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SERODETECTION OF ANTIBODIES FOR *MYCOBACTERIUM AVIUM* SUB-SPECIES *PARATUBERCULOSIS* IN SHEEP ALONG WITH ITS ASSOCIATED RISK FACTORS IN DISTRICT RAHIM YAR KHAN, PUNJAB, PAKISTAN

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Author's contribution
M.A.R conducted the experiments. W.Y designed the work and helped in conducting the experiment trial and also provide necessary facilities. M.K.I provided input in statistical data analysis. M.K provided technical assistance during research work and A.I.A drafted the manuscript, while SU revised the manuscript. All authors read and approved the final manuscript.

Key words:
ELISA, *mycobacterium avium* subsp. *paratuberculosis*, risk factors, sheep.

ABSTRACT

During this study identification of the antibodies of *Mycobacterium avium* sub-species *paratuberculosis* (MAP) prevalence and risk factors associated with paratuberculosis in sheep population of District Rahim Yar Khan, Punjab, Pakistan was carried out. For this purpose, 100 blood samples were randomly collected from slaughtered and marketed animals (irrespective of breed, age and sex) in district Rahim Yar Khan, Pakistan. Then samples were analyzed through ELISA for detection of MAP. Out of hundred animals 5.0 % were observed to be serological positive. In comparison to non-infected animals (control), the decreased in the total serum proteins ($P<0.05$) were noticed in the MAP infected animals. Furthermore, it was found that some risk factors can be responsible as a resource of spreading the paratuberculosis such as 92% malnutrition, 77% poor sanitation, 85% combine housing, 87% open grazing and 29% tick infestation. It was concluded that 5.0 % prevalence was occurred in the sheep population and might be some risk factors were also involved to spread the disease.

1. INTRODUCTION

Paratuberculosis also known as Johne's disease caused by *Mycobacterium avium* sub-species paratuberculosis (MAP) bacterium in small ruminants and distributed worldwide causes severe economical losses [1]. The bacterium principally affects the small intestine of ruminants, and also been observed in various non-ruminant species such as foxes, rabbits and birds. MAP is small rod shaped, gram positive and acid fast bacterium, and predominantly found in the endo- thelium Payer's Patches of intestines and mesenteric lymph nodes of effected animals [2]. Paratuberculosis is chronic disease having zoonotic importance and their etiological agents remain unaffected towards pasteurization temperature [3] and possibly may create problems regarding public health such as Crohn's disease in human [4]. It is more important to control the disease in animal population to avoid the public health problems. Previous literature recommended that prevalence of paratuberculosis and its causative bacteria MAP estimated via different methods. The molecular detection by PCR from various tissues and blood samples validated the prevalence of paratuberculosis in wild ruminants [5] (Pavlik et al., 2000), sheep and goat [6] and in cattle [7]. The occurrence of MAP also confirmed via

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histological, bacteriological and faecal examination by ELISA. The serological detection of MAP well proved by ELISA [9, 10, 11] in cattle, goat and in sheep. In addition to causative agent, the risk factors also responsible for spreading of diseases [12, 13, 14]. In our environment prevalence studies of paratuberculosis also conducted from various samples (serum, semen and tissues) by ELISA in cattle and buffalo [15, 16]. Despite of its economic consequence, paratuberculosis has been debated currently. Little is known about its prevalence and detection in small ruminant population of Pakistan more importantly in sheep.

Keeping in view the economic and public health importance of the disease, current study was planned to detect paratuberculosis in serum samples of sheep from slaughter houses and local market of Rahim Yar Khan via enzyme-linked sorbent immunoassay (ELISA) along with its associated risk factors.

2. MATERIALS AND METHODS

Ethical approval

The study to identify the antibodies of *Mycobacterium avium* sub-species *paratuberculosis* (MAP) prevalence and risk factors associated with paratuberculosis in sheep population was designed and performed according to the global standard. Approval was duly obtained from the Institutional Ethics and Research Committee, Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences, Lahore, Pakistan.

Experimental design

The study was conducted in periphery of district Rahim Yar Khan, Pakistan. The 100 animals were randomly selected. The 50 animals from slaughter houses and local market on the basis of probable clinical signs of paratuberculosis. While 50 animals were selected without clinical sign possibly carrier of paratuberculosis. The demographic data regarding animals were collected on a predesigned questionnaire from owners, employees on the farms regarding risk factors associated with paratuberculosis. 5 ml blood was aseptically collected from jugular vein into anticoagulated vacutainer. The 100 blood samples were collected and transferred into ice boxes and transported to the Department of Clinical Medicine, University of Veterinary and Animal Sciences Lahore, Pakistan for further processing. Then samples were centrifuged at 1500 rpm for serum separation. The serum was stored in cryovials at -80 ^{o}C for future use. The antibodies against *mycobacterium avium* sub spp. *paratuberculosis* were detected according to the protocol and procedure as recommended in commercially available indirect ELISA kit (IDEXX Paratuberculosis Screening, USA). In the final step optical density of control and samples were measured and recorded at 450 nm through ELISA plate reader (Biotech Elx 808, USA). The total proteins in the serum were measured through plate reader (Biotech Elx 808, USA) according to the method described in the commercially available kit (Nanjing Jiancheng Bioengineering Co. Ltd., China).The normal values of total serum protein of non-infected animals were matched with previously documented reference values [17].

Statistical analysis

The data about antibody detection by ELISA and associated risk factors were simply presented as percentage. The serum total protein mentioned as mean ±standard error (S.E), and t-test was applied for comparison between the serum total proteins of non-infected and infected animals using statistical software SPSS (16.0 version). $P<0.05$ was considered for significance level.

3. RESULTS

Out of 100 sheep the total number of ELISA positive sheep for the MAP was 05 sheep. The overall prevalence was 05% for paratuberculosis in sheep in district Rahim Yar Khan in different slaughtering and market animals irrespective of age, breed and sex (Table-1, Figure-1). The prevalence percentage further confirmed by detection of serum proteins. The mean indices of total serum protein were depicted in Figure-3. The overall decrease was observed in the total serum proteins ($P<0.05$) of sheep which were infected with MAP. The certain risk factors were also recorded from hundred animals might cause spreading of infection (Figure-2). The important risk factors observed during the current study for 5% prevalence of paratuberculosis were: 92% malnourished sheep vs 8% healthy, 77% poor vs 23% good sanitation, 29% tick vs 71% without tick infestation and 85% combine vs 15% separate housing (Table-2).

4. DISCUSSION

During the current study overall prevalence was 05% for paratuberculosis, while in other countries reported herd level prevalence were more than 50 % and prevalence at animal level had been described nearly 20% or minimally 3-5% [14].

The documented literature suggested that 8.5 % (47 animals) prevalence of paratuberculosis in Rajasthan state of India was positive through ELISA in goat kids [11]. Singh [18] observed 15.6 % prevalence by ELISA in serum samples collected from slaughterhouse of goats in Agra, India. Kumar *et al.,* [19] reported sero-prevalence with 35.4 and 58.6 percentages in goats belonging to farmers and commercial herds, respectively. Another reported study states 8.3% and 36.3% prevalence rates sourced from fecal and tissue samples, correspondingly, in kids of Barbari goat, Uthar Pradesh India [20]. In our environment locally based studies suggested 6-20 % prevalence rate from different samples detected by ELISA in buffalo, cows and sheep [15, 16, 21]. The divergence in frequency or prevalence of paratuberculosis examined in the current and previously reported studies possibly because of difference in management conditions, breeds and species of animals, individual immune response, population of animals in that specific area and environmental conditions. In current research based local conditions, there is less population of peoples and animals so might be less chance of spreading the infection that's why low prevalence rate was observed. In the study area the survival of the peoples depends upon the livestock specially goats and sheep. Most of the families are poor and some survive their life like nomads. They cannot manage the animals properly according to standards need for production of livestock. We observed that malnutrition and poor sanitation conditions are possible factors in transmission of MAP, so shortage of diet can cause to weaken the animals' ultimately lead to poor immunity and poor sanitation (fecal, urine and other wastes) at the farm lead to introduction of microbes through diet, water and respiration. It was also observed combined management of animals can be one factor; MAP may transmissible through respiration in the form of exchanging aerosols between the animal when managed combinable.

It was noticed possibly MAP infection transfer because of open grazing. Mostly open pastures are contaminated (urine and feces) by animals, those contaminates have infectious microbes, can be transmitted while animals grazing. It was seen that animals were infested with tick, possible play role in spreading of this melodious disease. It is also confirmed through documented literature that some important risk factors were associated with transmission of infection inside the herd or between herd to herd such as vehicles, equipment, contaminated food, water, neonatal environment, milk and colostrums [12, 13]. The present study coincides with previously reported studies on prevalence of paratuberculosis in sheep confirmed through histopathological examination in our country [20]. Our study also coincides with prevalence of MAP confirmed through plate ELISA and microbiological examination in goat [11, 21]

5. CONCLUSION

The lower rate (05 %) prevalence of paratuberculosis were observed in the slaughter and marketed animals along with few associated risk factors in sheep population located in District Rahim Yar Khan, Punjab, Pakistan.

6. RECOMMENDATIONS

A good surveillance system is very important to early reorganization of paratuberculosis outbreak and takes immediate measure for further control and transmission of the paratuberculosis. For preventive future preventive measures vaccination and quarantine of newly introduced animals should be regularly practiced. A strict bio security measures should be adopted at the farm. The waste materials should be properly handled because it is a source of infection.

7. CONFLICT OF INTEREST

The authors of this paper have no conflict of interest in relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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REFRENCES

[1] Windsor, P.A. and Whittington, R.J. (2010) Evidence for age susceptibility of cattle to Johne's disease. *The Vet. J.,* 184: 37-44.

[2] Chiodini, R.J. and Van Kruiningen, H. (1986) The prevalence of paratuberculosis in culled New England cattle. *Cornell Veterin.,* 76: 91-104.

[3] Ayele, W.Y., Svastova, P., Roubal, P., Bartos, M. and Pavlik, I. (2005) Mycobacterium avium subspecies

paratuberculosis cultured from locally and commercially pasteurized cow's milk in the Czech Republic. *Applied Environ. Microbiol.,* 71: 1210-1214.

[4] Feller, M., Huwiler, K., Stephan, R., Altpeter, E., Shang, A., Furrer, H., Pfyffer, G.E., Jemmi, T., Baumgartner, A. and Egger, M. (2007) Mycobacterium avium subspecies paratuberculosis and Crohn's disease: a systematic review and meta-analysis. *The Lancet Inf. Dis.,* 7: 607-613.

[5] Pavlik, I., Bartl, J., Dvorska, L., Svastova, P., Du Maine, R., Machackova, M., Ayele, W.Y. and Horvathova, A. (2000) Epidemiology of paratuberculosis in wild ruminants studied by restriction fragment length polymorphism in the Czech Republic during the period 1995–1998. *Vet. Microbiol.,* 77: 231-251.

[6] Chaudhry, Z.I., Zahid, B., Ali, M. and Iqbal, F. (2012) Detection of Mycobacterium avium subsp. paratuberculosis in fecal samples of sheep and goats from Lahore (Pakistan) by acid fast staining, fecal culture and PCR amplification. *Asian Pacific J. Trop. Biomedic.,* 1: 941-944.

[7] Khan, F.A., Chaudhry, Z.I., Ali, M.I., Khan, S., Mumtaz, N. and Ahmad, I. (2010) Detection of Mycobacterium avium subsp. paratuberculosis in tissue samples of cattle and buffaloes. *Trop. Anim. Health Produc.,* 42: 633-638.

[8] McKenna, S., Keefe, G.P., Barkema, H. and Sockett, D. (2005) Evaluation of three ELISAs for Mycobacterium avium subsp. paratuberculosis using tissue and fecal culture as comparison standards. *Vet. Microbiol.,* 110: 105-111.

[9] Kostoulas, P., Leontides, L., Enoe, C., Billinis, C., Florou, M. and Sofia, M. (2006) Bayesian estimation of sensitivity and specificity of serum ELISA and faecal culture for diagnosis of paratuberculosis in Greek dairy sheep and goats. *Prev. Vet. Med.,* 76: 56-73.

[10] Boelaert, F., Walravens, K., Biront, P., Vermeersch, J., Berkvens, D. and Godfroid, J. (2000) Prevalence of paratuberculosis (Johne's disease) in the Belgian cattle population. *Vet. Microbiol.,* 77: 269-281.

[11] Singh, S., Singh, A., Singh, P., Gupta, V., Kumar, S. and Vohra, J. (2007) Sero-prevalence of paratuberculosis in young kids using 'Bison type', Mycobacterium avium subsp. paratuberculosis antigen in plate ELISA. *Small Rumin. Res.,* 70: 89-92.

[12] Dore, E., Pare, J., Cote, G., Buczinski, S., Labrecque, O., Roy, J. and Fecteau, G. (2012) Risk factors associated with transmission of Mycobacterium avium subsp. paratuberculosis to calves within dairy herd: a systematic review. *J. Vet. Intern. Medic.,* 26: 32-45.

[13] Humblet, M.F., Boschiroli, M.L. and Saegerman, C. (2009) Classification of worldwide bovine tuberculosis risk factors in cattle: a stratified approach. *Vet. Res.,* 40: 1-24.

[14] Nielsen, S.S. and Toft, N. (2008) Ante mortem diagnosis of paratuberculosis: a review of accuracies of ELISA, interferon-γ assay and faecal culture techniques. *Vet. Microbiol.,* 129: 217-235.

[15] Abbas, M., Munir, M., Khaliq, S.A., Haq, M.I.U. and Tanveer, K.M. (2011) Detection of paratuberculosis in breeding bulls at Pakistani semen production units: a continuous source of threat. *ISRN Vet. Sci.,* 501235.

[16] Sikandar, A., Cheema, A., Younus, M., Aslam, A., Zaman, M. and Rehman, T. (2012) Histopathological and serological studies on paratuberculosis in cattle and buffaloes. *Pak. Vet. J.,* 32: 547-551.

[17] Cynthia, M. and Kahn, M. (2005) The merck veterinary manual. Philadelphia, USA. Pp. 131: 425.

[18] Singh, S. (1998) Diagnosis of paratuberculosis in goats. Ph. D. Thesis, Chandra Shekar Azad Univ. Agri. Technol., Kanpur, India.

[19] Kumar, P., Singh, S., Bhatia, A., Singh, A., Yadav, D., Sharma, G., Kumar, S., Sevilla, I. and Juste, R. (2004) Juvenile paratuberculosis in goats and its molecular characterization. Proceed. XXII Annual Conf. Indian Assoc. Vet. Microbiologists, Immunologists and Specialists in Inf. Dis., CSK Himachal Pradesh Krishi Vishvavidhalaya, Palampur, India ed. 14-15.

[20] Sikandar, A., Cheema, A., Adil, M., Younus, M., Zaneb, H., Zaman, M., Tipu, M. and Masood, S. (2013) Ovine paratuberculosis-A histopathological study from Pakistan. *J. Anim. Plant. Sci.,* 23: 749-753.

[21] Hajra S, Srivastav, A. and Singh S. (2004) Pathogenicity of S-5 strain of Mycobacterium avium subsp. paratuberculosis in experimental kids at early stage of the disease. Proceed. XXI Annual Conf. Indian Assoc. Vet.

Pathologists, WB Univ. Anim. & Fishery Sci., Kolkata, India ed. Pp. 93.

Table 1: Overall prevalence percentage of paratuberculosis in sheep. Positive cut off titer-0.350 optical density (OD).

Number of animals	Positive animals	Negative animals
100	5 (5%)	95 (95%)

Table 2: Percentage of associated risk factors to paratuberculosis.

Associated risk factors	Frequency	Percent (%)
Malnourished	92	92%
Healthy	8	8%
Poor sanitation	77	77%
Good sanitation	23	23%
Tick infestation	29	29%
Without tick infestation	71	71%
Combine housing	85	85%
Separate housing	15	15%

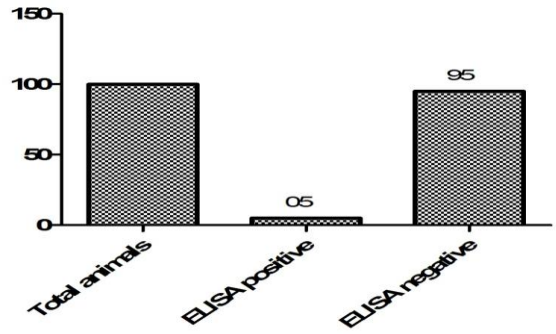

Figure 1: ELISA negative and positive

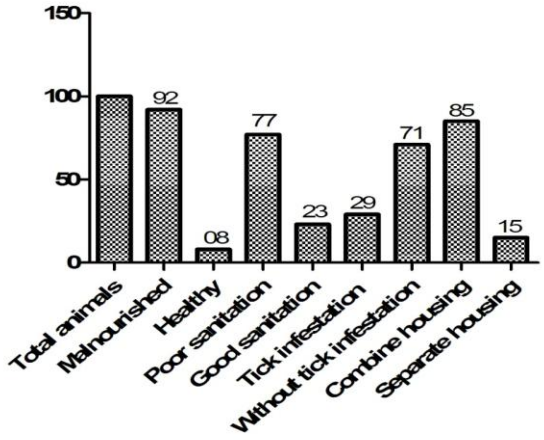

Figure 2: Animals (n=100) with associated risk factors to paratuberculosis.

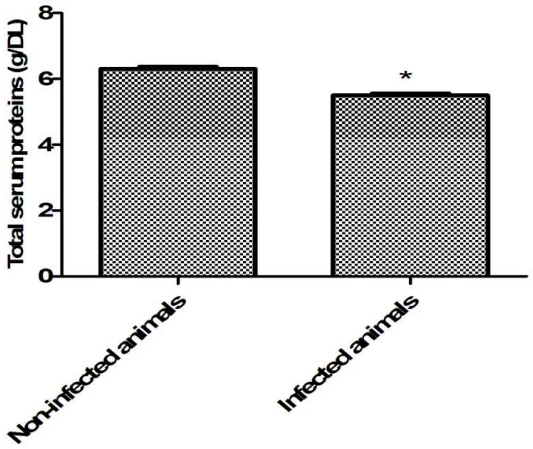

Figure 3: Mean serum total protein (g/DL) of non-infected and infected animals (n=5). Significance level P<0.005 indicated as*.