Seroprevalence of Johne's Disease in Dairy Cattle

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A B S T R A C T

Johne`s disease is economically and zoonotically an important disease in dairy animals and required continuous surveillance. The study was carried out to investigate the prevalence of Paratuberculosis (Johne's disease) in one of dairy cattle farm in Egypt. A total of 964 serum samples were collected from 964 dairy cattle and were examined by ELISA. Out of them 155 (16.1%) samples reacted positively. The results revealed that the age and lactation period have significant effect of prevalence of Johne's disease in dairy cattle. The disease was mostly prevalent in animals over 6 years of age and in early stage of lactation. Furthermore, the disease is more prevalent in heavy lactating animals in comparison with other examined cattle. In contrast, the pregnancy stage showed no significant effect on occurrence of the disease in dairy cattle. The result of the current study demonstrate that *Mycobacterium avium subsp. paratuberculsosis* (MAP) is circulating in dairy farm in Egypt and further studies require to reveal the epidemiology of the disease

Keywords: *Johne's disease, Cattle, prevalence, ELISA.*

1. INTRODUCTION

Paratuberculosis or Johne's disease (JD) is a chronic debilitating, enteropathy disease of cattle and ruminants that is caused by bacterium *Mycobacterium avium subsp. paratuberculosis* (MAP) (Selim and Gaede, 2015).
The disease is responsible for economic losses worldwide in terms of medication, premature culling and mortality. Fecal oral transmission is the primary mode of spread of MAP in herds, while contaminated feed, water, soil and in-utero transmission is also possible. MAP is also secreted through milk, colostrum and the calves at younger age got infected by this mean of transmission (Lu et al., 2008)**.** The MAP is also of interest due to the possible role in causation of inflammatory bowel disease of humans known as Crohn's disease. Milk and its products are a potential source of infection to humans (Hruska et al., 2011)**.**
MAP has prolong incubation period, it disturbs the productive efficiency and the animals remain infected sub clinically (Gupta et al., 2012). Animals in advanced clinical disease become weak, emaciated, and suffer from profuse diarrhea. Intermandibular oedema or bottle jaw is characteristic of this stage and animals eventually die of dehydration and cachexia (Tiwari et al., 2006).
It was first reported in cattle in Europe in 1895 and has spread throughout the developed and parts of the developing world, particularly in the bovine dairy industries (Benazzi et al., 1995).

In the USA, paratuberculosis is present in 5-10% of dairy animals and in 33% of dairy herds (Dorshorst et al., 2006)**,** while in England the prevalence rate is 7.3% in dairy herds (Woodbine et al., 2009)**..**

Diagnosis of paratuberculosis is established by direct detection of causative agent using selective media or indirect methods for diagnosis of paratuberculosis are based on the detection of specific antibodies in blood sera or milk, or on the measuring of cellular immunity. The detection of antibodies using absorbed enzyme-linked immunosorbent assay (ELISA) method is considered the method of choice for the diagnosis of paratuberculosis, because of the rapidity of the test and relatively low expenses (Vidić et al., 2011).

Paratuberculosis is a challenging and econom-ically important disease not only for the dairy industry, but also from a public health perspective. Thus, it becomes essential to keep on monitoring the status of disease in animals in Egypt. Therefore, this study was undertaken to investigate the epidemiology of paratuberculosis in dairy cattle using ELISA test.

2. MATERIAL AND METHODS

2.1 Ethic statement

Blood collections were performed under owner's consent, and the study was approved by the Internal Ethics Review Committee of Faculty of Veterinary Medicine, Benha University.

2.2 Samples collection and preparation

The study was conducted to determine the prevalence of Paratuberculosis (Johne's disease) in one of dairy cattle in Egypt. The farm consisted of 964 cows with age ranged between 1-10 years. The history of each cow was recorded in farms including age, number of lactations, stage of lactation and pregnancy. A total 964 serum samples were collected from all examined cow to study the prevalence of the disease. Blood samples were collected from individual animal using vacuum tube, sera were separated by centrifugation at 4025xg in 1.5 ml Eppendorf tube and stored at -20ºC until serological analysis.

2.3 Serological examination using ELISA

All collected serum samples were examined serologically using IDEXX Paratuberculosis ELISA test (IDEXX Laboratories, Inc., Westbrook, ME) to investigate the antibodies against MAP in examined cattle followed by statistical analysis. The test was performed according the manufacturer's instructions.

The ELISA results were measured as the sample optical density (OD) and were recorded as a sample-to-positive (S/P) ratio, where S/P = (OD of unknown sample − OD of negative-control sample)/ (OD of positive-control sample − OD of negative-control sample). Samples with an S/P ratio <0.2 were considered negative, whereas samples with an S/P ration ≥0.25 was considered a positive.

2.4 Statistical analysis

The serological results were analyzed by Chi-square test using SPSS V17. The results were considered significant at a probability level ≤0.05

3. RESULTS

Age-wise seroprevalence of paratuberculosis is shown in table 1. The results revealed that the prevalence of the disease was significant difference between different age of examined animal (P=0.001) and the prevalence was higher in age stage (>6 years) in comparison with young. Regarding to seroprevalence of the disease in relation to stage of lactation, the results showed that the disease was more prevalent in late stage of lactation with significant difference with other stages (P=0.002). as in Tab 2. Furthermore, the heavy lactating animals showed higher seropositive rate with significant difference (P=0.04) in comparison with others examined cattle as in Tab. 3. In contrast, the pregnancy had no significant effect on the prevalence of the disease in examined cattle (P=0.1) as in Tab. 4.

Table 1: Age related seroprevalence of Johne`s disease in cattle based on ELISA

parameter	No of examined animal	No of positive (%)	P value
<2 years	180	14 (7.8%)	0.001
2-3 years	264	39 (14.8%)	
3-6 years	403	75 (18.6%)	
>6 years	117	27 (23.1%)	
Total	964	155 (16.1)	

Table 2: Lactation stage related seroprevalence of Johne's disease

Parameter	No of examined animal	No of positive (%)	P value
Early stage (<3months)	210	42 (*20%*)	0.002
Mid stage (3-6 months)	62	10 (*16.1%*)	
Late stage (>6 months)	269	53 (*19.7%*)	
Dry stage	140	23 (*16.4%*)	
Total	681	128 (*18.8%*)	

Table 3: Relation between milk yield and prevalence of Johne's disease

Milk yield (L)	No of examined animal	No of positive (%)	P value
<20	244	54 (*22.1%*)	0.04
20-30	233	39 (*16.7%*)	
>30	64	12 (*18.8%*)	
Dried cows	140	23 (*16.4%*)	
Total	681	128 (*18.8%*)	

Table 4: Relation between pregnancy state and seroprevalence of Johne's disease

Pregnancy state		No of examined animal	No of positive (%)	P value
Non-pregnant		203	44 (*21.7%*)	0.1
Pregnant	1st trimaster	33	3 (*9%*)	
	2nd trimaster	185	19 (*10.3%*)	
	3rd trimaster	290	48 (*16.6%*)	
Total		711	114 (*16%*)	

4. DISCUSSION

Paratuberculosis (Johne's disease) is one of the most economically important diseases of dairy cattle and caused by MAP, which cause severe economic losses in dairy production farms and believed to be a potential public health hazard. Infected cows usually suffer from weight loss, diarrhea, decreased milk production and even death. Diagnosis of MAP usually based on detection of MAP itself or the host's immune response against it (Timms et al., 2011).

At present, the ELISA is the most sensitive and specific test for serum antibodies to MAP in cattle (Speer et al., 2006). Despite of low sensitivity and specificity, ELISA is considered a method of choice for diagnosis of Johne's disease positive herds. This is due to the ease of sample collection, rapid procedure, low cost and possibility of testing a large number of samples in a short time (Abraham et al., 2014; Gupta et al., 2012).

A total number of 964 animals tested by ELISA. Out of them 155 animals reacted positively with ELISA, while 809 animals reacted negatively in the farm with a prevalence rate 16.1%. The prevalence of paratuberculosis was in parallel to that recorded by (Abraham et al., 2014) who reported that the prevalence of paratuberculosis among dairy cattle was (16%). In another side, the obtained results are different from the result obtained by (Fawzy et al., 2013), who found that, the highest prevalence rate of the disease among the examined farms, that may be attributed to size of sample collected and method of diagnosis and the prevalence rate was 46.6%. The high percentages may be due to bias by investigating paratuberculosis-suspected farms. In contrast, the lower prevalence was in study conducted in Sharkya and Alexandria governorates by Abdel-Mughni and Abdel-Rashid. (2012) showed that prevalence 9.3% and 11.04% in Alex. desert road and Sharkya farm, respectively. Also, Riad et al. (2016) reported prevalence (10.9%). Age-wise sero-prevalence of paratuberculosis is shown in Tab 1. The results revealed that the prevalence of the disease was significant difference between different age of examined animal (P=0.001) and the prevalence was higher in age stage (3-6 years) in comparison with other ages. Similar to our findings, other studies reported a relatively higher prevalence of disease in older age animals than that of young ones (Hussain et al., 2018; Woodbine et al., 2009).

Also, the obtained results revealed that the disease was more prevalent in early stage of lactation with significant difference with other stages. This result was in contrast to that reported by (Hussain et al., 2018), the prevalence was higher in late lactation. In addition, the heavy lactating animals showed higher seropositive rate with significant difference in comparison with others examined cattle. These results also reported by (Hussain et al., 2018), showed that the disease was more prevalent in high producing animals. The stress of high milk production might make the animal more vulnerable to the MAP or there may be some genetic association in high producing animals.

5. CONCLUSION

The present study reports the circulation of MAP among dairy cattle in Egypt and play as an important role in animals suffer from chronic diarrhea. In addition, the study revealed that the occurrence of the disease increases with age and in heavy producing animals.

6. REFERENCES

Abdel-Mughni. and Abdel-Rashid ,F. 2012. Bacteriological, molecular and biochemical studies on cattle infected with paratuberculosis The Scientific Conference of Provincial Labs: 10-11 .

Abraham, A., Tomas, N., Joseph, S., Raghavan, K., 2014. Comparitive study on the efficacy of ELISA and IS900 PCR for the diagnosis of paratuberculosis in goats. Biotechnology in Animal Husbandry 30, 661-667.

Benazzi, S., Berrada, J., Schliesser, T., 1995. First report of paratuberculosis (Johne's disease) in sheep in Morocco. Journal of Veterinary Medicine, Series B 42, 339-344.

Dorshorst, N.C., Collins, M.T., Lombard, J.E., 2006. Decision analysis model for paratuberculosis control in commercial dairy herds. Preventive veterinary medicine 75, 92-122.

Fawzy, A., Prince, A., Hassan, A., Fayed, A., Zschöck, M., Naga, M., Omar, M., Salem, M., El-Sayed, A., 2013. Epidemiological studies on Johne's disease in ruminants and Crohn's disease in humans in Egypt. International Journal of Veterinary Science and Medicine 1, 79-86.

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.

Hruska, K., Slana, I., Kralik, P., Pavlik, I., 2011. Mycobacterium avium subsp. paratuberculosis in powdered infant milk: F57 competitive real time PCR. Veterinarni Medicina 56, 226-230.

Hussain, S.M., Javed, M.T., Rizvi, F., Qamar, M., 2018. Prevalence of paratuberculosis in cattle and buffaloes in Punjab Pakistan. Pakistan Journal of Agricultural Sciences 55.

Lu, Z., Mitchell, R., Smith, R.L., Van Kessel, J., Chapagain, P., Schukken, Y., Grohn, Y., 2008. The importance of culling in Johne's disease control. Journal of theoretical biology 254, 135-146.

Riad, E.M., Amany, N. dapgh ., and Dalia M. Mohsen 2016. Evaluation of ELISA and The Conventional Methods Used in JOHNE' S Disease Diagnosis in Cows . Egypt. J. Chem. Environ. Health, 2 (2):324-334(2016) On line ISSN: 2536-9164.

Selim, A., Gaede, W., 2015. Comparative evaluation of PCR assay for direct detection of Mycobacterium avium subsp. paratuberculosis in Ruminant. Asian J Anim Vet Adv 10, 761-771.

Speer, C., Scott, M.C., Bannantine, J.P., Waters, W.R., Mori, Y., Whitlock, R.H., Eda, S., 2006. A novel enzyme-linked immunosorbent assay for diagnosis of Mycobacterium avium subsp. paratuberculosis infections (Johne's Disease) in cattle. Clin. Vaccine Immunol. 13, 535-540.

Timms, V.J., Gehringer, M.M., Mitchell, H.M., Daskalopoulos, G., Neilan, B.A., 2011. How accurately can we detect Mycobacterium avium subsp. paratuberculosis infection? Journal of microbiological methods 85, 1-8.

Tiwari, A., VanLeeuwen, J.A., McKenna, S.L., Keefe, G.P., Barkema, H.W., 2006. Johne's disease in Canada: Part I: Clinical symptoms, pathophysiology, diagnosis, and prevalence in dairy herds. The Canadian Veterinary Journal 47, 874.

Vidić, B., Savić, S., Prica, N., 2011. Paratuberculosis in cattle: The outspread, diagnostics and control. Biotechnology in Animal Husbandry 27, 335-344.

Woodbine, K.A., Schukken, Y.H., Green, L.E., Ramirez-Villaescusa, A., Mason, S., Moore, S.J., Bilbao, C., Swann, N., Medley, G.F., 2009. Seroprevalence and epidemiological characteristics of Mycobacterium avium subsp. paratuberculosis on 114 cattle farms in south west England. Preventive veterinary medicine 89, 102-109.