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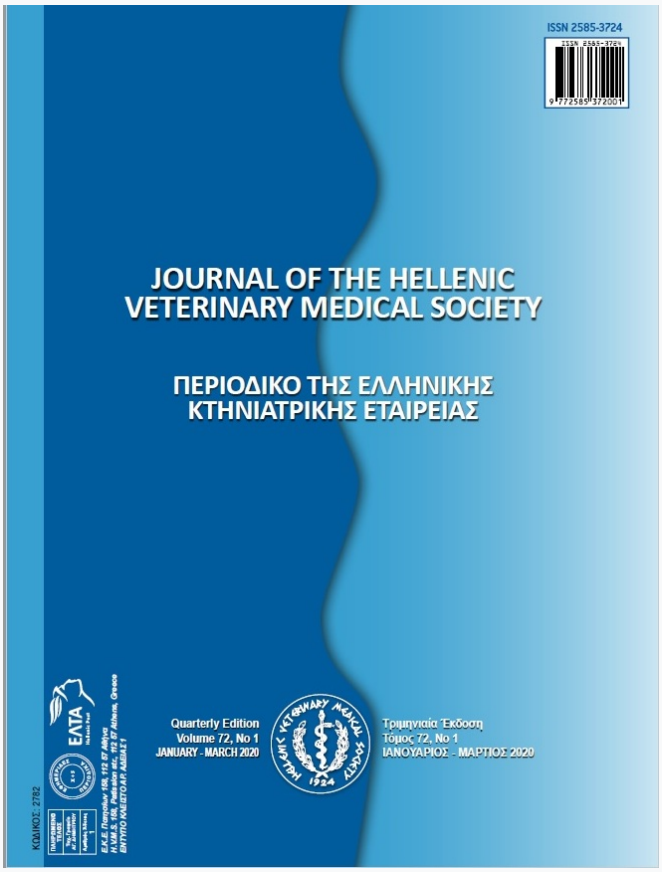

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Research article
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Seroprevalence and associated risk factors for bovine paratuberculosis in dairy cattle

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ABSTRACT: Paratuberculosis is an economically important disease in dairy cows and requires continuous surveillance. The study aimed to investigate the seroprevalence of bovine paratuberculosis (Johne's disease) in one of dairy farm in Egypt. A total of 964 dairy cattle were blood sampled and examined with an ELISA method. One-hundred fifty-five (16.1%) samples reacted positively. The results revealed that age was significantly associated with the prevalence of paratuberculosis in dairy cattle, particularly in animals over 6 years of age. Furthermore, the lactation period, milk yield and pregnancy had non-significant effect on appearance of paratuberculosis in cattle.

Keywords: *Paratuberculosis, Cattle; Seroprevalence: Risk factors; ELISA.*

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INTRODUCTION

Paratuberculosis or Johne's disease (JD) is a chronic debilitating, enteropathy disease of ruminants that is caused by bacterium *Mycobacterium avium subsp. paratuberculosis* (MAP) (Selim and Gaede, 2015; Selim et al., 2019a). The disease is responsible for economic losses worldwide in terms of medication, early culling, reduction in production, severe weight losses and reduced in body weight. The disease can be transmitted through multiple routes either horizontal route by direct transmission through ingestion of contaminated food with feces of adult infected cows or vertical route from dam to calf (Selim et al., 2013). Moreover, milk and its products being a potential source of infection for MAP in humans, causing Crohn's disease or an inflammatory bowel disease (Hruska et al., 2011).

Paratuberculosis has a prolonged incubation period and present slowly in various clinical phases. The infected animals remain in subclinical phase for long period without obvious clinical signs (Gupta et al., 2012). However, animals in advanced clinical phase become weak, emaciated, and suffer from profuse watery diarrhea. Intermandibular oedema or bottle jaw is characteristic of this stage and animals eventually die of dehydration signs and cachexia (Tiwari et al., 2006). Bovine paratuberculosis is worldwide disease, was first reported in European cattle at 1895 then has spread throughout the developed and parts of the developing world, particularly in the bovine dairy industries (Benazzi et al., 1995). Moreover, the herd prevalence level is high could be reach to 50%. Diagnosis of paratuberculosis is established by direct detection of causative agent using selective media or indirectly through detection and estimation 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; Selim et al., 2018; Selim et al., 2019b).

In Egypt, the epidemiological information about paratuberculosis is scarce despite few studies have been reported the seroprevalence of MAP among dairy cattle in some localities and detected MAP-DNA in feces of clinical infected cattle (Selim et al., 2019a; Selim et al., 2019b). Paratuberculosis is a challenging and economically 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 aimed to investigate the seroprevalence of bovine paratuberculosis among dairy cattle and estimate the associate risk factors for MAP infection.

MATERIAL AND METHODS

Ethics statement

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

Samples collection and preparation

A total 964 serum samples were collected from 964 cattle to study the seroprevalence of the disease in one of dairy farm in Egypt. All data related to examined animals such as age, lactation period, milk yield and pregnancy stage were collected from farm's owner. Blood samples (5ml) were collected from each animal using vacutainer tube. Serum was separated by centrifugation at 4025 x g for 10 min and stored at -20°C until further serological analysis.

Serological examination using ELISA

The antibody titer against MAP infection was determined using the commercially available IDEXX Paratuberculosis ELISA kit (IDEXX Laboratories, Inc., Westbrook, ME) according the manufacturer's instructions. The optical density (OD) was measured at 450nm using ELISA reader plate (BioTek, USA). The results were expressed based on sample/positive (S/P) ratio.

S/P = (OD of unknown sample - OD of negative-control sample)/(OD of positive-control sample - OD of negative-control sample). Samples having an S/P ratio < 0.2 were considered negative, whereas samples with an S/P ration ≥0.25 were considered a positive.

Statistical analysis

The data were analyzed by the Chi-square using SPSS V17 (IBM, USA). The results were considered significant at a probability level ≤0.05. The relation between each variable and seroprevalence of MAP infection was determined using univariant and then multivariant logistic regression model.

RESULTS

Out of 964 examined sera, the seropositive

cases of MAP infection among dairy cattle were 155 (16.1%). The logistic regression analyses of obtained results showed that the results differed according to the studied factors. The seroprevalence of bovine paratuberculosis was signific antly differed between age groups of examined cattle. The disease was more prevalent in old cattle >6 years of age (23.1%, 95%CI: 16.01-31.96) in comparison with other age groups, as shown in (Table 1). Moreover, the seroprevalence of bovine paratuberculosis showed non-significant relation with stage of milk production or milk yield, (Table 2).

In addition, the pregnancy status of cattle showed significant relationship with number of seropositive MAP cases, where the seroprevalence of MAP infection was more prevalent in non-pregnant cattle (21.7%, 95%CI: 16.34-28.1) and during late stage of pregnancy at third trimester (16.6%, 95%CI: 12.56-21.45), (Table 3).

Two risk factors were fitted for multivariant logistic regression model, (Table 4). The results revealed that the risk of MAP infection increased in cattle of >6 years old (OR=3.5, 95%CI: 1.76-7.08), cattle of middle age (OR=2.7, 95%CI: 1.48-4.94) and in heavy pregnant animal during third trimester of pregnancy (OR=1.9, 95%CI: 0.58-6.79).

Table 1: Age related seroprevalence of bovine paratuberculosis in cattle based on ELISA

Factor	No of examined animals	No of positive (%)	95%CI	P value
Age				
<2 years	180	14 (7.8%)	4.48-12.97	
2-3 years	264	39 (14.8%)	10.8-19.76	0.001*
3-6 years	403	75 (18.6%)	15-22.83	
>6 years	117	27 (23.1%)	16.01-31.96	
Total	964	155 (16.1)	13.85-18.59	

*The results are significant at $p < 0.05$

Table 2: The relation of milk production and stages with seroprevalence of bovine paratuberculosis

Factor	No of examined animals	No of positive (%)	95%CI	P value
Lactation stage				
Early stage (<3months)	210	42 (*20%*)	14.94-26.18	
Mid stage (3-6 months)	62	10 (*16.1%*)	8.41-28.13	0.7*
Late stage (>6 months)	269	53 (*19.7%*)	15.22-25.06	
Dry stage	140	23 (*16.4%*)	10.91-23.85	
Milk yield				
<20	244	54 (*22.1%*)	17.19-27.97	
20-30	233	39 (*16.7%*)	12.31-22.3	0.4*
>30	64	12 (*18.8%*)	10.47-30.85	
Dried cows	140	23 (*16.4%*)	10.91-23.85	

The results are not significant at $p > 0.05$

Table 3: Relation between pregnancy state and seroprevalence of bovine paratuberculosis

Pregnancy state		No of examined animal	No of positive (%)	95%CI
Non-pregnant		203	44 (*21.7%*)	16.34-28.1
pregnant	1st trimester	33	3 (*9%*)	2.38-25.47
	2nd trimester	185	19 (*10.3%*)	6.46-15.8
	3rd trimester	290	48 (*16.6%*)	12.56-21.45
	Total	711	114 (*16%*)	13.45-18.98

The results are significant at P=0.01

Table 4: Risk factors associated with MAP seropositivity in dairy cattle

Risk factors	OR	95% of OR	P value
Age			
<2 years		ref	
2-3 years	2.03	1.06-3.86	0.03
3-6 years	2.7	1.48-4.94	0.001
>6 years	3.5	1.76-7.08	0.001
Pregnancy status			
1st trimaster		ref	
2nd trimaster	1.1	0.31-4.10	0.8
3rd trimaster	1.9	0.58-6.79	0.2
non-pregnant	2.7	0.80-9.49	0.1

OR; Odds ratio
CI; Confidence interval
The result is significant at $P < 0.05$

DISCUSSION

Johne's disease is one of the most economically important diseases of dairy farm and believed to be a potential public health hazard. The 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; El-haig et al., 2018; Selim et al., 2020b).

The ELISA is the most sensitive and specific test for detection of serum antibodies against MAP-infection in cattle (Speer et al., 2006). ELISA is considered a method of choice for diagnosis of Johne's disease in 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 (Gupta et al., 2012; Abraham et al., 2014; Ali et al., 2019; Selim and Ali, 2020).

A total 964 cattle were examined for anti-MAP antibodies by ELISA. Out of them 155 (16.1%) animals reacted positively. The prevalence of bovine paratuberculosis came in accordance with other previous rate 15% in lower saxony, Germany (Böttcher, 1997). In contrast, the obtained seroprevalence rate was higher than previous rate, 2.4 % in Italy (Lillini et al., 2005), 5.5% in Germany (Donat et al., 2005) and 12.6% in Bhutan, Austria (Gurung et al., 2018) but lower than seroprevalence rate in Austria, 19% (Dreier et al., 2006). The difference of seroprevalence rate in different studies may be attributed to size of sample collected and method of diagnosis (Gurung et al., 2018; Selim et al., 2020a).

The seroprevalence of bovine paratuberculosis had significant disparity between different age groups of examined cattle. The disease was more prevalent among elder cattle (>6 years old) in comparison with young ones. Similar to our findings, other studies reported a relatively higher prevalence of disease in older age animals than that of young ones (Woodbine et al., 2009; Hussain et al., 2018). We believe that the disease was more frequent among animals due to frequent exposure of infection during life and decrease the immunity of animals with advancing of age (Harris and Barletta, 2001; Hailat et al., 2010).

Interestingly, the results of the present study revealed a non-significant relation between stage of milking or milk yield in dairy cattle and seropositivity to MAP infection. The result was disagree with (Hussain et al., 2018), they reported the highest prevalence rate of paratuberculosis in late stage of lactation, that

could be attributed to stress of lactation and increase susceptibility to get MAP infection (Rathnaiah et al., 2017). Further, the seroprevalence of MAP infection more common in lactating animals may be attributed to milk production considered as a stress factor on animals or due to some genetic association in high producing animals (Sorge et al., 2011).

In addition, the assessment of risk factors associated with MAP infection revealed strong relationship between age and pregnancy status of animals and seroprevalence of MAP infection. Similar results were concluded by (Sergeant and Baldock, 2002; Khan et al., 2010; Selim et al., 2019b).

CONCLUSIONS

The present study confirmed the presence of antibodies of MAP among dairy cattle in Egypt and give attention to necessary for application of large-scale screening for bovine paratuberculosis in Egypt. The present study revealed strong association between seroprevalence of MAP and age and pregnancy status of animals. Thus, the periodical screening of old or pregnant animals is very important to identify the infected animals and for early control.

CONFLICT OF INTEREST

None declared by the authors.

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