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Abattoir based Sero-Survey of *Mycobacterium avium* Subspecies Paratuberculosis in Bovines in District Faisalabad-Pakistan

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Authors' Contribution
RA collected blood samples, performed ELISA and wrote manuscript. MKM, IH and MS helped in experimental work. HS and JM helped in data analysis. AIA and AI helped in manuscript writing and statistical analysis.

Key words
Bovine paratuberculosis, Cattle and buffalo, Prevalence, Odds ratio, ELISA

ABSTRACT

Bovine paratuberculosis is a chronic disease of cattle and buffaloes, causing progressive weight loss, persistent diarrhea and finally death. Due to zoonotic nature of the disease, workers in slaughterhouses are at a high risk of infection. In this study, the status of paratuberculosis was assessed in a slaughterhouse located in district Faisalabad, Pakistan. A total of 455 blood samples were collected from slaughtered cattle and buffaloes at random and then tested by a commercially available ELISA kit for the detection of antibodies against *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Data were analyzed using chi square for ELISA and odds ratio for finding association of species, gender, body weight, age and body condition score (BCS) with prevalence of antibodies against MAP. Seropositivity against MAP was significantly ($P<0.05$) detected. Male and female animals were equally susceptible to the disease (OR: 0.908, 95% CI= 0.6111-1.350). Age groups were not found associated with chances of being seropositive. Seropositivity was significantly ($P<0.05$) higher in animals under 300kg body weight. Chances of being seropositive were found more in animals belonging to BCS 1 (OR: 13.25, 95%CI=6.29-27.88) and BCS 3 (OR: 4.78, 95%CI=2.36-9.70) than BCS 4 and above. In conclusion, gender, body weight and body condition score of the animals are positively associated with the occurrence of the disease. These data will not only help in screening of animals but will also be instrumental in future for isolation of MAP to make vaccine against paratuberculosis using local isolates.

INTRODUCTION

Livestock being a major sector contributes 11% in the GDP which is more than the share contributed by the crop sector (i.e., 10.3%) thus playing a vital role in the economy of Pakistan (Chandio *et al*., 2016). The causative agent of paratuberculosis is *Mycobacterium avium subsp. Paratuberculosis* (MAP). Animals affected with paratuberculosis exhibit clinical signs such as reduced milk production, intermittent diarrhea, chronic weight loss and eventually results in death of the animals. On post-mortem examination, granulomatous lesions may be observed in liver and lymph nodes of the affected animals (Singh *et al*., 2016).

Paratuberculosis effects all ruminants species including cattle and calves less than six months of age are more susceptible as compare to other age group while it has been estimated that a single exposure of infection can result in the development of the disease in one third of the exposed calves (Windsor and Whittington, 2010). In older animals due to the well-developed immune system

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the production of disease requires higher doses of MAP (Hines II *et al*., 2007). Paratuberculosis is most commonly transmitted via ingestion of contaminated colostrum, milk and feces, which are contaminated by MAP either by intra-mammary shedding or indirectly. They further reported that MAP is also transmitted vertically or transplacentally (Kirkeby *et al*., 2016; Van Kooten *et al*., 2006).

Paratuberculosis being a major threat to meat and dairy animals has a global economic importance and its control poses a global challenge for the livestock sector (Hines II *et al*., 2007). Furthermore some research studies indicated its potential as public health hazard by demonstrating an association between MAP and human Crohn's disease (Feller *et al*., 2007; Naser *et al*., 2004). The control strategies of paratuberculosis are mainly based upon screening and slaughtering policy and adopting good management practices (Garry, 2011). This study was designed to evaluate the prevalence of paratuberculosis in bovines in Faisalabad-Pakistan.

MATERIALS AND METHODS

Study area and sample collection

The present study was designed at a slaughter house located in city area of district Faisalabad, which is the 3rd largest city of Pakistan with a human population of 2,506,595 and is located with GPS coordinates of 31° 25' 15.7620'' N and 73° 5' 21.4584'' E. Blood samples (n=455) were randomly collected from slaughterd cattle (n=172) and buffaloes (n=283) from slaughter house in district Faisalabad. A questionnaire containing history of slaughtered animals, age, BCS, sex and body weight was filled. The blood samples were collected directly from jugular vein, all tubes were places in ice bags and immediately transported to Bacteriology Lab, Institute of Microbiology, University of Agriculture, Faisalabad for further analysis.

Serum separation and detection of antibodies against MAP

The serum was separated by centrifugation @ 1500 RPM as described by Adkins *et al*. (2002). Sera were stored at -20°C for a temporary period. Then, serum samples were thawed at room temperature and antibodies against MAP were detected by using a commercial ELISA kit (LSI, Vet, Ruminant Serum paratuberculosis Screening, France).

Statistical analysis

The data were analyzed by Chi Square test using SPSS 20.0 version and odds ratios were also calculated using OpenEpi statistical software (version 3.01).

RESULTS

In this serological study MAP was not detected significantly ($p>0.05$) in cattle and buffaloes. Out of 283 blood samples collected from buffaloes, 90 samples (31.8%) were found positive for paratuberculosis while out of 172 blood samples collected from cattle, 54 samples (31.4%) samples reacted positively for paratuberculosis.

A total of 68 samples were collected from cattle under 4 years of age and 23 (33.8%) were positive for MAP and for buffalo under same age, 115 samples were collected and 34 (29.6%) were positive for MAP. A total of 18 samples were collected from cattle having age >6 years, and 6 (33.3%) were positive and 33 samples were collected from buffaloes with same age and 10 (30.3%) were positive for MAP. Seropositivity was not different among different age groups of cattle (chi square=0.420, df=2, P=0.809) and buffaloes (chi square=0.620, df=2, P=0.733). The seropositivity was not dependent upon age groups (chi square=0.046, df=2, P=0.977) as shown in Table I. Paratuberculosis has a significant effect on body weight, Seropositivity was significantly ($p<0.05$) higher in animals under 300kg body weight and decreased significantly ($P<0.001$) with the increase in BCS of animals as shown in Table II and III, respectively. Gender of the animals was not significantly associated with the occurrence of the disease ($x^2=0.227$, df=1, $P>0.05$) (Table IV).

Table I. Age wise prevalence of paratuberculosis in cattle and buffaloes.

Age group (years)	Cattle			Buffalo		
	Positive/tested	Prevalence %	95% confidence interval	Positive/tested	Prevalence %	95% confidence interval
<=4	23/68	33.8	22.8-46.3	34/115	29.6	21.4-38.8
5 - 6	25/86	29.1	19.8-39.9	46/135	34.1	26.1-42.7
> 6	6/18	33.3	13.3-59.0	10/33	30.3	15.6-48.7
Total	54/172	31.4	24.5-38.9	90/283	31.8	26.4-37.6

Table II. Body weight wise prevalence of paratuberculosis in cattle and buffaloes.

Body weight (kg)	Positive/ tested	Prevalence %	95% Confidence interval
<= 200	66/195	33.8[a]	27.2-41.0
201-300	58/151	38.4[a]	30.6-46.7
> 300	20/109	18.3[b]	11.6-26.9
Total	144/455	31.6	27.4-36.1

Table III. BCS wise prevalence of paratuberculosis in cattle and buffalo.

BCS groups	Body condition score (BCS)		
	Positive/ tested	Prevalence %	95% Confidence interval
1	65/117	54.7[a]	46.1-64.7
2	69/222	31.1[b]	25.1-37.6
3	10/116	8.6[c]	4.2-15.3
Total	144/455	31.6	27.4-36.1

Values with different superscripts differ significantly (chi square=59.381, df=2, P<0.001)

Table IV. Sex wise prevalence of paratuberculosis in cattle and buffaloes.

Sex of animals	Buffalo and cattle			
	+ve	-ve	P value	Odds Ratio (95% CI)
Male	66	150	0.227	0.908 (0.6111, 1.350)
Female	78	161		

DISCUSSION

Paratuberculosis is an infectious disease of dairy animals and is also zoonotic in nature (McNees *et al*., 2015). Submandibular edema, emaciation, progressive weight loss and chronic diarrhea are the signs which are shown by infected dairy animals (Garcia and Shalloo, 2015; Mortier *et al*., 2015). Antibodies can be detected against MAP using indirect ELISA (Vazquez *et al*., 2013). In the present study, blood samples were collected from a slaughterhouse located in the urban area where the animals were brought for slaughtering from periurban and rural areas. This is convenient sampling (Etikan *et al*., 2016) but the results revealed the prevalence of the disease in nearby areas and its association with other factors including age, sex, BCS and body weight.

In the present study overall prevalence of the paratuberculosis in cattle and buffaloes was found to be 31.8% and 37.8%, respectively; which is higher than the findings of McKenna *et al*. (2004) where the prevalence recorded in culled dairy cattle in abattoir was 16.1% (95% CI: 13.8 to 18.3%). Singh *et al*. (2008) reported a lower prevalence of 2.71% in Punjab, India. A low individual level prevalence (4.03%) than our study was also recorded in dairy herds in Spain (Diéguez *et al*., 2007), 28.6% herd in dairy cattle was documented in Cyprus (Slana *et al*., 2009), 2.77 % in Slovenia (Ocepek *et al*., 2002), 18% in dairy cattle in Belgium (Boelaert *et al*., 2000), 3.07% in Urmia state of Iran (Yousof *et al*., 2003) and 1.6% to 55% in the united States of America (Fernández-Silva *et al*., 2014).

In the present study, seropositivity was not dependent upon age groups (chi square=0.046, df=2, P=0.977). The results of our study are similar with the study conducted by Vazquez *et al*. (2013) in Spain wherein they found a high prevalence of 16.5% at 3-4 years and low prevalence at 7-8 years of age. Two years old calves have been reported to be effected by paratuberculosis (Stabel, 2006) but no significant difference has been recorded among age different groups with reference to paratuberculosis also not significant (Cetinkaya *et al*., 1996) which is similar with our finding. As per findings of Muskens *et al*. (2003) the prevalence of the paratuberculosis is not effected by season or months of year.

Paratuberculosis has a significant effect on body weight, seropositivity was significantly ($p<0.05$) higher in animals under 300kg body weight in the present findings which are similar with the findings of Mato *et al*. (2017) where animals also lose body weight as the disease progresses. According to Johnson *et al*. (2001) loss of body weight is the sign of paratuberculosis. BCS is in inverse relation with the onset of the disease in the present findings and same were observed by Lombard *et al*. (2005). But the present findings are not in accordance with McKenna *et al*. (2004), who did not find any association of BCS with paratuberculosis.

Gender of the animals was not significantly associated with the occurrence of the disease. Both male and female animals were equally susceptible for paratuberculosis in the present study. But our findings are not in accordance to Verdugo *et al*. (2014), female animals were more positive than male animals in their findings. This is because MAP multiplies fast in erythritol sugar found in uterus in the females. In the present study, male animals are mostly slaughtered at slaughter house and also due to lack of awareness to the farmers, female animals are still standing at farms in weak emaciated conditions without good productivity.

CONCLUSIONS

In conclusion, body weight and body condition score of the dairy animals are positively associated with occurrence of the disease while age, sex and species are not significantly affected by paratuberculosis. The disease imposes huge economic losses to the dairy farmers in reducing milk yield, weak calves, loss body weight and delayed calving, so the data of the present study will not only help in screening of animals through ELISA but will be instrumental in future for isolation of MAP to make a vaccine against paratuberculosis using local isolates.

Statement of conflict of interest

Authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

Adkins, J.N., Varnum, S.M., Auberry, K.J., Moore, R.J., Angell, N.H., Smith, R.D., Springer, D.L. and Pounds, J.G., 2002. Toward a human blood serum proteome analysis by multidimensional separation coupled with mass spectrometry. *Mol. Cell. Proteom.,* **1**: 947-955. https://doi.org/10.1074/mcp.M200066-MCP200

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. https://doi.org/10.1016/S0378-1135(00)00312-6

Cetinkaya, B., Egan, K., Harbour, D. and Morgan, K., 1996. An abattoir-based study of the prevalence of subclinical Johne's disease in adult cattle in south west England. *Epidemiol. Infect.,* **116**: 373-379. https://doi.org/10.1017/S0950268800052705

Chandio, A.A., Yuansheng, J. and Magsi, H., 2016. Agricultural sub-sectors performance: an analysis of sector-wise share in agriculture GDP of Pakistan. *Int. J. Econ. Finan.,* **8**: 156-163. https://doi.org/10.5539/ijef.v8n2p156

Diéguez, F.J., Arnaiz, I., Sanjuán, M.L., Vilar, M.J., López, M. and Yus, E., 2007. Prevalence of serum antibodies to *Mycobacterium avium* subsp. paratuberculosis in cattle in Galicia (northwest Spain). *Prev. Vet. Med.,* **82**: 321-326. https://doi.org/10.1016/j.prevetmed.2007.08.006

Etikan, I., Musa, S.A. and Alkassim, R.S., 2016. Comparison of convenience sampling and purposive sampling. *Am. J. Theoret. appl. Stat.,* **5**: 1-4. https://doi.org/10.11648/j.ajtas.20160501.11

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. *Lan. Infect. Dis.,* **7**: 607-613. https://doi.org/10.1016/S1473-3099(07)70211-6

Fernández-Silva, J.A., Correa-Valencia, N.M. and Ramírez, N.F., 2014. Systematic review of the prevalence of paratuberculosis in cattle, sheep, and goats in Latin America and the Caribbean. *Trop. Anim. Hlth. Prod.,* **46**: 1321-1340. https://doi.org/10.1007/s11250-014-0656-8

Garcia, A. and Shalloo, L., 2015. Invited review: The economic impact and control of paratuberculosis in cattle. *J. Dairy Sci.,* **98**: 5019-5039. https://doi.org/10.3168/jds.2014-9241

Garry, F., 2011. Control of paratuberculosis in dairy herds: Veterinary clinics. *Fd. Anim. Pract*., **27**: 599-607. https://doi.org/10.1016/j.cvfa.2011.07.006

Hines II, M.E., Stabel, J.R., Sweeney, R.W., Griffin, F., Talaat, A.M., Bakker, D., Benedictus, G., Davis, W.C., de Lisle, G.W. and Gardner, I.A., 2007. Experimental challenge models for Johne's disease: a review and proposed international guidelines. *Vet. Microbiol.,* **122**: 197-222. https://doi.org/10.1016/j.vetmic.2007.03.009

Johnson, Y., Kaneene, J., Gardiner, J., Lloyd, J., Sprecher, D. and Coe, P., 2001. The effect of subclinical *Mycobacterium paratuberculosis* infection on milk production in Michigan dairy cows. *J. Dairy Sci.,* **84**: 2188-2194. https://doi.org/10.3168/jds.S0022-0302(01)74665-6

Kirkeby, C., Græsbøll, K., Nielsen, S.S., Christiansen, L.E., Toft, N., Rattenborg, E. and Halasa, T., 2016. Simulating the epidemiological and economic impact of paratuberculosis control actions in dairy cattle. *Front. Vet. Sci.,* **3**: 90-99. https://doi.org/10.3389/fvets.2016.00090

Lombard, J.E., Garry, F.B., McCluskey, B.J. and Wagner, B.A., 2005. Risk of removal and effects on milk production associated with paratuberculosis status in dairy cows. *J. Am. Vet. Med. Assoc.,* **227**: 1975-1981. https://doi.org/10.2460/javma.2005.227.1975

Mato, I., Pesqueira, N., Factor, C., Camino, F., Sanjuán, M.L., Yus, E. and Diéguez, F.J., 2017. Effect of *Mycobacterium avium* subsp. paratuberculosis serostatus on carcass weight and conformation and fat cover scores. *Spanish. J. agric. Res.,* **15**: 0502-0509. https://doi.org/10.5424/sjar/2017151-10266

McKenna, S.L., Keefe, G.P., Barkema, H.W., McClure, J., VanLeeuwen, J.A., Hanna, P. and Sockett, D.C.,

2004. Cow-level prevalence of paratuberculosis in culled dairy cows in Atlantic Canada and Maine. *J. Dairy Sci.,* **87**: 3770-3777. https://doi.org/10.3168/jds.S0022-0302(04)73515-8

McNees, A.L., Markesich, D., Zayyani, N.R. and Graham, D.Y., 2015. *Mycobacterium paratuberculosis* as a cause of Crohn's disease. *Exp. Rev. Gastroenterol. Hepatol.,* **9**: 1523-1534. https://doi.org/10.1586/17474124.2015.1093931

Mortier, R.A., Barkema, H.W. and De Buck, J., 2015. Susceptibility to and diagnosis of *Mycobacterium avium* subspecies *paratuberculosis* infection in dairy calves: a review. *Prev. Vet. Med.,* **121**: 189-198. https://doi.org/10.1016/j.prevetmed.2015.08.011

Muskens, J., Elbers, A., Van Weering, H. and Noordhuizen, J., 2003. Herd management practices associated with paratuberculosis seroprevalence in Dutch dairy herds. *Zoonos. Publ. Hlth.,* **50**: 372-377. https://doi.org/10.1046/j.1439-0450.2003.00697.x

Naser, S.A., Ghobrial, G., Romero, C. and Valentine, J.F., 2004. Culture of *Mycobacterium avium* subspecies *paratuberculosis* from the blood of patients with Crohn's disease. *Lancet,* **364**: 1039-1044. https://doi.org/10.1016/S0140-6736(04)17058-X

Ocepek, M., Krt, B., Pate, M. and Pogacnik, M., 2002. Seroprevalence of paratuberculosis in Slovenia between 1999 and 2001. *Slovenia Vet. Res.,* **39**: 179-185.

Singh, S., Singh, A., Singh, R., Sharma, S., Shukla, N., Misra, S., Singh, P., Sohal, J., Kumar, H. and Patil, P., 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. *Comp. Immunol. Microbiol. Infect. Dis.,* **31**: 419-433. https://doi.org/10.1016/j.cimid.2007.06.002

Singh, S.V., Kuenstner, J.T., Davis, W.C., Agarwal, P., Kumar, N., Singh, D., Gupta, S., Chaubey, K.K., Kumar, A. and Misri, J., 2016. Concurrent resolution of chronic diarrhea likely due to Crohn's disease and infection with *Mycobacterium avium paratuberculosis*. *Front. Med.,* **3**: 49-59. https://doi.org/10.3389/fmed.2016.00049

Slana, I., Liapi, M., Moravkova, M., Kralova, A. and Pavlik, I., 2009. *Mycobacterium avium* subsp. paratuberculosis in cow bulk tank milk in Cyprus detected by culture and quantitative IS900 and F57 real-time PCR. *Prev. Vet. Med.,* **89**: 223-226. https://doi.org/10.1016/j.prevetmed.2009.02.020

Stabel, J., 2006. Host responses to *Mycobacterium avium* subsp. *paratuberculosis*: a complex arsenal. *Anim. Hlth. Res. Rev.,* **7**: 61-70. https://doi.org/10.1017/S1466252307001168

Van Kooten, H., Mackintosh, C. and Koets, A., 2006. Intra-uterine transmission of *paratuberculosis* (Johne's disease) in farmed red deer. *N. Z. Vet. J.,* **54**: 16-20. https://doi.org/10.1080/00480169.2006.36598

Vazquez, P., Garrido, J.M. and Juste, R.A., 2013. Specific antibody and interferon-gamma responses associated with immunopathological forms of bovine *paratuberculosis* in slaughtered Friesian cattle. *PLoS. One,* **8**: e64568. https://doi.org/10.1371/journal.pone.0064568

Verdugo, C., Pleydell, E., Price-Carter, M., Prattley, D., Collins, D., de Lisle, G., Vogue, H., Wilson, P. and Heuer, C., 2014. Molecular epidemiology of *Mycobacterium avium* subsp. *paratuberculosis* isolated from sheep, cattle and deer on New Zealand pastoral farms. *Prev. Vet. Med.,* **117**: 436-446. https://doi.org/10.1016/j.prevetmed.2014.09.009

Windsor, P.A. and Whittington, R.J., 2010. Evidence for age susceptibility of cattle to Johne's disease. *Vet. J.,* **84**: 37-44. https://doi.org/10.1016/j.tvjl.2009.01.007

Yousof, B.G., Farajivand, A. and Ramin, A., 2003. Study on the prevalence of subclinical cattle Johne's Disease in the *Urmia abattoir*. *Iran. J. Vet. Res.,* **7**: 123-131.