SEROLOGICAL DETECTION OF JOHNE'S DISEASE IN CATTLE USING ELISA TECHNIQUES

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Abstract

This study was designed to diagnose Johne's disease infection in serum of cattle. Ninghty two blood samples were taken from cattle of Ninevah province and tested for detection of antibodies against Johne's disease. Indirect ELISA Kit for *Mycobacterium avium* subspp. *Paratuberclosis* used to examined animals serum samples, Clinically cattle show emaciation, decrease in milk production and some cases of persistence diarrhea. ELISA test show that 1.08 % of serum samples were positive and all the seropositive animals were more than 2 years aged. In conclusion the cattle were infected by Johne's disease and animals above 2 years were more susceptible to infection.

Introduction

Johne's disease is a chronic progressive disease of ruminants caused by *Mycobacterium avium* subspp. *Paratuberclosis* (MAP) (1), the causative agent is gram positive, acid fast bacteria. the name Johne's disease comes from A.M. Johne's and L. Frothingham which are firstly reported the disease in 1894 in Germany(2). Johne's disease cause high economic loss due to decrease in cattle production which suffer from persisting diarrhea with continuously loss of weight and finally death or culling of infected cattle(3,4) also there are some evidence that the bacteria is associated with human Crohn's disease (5,6).

Fecal –oral rote is the ordinary rote of transmission of disease, by ingestion of contaminated food and \ or water with feces of infected animals ,wild ruminant and monogastric with other wild life may play an important role in epidemiology of the disease(7,8).Younger calf (under 6 months age) are most susceptible for infection which mostly occur during the first 6 months of life, but the clinical sings delay for 6 months to 15 years for appear. The animals may still in subclinical state and shedding bacteria before appearance of clinical disease (1,3,4). Because no effective vaccination are found, the control plays an important role in decreasing the spread of the pathogen within heard by (culling) reduction pathogen loads within infected herds (9), culture from feces or tissue to isolate *Mycobacterium avium* subspp. *Paratuberclosis* is a standard method for diagnosis of infected animals but high cost and longer period ranged 3-5 months which is required for bacterial colony to appear and the irregular shedding of the

pathogen from subclinical infected animals, all these factors make culture less sensitive (10). So that ELISA which is rapid, inexpensive, with sufficient specificity and sensitivity is used alone or with fecal culture to screen and detect the infected cases in cattle herds (11,4).Due to the absence of information about cattle Paratuberclosis infection in Iraq, this study was under taken to screen the presence of clinical, subclinical infection in cattle by detecting antibodies in serum against Johne's disease.

Materials And Methods

1- Samples: Ninghty two samples were taken from cattles in 3 area of Ninevah province (shalalat , abattoirs, kokgaly). the animals from both sex, aged between 2-4 years, clinical signs which appear in animals are also recorded, All sample were taken in the period between December 2007- April 2008. The blood samples were collected from jugular vein , 5 ml of blood was collected from each animal and allowed to clot. The serum was taken after centrifugation at 3000Xg for 10 min . Sera were subsequently stored at -20C° until used (12).

2- ELISA test: Detection of *Mycobacterium avium* subspp. *Paratuberclosis* antibodies was done by the ELISA test using Paratuberclosis Indirect ELISA Kit (ID.VET- France). This test was done according to the procedure of the company, in order to avoid cross-reaction, samples were pre-incubated in neutralized buffer containing *Mycobacterium phlie* before being transferred to the coated plate. Any anti-MAP antibodies present from an antibody–antigen complex with the MAP epitopes. An anti-ruminant IgG-peroxidase (Po) conjugate was added to the microwells. to fix to the anti –Map antibodies, forming an antigen –antibody conjugate –peroxides complex.

After washing in order to eliminate the excess conjugate, the substrate solution (TMB) was added. The resulting coloration depended on the quantity of specific antibodies present in the specimen to be tested :

- in the presence of antibodies, a blue solution appeared which became yellow after addition of the stopping solution
- in the absence of antibodies, no coloration appeared.

The microplate was read by spectrophotometer (ELISA reader) at 450 nm that was done in the Virology Lab – College of Veterinary Medicine –University of Mosul.

Results

Most animals showed clinical signs of emaciation, decrease in milk production in cows, with some sporadic cases of persistence diarrhea. Cattle serum samples showed that 1.08 % had significant antibodies titer to infection with *Mycobacterium avium* subspp. *Paratuberclosis* in all area of sample source, while 98.92% of serum samples were negative, all positive samples appeared to be collected from kokgaly (table 1).

Table (1) Results of ELISA test.

Cattle sample area	Number of tested samples	Percentage of Positive samples %	Percentage of Negative samples %
kokgaly	28	3.6	96.4
shalalat	39	0	0
abattoirs	25	0	0

Relationship between age ,sex show that 2.38% of male aged more than 2 year appear positive while all females appear negative in all ages.(Table 2).

Table (2) Results of Relationship between age ,sex and ELISA test

Cattle sample age and sex	Number of tested samples	Percentage of Positive samples %	Percentage of Negative samples %
≥2 , Male	42	2.38	97.62
≤ 4 , Females	50	0	0

Discussion

This study was done to screen the presence of cattle infection by detecting antibodies against Johne's disease using ELISA test, which is very sensitive and useful tool to diagnose and control Johne's disease (13). Serum sample show that 1.08% of Cattle were infected with *Mycobacterium avium* subspp. *Paratuberclosis* this result is closer
to that 2% recorded by Haji hajikolaei in AHWAZ–Iran.(14), All of the seropositive samples
found in kokgaly and this partly because overcrowding of animals in the area, and transport infection from contaminated food or water by feces of infected animals(15,7).

Large percentage of seronegative samples appeared may due to the delay in development of humeral response in the infected animals, which progresses to reach high level in the late stage of the disease with appearance of clinical sings (10,16,17,18). most seronegative sample is collected from asymptomatic cattle, thus its possible that the titer of antibodies is still low and undetectable by our ELISA assay.

Males were more infected than females, since most samples of male were collected from kokgaly area in which all of the seropositive samples were found, animals above 2 years old were positive. This result agree with Nielsen S.S (19), who said "that highest probability of test positive ELISA was from 2.5-4.5 years of age " thus because younger animals were infected in the early stage of life by ingestion contaminated milk (20), which lead to the rapid developed of cell-mediated immunity followed by increase of humeral antibodies titer with the development of the animals age (21) .

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التحري مصلياً عن مرض جونز في الأبقار باستخدام تقنية الاليزا

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الملخص

صممت هذه الدراسة لغرض الوقوف على تواجد الإصابة بمرض جونز في الأبقار، جمعت ٩٢ عينة من دم الأبقار في محافظة نينوى وفحصت لغرض الكشف عن الأجسام المضادة لمرض جونز باستخدام فحص الاليزا غير المباشر لبكتريا *Mycobacterium avium* subspp. *Paratuberclosis* . سريريا أظهرت الأبقار علامات الهزال وقلة إنتاج الحليب وبعض الحالات عانت من الإسهال المستمر، اظهر فحص الاليزا أن 1.08 % من العينات كانت موجبة مصليا بالإضافة لذلك فان معظم الحيوانات الموجبة كان عمرها يزيد عن السنتين. يستنتج من ذلك بأن الأبقار المصابة بمرض جونز و الحيوانات التي يزيد عمرها عن السنتين هي أكثر عرضة للإصابة بهذا المرض.