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Determination of sero-prevalence of paratuberculosis (Johne's disease) in sheep (*Ovis aries*) in Bikaner district of Rajasthan

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Abstract
Paratuberculosis is an infectious disease of ruminants caused by *Mycobacterium avium ssp.* Paratuberculosis (MAP). This bacterium causes severe granulomatous enteritis, resulting in wasting in small ruminants and diarrhoea in about 20%of case in the end stage of the disease. In view of the epidemiology of Paratuberculosis in India the topic on the prevalence of this disease in the sheep population of Bikaner District of Rajasthan state was carried out. Age-wise and Sex-wise prevalence of Paratuberculosis carried out along with the overall prevalence of the disease using Indigenous ELISA. A total number of 100 sheep sera samples were screened. The age-wise prevalence was detected as 18.75 percent in less than 6-month age group, 21.05 percent in 6-12 months age group and 43.75 percent in more than 12 months age group. Sex-wise sero-prevalence was detected as 18 percent and 13 percent in male and female sheep respectively. An overall seroprevalence of Paratuberculosis was found to be 31 percent. The current findings revealed that Paratuberculosis infections is more prevalent in Bikaner District of Rajasthan.

Keywords: Paratuberculosis, sero-prevalence, indigenous ELISA

Introduction
As per 20th Livestock census (2019) [1], sheep population in Rajasthan is 74.26 million. In Rajasthan Sheep population has increased by 14.13 percent over previous Livestock Census (2012). About 13.8 percent of the total livestock population is contributed by Sheep. Paratuberculosis is a contagious, chronic and fatal infectious disease of ruminant caused by *Mycobacterium avium* sp. Paratuberculosis (MAP) that primarily affect the small intestine of ruminants. Paratuberculosis is prevalent worldwide and has a significant financial impact on animal husbandry (Ott *et al*., 1999) [8]. In India assessment of the disease at several Government farms in most states has been based on single intradermal Johnin test, and faecal and tissue smear examination. Sero-prevalence of disease has also been reported based on an in-house development ELISA (Rajukumar *et al.*, 2001; Sivakumar *et al.*, 2007) [9, 5].

Material and Methods
In present investigation 100 sheep were screened for prevalence of Paratuberculosis (Johne's disease). Serum samples for the present study were collected from veterinary hospitals, local abattoir and farms in Bikaner district of Rajasthan. For these studies, about 10 ml of blood was collected in a test tube without anticoagulant for separation of serum. The serum was separated then serum samples were transferred to sterilized pyrex tubes with help of pasture pipette and were stored at -20 °C till further analysis. The collected serum samples were examined by using Indigenous ELISA kit developed by CIRG, Makhdoom, Mathura.

Results and Discussion
Age related sero-prevalence of John's disease in sheep
Sheep sampled for the current study were divided into three age groups (l) Less than 6 month of age, group (ll) 6 to 12 month of age and group (lll) more than 12 month of age, to determine the possible association of John's disease with age.

Out of 100 sheep sampled for study, 16were less than 6 months of age, 38 were 6 to 12 month of age and 46 were more than 12 months of age (Table 1).

Analysis of age-related data of John's disease positive sheep (n = 31) indicates towards higher sero-prevalence in sheep of age group-III (>12 months) i.e., 43.47 percent (20/46) followed by 21.05 percent (8/38) in group-II (6-12 months) and 18.75 percent in group-I (3/16).

Table 1: Age wise sero-prevalence of John's disease in sheep

S. No.	Age (Months)	No of positive for John's disease in each group
1.	<6	18.75 percent (3/16)
2.	6-12	21.05 percent (8/38)
3.	>12	43.47 percent (20/46)

Singh *et al*. (2007) [14] recorded 8.5 percent seroprevalence with Plate ELISA testing in kids. Young animals are known to be at greater risk of infection due to their 'open gut' specifically the existence of specialized lymphoid tissue (Peyer's patches) that early in life tolerates maternal antibodies. While very young animals are still thought to be most vulnerable to MAP infection, dose dependency is clear, with older animals susceptible to higher doses of infection (Windsor and Whittington, 2010; Mortier *et al*, 2013; Imada *et al*., 2020) [15, 7, 6].

Animals are most often infected by faecal-oral pathways, but infection occurs mostly in young lamb either by the ingestion of bacilli by contaminated ewe's milk or through the ingestion of contaminated surface (Cocito *et al*., 1994) [4]. This may be the cause of higher seroprevalence in goats of Johne's disease. Singh and Vihan (2004) [3] described milk from ewe to offspring as an imported transmission vehicle of Johne's disease bacilli. It is therefore a possible cause of disease transmission and infection in very young lamb, and Paratuberculosis is therefore a serious endemic disease.

Traditionally, Johne's disease is considered the disease of adult animals, but the disease was also documented in lamb within 90 days of infection with native Johne's disease isolate 5S (Bison type) on the basis of marked lesion, culture isolation and PCR. Continued infection by infected ewe in newborn lamb helps sustain the cycle of infection in herds at various stages of foetus or young lamb's development (Singh and Vihan, 2004) [3].

The prevalence of antibodies in adult animals is not always indicative of infection, as there is always a high probability of these animals receiving vaccination once during a lifetime.

Sex related sero-prevalence of John's disease in sheep

Out of 100 sampled sheep there were 59 male and 41 females (Table 2). Out of 31 positive sheep from the studies area, 18.00 percent (18/100) male and 13.00 percent (13/100) female were found positive.

Table 2: Sex wise sero-prevalence of John's disease in sheep

S. No.	Sex	Prevalence (percent)
1.	Male	18.00 percent (18/100)
2.	Female	13.00 percent (13/100)

The organism has been isolated from contaminated bulls' genitals and semen. If the pregnant female is a strong shedder of the organism, fetal infection is more likely to be associated with advanced infection level (Constable *et al*., 2017) [5].

Over-all Sero-prevalence of John's disease in the sheep

Out of 100 sheep serum samples collected from the studies area, 31 percent (31/100) were found positive (Table-3), for John's disease on the basis of indigenous ELISA kit (Developed by CIRG, Makhdoom).

Table 3: Overall sero-prevalence of John's disease in the sheep

S. No.	Disease	Prevalence
1.	John's disease	31 percent (31/100)

Findings of the present study was lower than the findings of Biswal *et al*. (2018) [2] who reported that around 68.19 percent of sheep serum samples investigated for John's disease as seropositive. However, it was higher than the sero-prevalence reported by Shabana *et al*. (2020) [11] who found that the sero-prevalence of John's disease was 11.12 percent in sheep.

Biswal *et al*. (2018) [2] and Celebi *et al*. (2014) [3] recorded a very high prevalence of John's disease in sheep, with 68.17 Percent, 57.70 Percent respectively, which was higher than the present result.

Safi (2015) [10] reported that the overall prevalence of paratuberculosis by indirect ELISA test in the Bikaner district was 25 percent. Variation in the prevalence of infections of John's disease in different regions was observed on the basis of these facts. Its reliance on management and environmental determinants can be due to this difference in prevalence.

Conclusion

The present results show that infection with John's disease is prevalent in the district of Bikaner. However, to allow early detection of John's disease among sheep and other susceptible animal species, the adoption of the year-round surveillance system is important because some livestock owners used to keep different animal species in one flock/herd, which increases the transmission of the disease between species.

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