, S., Chaubey, K. K., Singh, S. V., ... Deb, R. (2014). Economic losses in a commercial dairy farm due to the outbreak of Johne's disease in India. *Research Journal for Veterinary Practitioners, 2*, 73–77. http://dx.doi.org/10.14737/journal.rjvp

Sevilla, I., Singh, S. V., Garrido, J. M., Aduriz, G., Rodriguez, S., Geijo, M. V., ... Juste, R. A. (2005). Molecular typing of *Mycobacterium avium subspecies paratuberculosis* strains from different hosts and regions. *Revue Scientifique et Technique de l'OIE, 24*, 1061–1066. http://dx.doi.org/10.20506/rst.issue.24.3.30

Shroff, S., Chandel, B. S., Dadawala, A. I., Singh, S. V., Bhagat, A. G., Chauhan, H. C., Gupta, S., & Chaubey, K. K. (2013). Evaluation of an Indigenous vaccine based on goat adapted *Mycobacterium avium* subspecies *paratuberculosis* in patanwadi breed of sheep naturally infected with clinical Johne's disease in north Gujarat. *Research Opinion in Animal & Veterinary Sciences, 3*, 322–329.

Singh, A. V., Chauhan, D. S., Singh, A., Singh, P. K., Sohal, J. S., & Singh, S. V. (2015). Application of IS*1311* locus 2 PCR REA assay for the specific detection of 'Bison type' *Mycobacterium avium* subspecies *paratuberculosis* isolates of Indian origin. *Indian Journal of Medical Research, 141*, 55–61.

Singh, A. V., Singh, S. V., Singh, P. K., & Sohal, J. S. (2010). Genotype diversity in Indian isolates of *Mycobacterium avium* subspecies *paratuberculosis* recovered from domestic and wild ruminants from different agro-climatic regions. *Comparative Immunology, Microbiology and Infectious Diseases, 33*, e127–e131.

Singh, A. V., Singh, S. V., Singh, P. K., Sohal, J. S., Swain, N., Rajindran, A. S., & Vinodh, O. R. (2009). Multiple tests based prevalence estimates of *Mycobacterium avium* subspecies *paratuberculosis* infection in elite farms of goats and sheep. *Indian Journal of Small Ruminant, 15*, 178–182.

Singh, P. K., Singh, S. V., Kumar, H., Sohal, J. S., & Singh, A. V. (2010). Diagnostic application of IS900 PCR using blood as a source sample for the detection of *Mycobacterium avium* subspecies *paratuberculosis* in early and subclinical cases of caprine paratuberculosis. *Veterinary Medicine International, 2010*, 1–8.

Singh, S. V. (1998). *Diagnosis of paratuberculosis in goats* (Ph.D. thesis). Submitted to CSA University of Agriculture & Technology, Kanpur, India.

Singh, S. V., Kumar, N., Sohal, J. S., Singh, A. V., Singh, P. K., Agrawal, N. D., ... Rawat, K. D. (2014). First mass screening of the human population to estimate the bio-load of *Mycobacterium avium* subspecies *paratuberculosis* in North India. *Journal of Biological Sciences, 14*, 237–247. http://dx.doi.org/10.3923/jbs.2014.237.247

Singh, S. V., Singh, A. V., Singh, P. K., Gupta, S., Singh, H., Singh, B., ... Sohal, J. S. (2013). Evaluation of 'Indigenous vaccine' developed using 'Indian Bison Type' genotype of *Mycobacterium avium* subspecies *paratuberculosis* strain 'S5' of goat origin in a sheep flock endemic for Johne's disease: A three years trial in India. *World Journal of Vaccines, 3*, 52. http://dx.doi.org/10.4236/wjv.2013.32009

Singh, S. V., Singh, A. V., Singh, P. K., Sohal, J. S., & Singh, N. P. (2007). Evaluation of an indigenous ELISA for diagnosis of Johne's disease and its comparison with commercial kits. *Indian Journal of Microbiology, 47*, 251–258. http://dx.doi.org/10.1007/s12088-007-0046-2

Singh, S. V., Singh, A. V., Singh, R., Sharma, S., Shukla, N., Mishra, S., ... Sandhu, K. S. (2008). Sero-prevalence of Bovine Johne's disease in buffaloes and cattle population of North India using indigenous ELISA kit based on native *Mycobacterium avium* subspecies *paratuberculosis* Bison type genotype of goat origin. *Comparative Immunology, Microbiology and Infectious Diseases, 31*, 419–433. http://dx.doi.org/10.1016/j.cimid.2007.06.002

Singh, S. V., Singh, P. K., Gupta, S., Chaubey, K. K., Singh, B. , Kumar, A., ... Kumar, N. (2013). Comparison of microscopy and blood-PCR for the diagnosis of clinical Johne's disease in domestic ruminants. *Iranian Journal Of Veterinary Research, 14*, 345–349.

Singh, S. V., Singh, P. K., Kumar, N., Gupta, S., Chaubey, K. K., Singh, B., … Dhama, K. (2015). Evaluation of goat based 'Indigenous vaccine' against Bovine Johne's Disease in endemically infected native cattle herds. *Indian Journal of Experimental Biology, 53*, 16–24.

Singh, S. V., Singh, P. K., Singh, A. V., Sohal, J. S., Kumar, N., Chaubey, K. K., … Dhama, K. (2014). 'Bio-load' and bio-type profiles of *Mycobacterium avium* subspecies *paratuberculosis* infection in the domestic livestock population endemic for Johne's disease: A survey of 28 years (1985-2013) in India. *Transboundary and Emerging Diseases, 61*, 43–55. http://dx.doi.org/10.1111/tbed.12216

Sohal, J. S., Singh, S. V., Tyagi, P., Subhodh, S., Singh, P. K., Singh, A. V., … Singhsandhu, K. (2008). Immunology of mycobacterial infections: With special reference to *Mycobacterium avium* subspecies *paratuberculosis*. *Immunobiology, 213*, 585–598. http://dx.doi.org/10.1016/j.imbio.2007.11.002

Sonawane, G. G., & Tripathi, B. N. (2013). Comparison of a quantitative real-time polymerase chain reaction (qPCR) with conventional PCR, bacterial culture and ELISA for detection of *Mycobacterium avium* subsp. *paratuberculosis* infection in sheep showing pathology of Johne's disease. *SpringerPlus, 2*, 45–53. http://dx.doi.org/10.1186/2193-1801-2-45

Stau, A., Seelig, B., Walter, D., Schroeder, C., & Ganter, M. (2012). Seroprevalence of *Mycobacterium avium* subsp. *paratuberculosis* in small ruminants in Germany. *Small Ruminant Research, 105*, 361–365. http://dx.doi.org/10.1016/j.smallrumres.2012.03.008

Vary, P. H., Andersen, P. R., Green, E., Hermon-Taylor, J., & McFadden, J. J. (1990). Use of highly specific DNA probes and the polymerase chain reaction to detect *Mycobacterium avium* ssp. *paratuberculosis* in Johne's disease. *Journal of Clinical Microbiology, 28*, 933–937.

VinodhKumar, O. R., Gunaseelan, L., Ronald, B. S., & Sakthivelan, S. M. (2013). Slaughterhouse prevalence of ovine paratuberculosis in Southern India. *Tropical Animal Health and Production, 45*, 1063–1069. http://dx.doi.org/10.1007/s11250-012-0321-z

Whipple, D. L., Callihan, D. R., & Jarnagin, J. L. (1991). Cultivation of *Mycobacterium paratuberculosis* from bovine fecal specimens and a suggested standardized procedure. *Journal of Veterinary Diagnostic Investigation, 3*, 368–373. http://dx.doi.org/10.1177/104063879100300424

Whitlock, R. H., Sweeney, R. W., Fyock, T., & Smith, J. (2005). MAP super-shedders: another factor in the control of Johne's disease. *8th International Colloquium on Paratuberculosis* (p. 164), Copenhagen.

Whittington, R. J., Marsh, I. B., & Whitlock, R. H. (2001). Typing of IS 1311 polymorphisms confirms that bison (*Bison bison*) with paratuberculosis in Montana are infected with a strain of *Mycobacterium avium* subsp. *paratuberculosis* distinct from that occurring in cattle and other domesticated livestock. *Molecular and Cellular Probes, 15*, 139–145. http://dx.doi.org/10.1006/mcpr.2001.0346

Cogent Food & Agriculture **(ISSN: 2331-1932) is published by Cogent OA, part of Taylor & Francis Group.**