

Serodiagnosis of Johne's disease by indirect ELISA in ovine

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(Received August 11, 2008; Accepted October 5, 2009)

Abstract

The study included collection of 92 serum samples from local Awassii breed in Mosul and Karakosh regions, some of them show clinical signs for John's disease, all samples were assayed using indirect Enzyme-Linked Immunosorbent Assay (ELISA) to detect antibodies against *Mycobacterium avium* subsp. *paratuberculosis* (Map). The results showed that 7/92 (7.6%) samples were positive for antibodies against (Map), and 7/89 (7.9%) were positive in female and 0/3 (0%) in male.

Keywords: *Mycobacterium*, Paratuberculosis, Ovine Johne's disease, Indirect ELISA, Serodiagnosis.

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التشخيص المصلي لمرض جونز في الأغنام باستخدام اختبار الاليزا غير المباشر

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فرع الأحياء المجهرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

تضمنت الدراسة جمع ٩٢ عينة مصل من الأغنام المحلية (العواسي) من مناطق متفرقة من مدينتي الموصل وقره قوش والتي ظهرت على بعضها العلامات السريرية الخاصة بمرض جونز وتم فحص جميع العينات باستخدام اختبار الاليزا غير المباشر للتحري عن الأجسام المضادة الخاصة بالجرثومة *Mycobacterium avium* subsp. *paratuberculosis* (Map). أظهرت النتائج أن ٩٢/٧ عينة كانت موجبة للأجسام المضادة بنسبة 7.6%. كما أن الحالات الموجبة في الإناث كانت 89/7 بنسبة 7.9% في حين كانت في الذكور ٣/٠ بنسبة ٠%.

Introduction

Johne's disease is also known as "paratuberculosis" because it is caused by *Mycobacterium avium* subspecies *paratuberculosis* (Map). It is a contagious disease in dairy cattle, sheep, goats and other ruminants (1). The clinical manifestation of paratuberculosis in sheep tends to prevail at younger age than in cattle. Chronic weight loss is the primary clinical sign of paratuberculosis in sheep and goats. Affected sheep will experience progressive weight loss over a period of weeks to months and eventually die (2). Only 10-20% of clinical cases present with diarrhoea or clumping of faeces in the advanced stage of the disease (3). Johne's disease is commonly found in dairy cattle herds and sheep flocks but identification of individual sheep with

subclinical infections is difficult. Animals apparently are infected when young but, while shedding the organism via faeces, these animals may not show clinical symptoms for several years. Infected animals may have reduced feed efficiency without obvious clinical signs of disease. (4,5). In sheep flocks, the fecal culture detects less than 12 % of clinical cases and requires up to 12 months of incubation, making it an impractical diagnostic test (6,7). Three different tests are currently available for measuring antibodies against *Mycobacterium avium* subsp. *paratuberculosis* in the serum of infected animals. These are the complement fixation test (CFT), the agar gel immunodiffusion (AGID) test and enzyme-linked immunosorbent assay (ELISA). ELISA or AGID are still the main options in live animals (8). Among various

serological tests for Johne's disease, ELISA-based tests are widely used and can be conducted rapidly and require limited expertise (9).

Materials and methods

A total of 92 blood samples, 3 samples from males and the others from females were collected from local Awassi breed sheep that some of them showed clinical signs for John's disease including emaciation, unresponsive to dewormers and antibiotics. Appetite was often good, in spite of weight loss, all animals wer not previously vaccinated against Johne's disease and they were >2 years old. The samples were collected through April 2008 from different regions, 69 samples represent 3 flocks containing 580 sheep from Mosul and 23 samples represent 2 flocks containing 264 sheep from Karakosh. Samples were submitted to the department of microbiology (College of Veterinary Medicine, Mosul, Iraq); within 24 h after bleeding, serum samples were separated and stored at – 20°C until they were assayed (2,10).

A commercial ELISA kit (ID SCREEN®, Paratuberculosis Indirect) for detection of antibodies against Mycobacterium avium subsp. paratuberculosis in ovine serum samples was used, the kit has been supplied from (ID Vet (innovative diagnostics)-France). The principle of the test depends on indirect ELISA. Sera were tested according to the manufacturer's instructions for ovine, the absorbance reading O.D in all ELISA plate wells were measured at 450 nm using an automated ELISA reader. ELISA optical density (OD) readings were transformed to Serum/Positive percentage (S/P%) according to a specific equation cited by manufacturer. The sample considered positive if it gives S/P % ≥ 70%, 60% < S/P % < 70% considered doubtful, S/P % ≤ 60% considered negative. $S/P\% = \frac{OD\ sample - ODNC}{ODPC - ODNC}$.

Results

The results showed that 7/92 samples 7.6% were positive for antibodies against Mycobacterium avium subsp. paratuberculosis, P<0.001. The results are detailed in table 1. Also the results showed no positive results in males 0/3 (0.0%) when compared with females 7/89 (7.9%). The distribution of S/P % values for samples, positive control and negative control are given in figure 1.

Table 1: Positive samples according to Mosul regions

Region	No. of samples	Positive	%
Mosul	69	5	7.3
Karakosh	23	2	8.7
Total	92	7	7.6

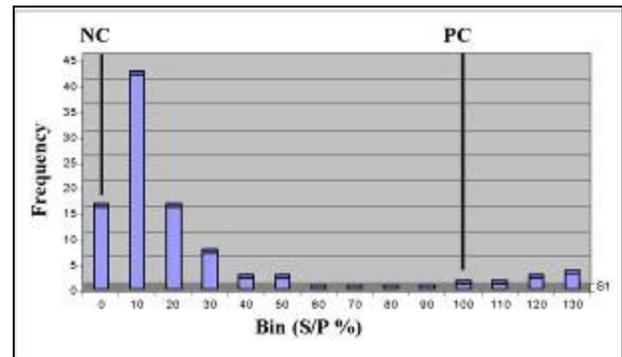


Figure 1: Distribution of ELISA S/P % values for (n=92), positive controls (PC) and negative controls (NC). ID SCREEN®, Paratuberculosis Indirect ELISA kit, ID Vet (innovative diagnostics).

Discussion

The aim of this study was to investigate Johne's disease in sheep by serodiagnosis using ELISA as there are indications for M. avium subsp. paratuberculosis infection and unresponsive to dewormers and antibiotics. All sheep were selected >2 years old as the clinical signs are commonly not evident until at least 18 months of age (6). As the cultivation of sheep strains of M. avium subsp. paratuberculosis using culture media (Herrold's egg yolk medium) has been extremely difficult to perform, the selection of ELISA in this study was based on the studies by (6,12,13). Among the antibody tests, ELISA is more sensitive than AGID and CFT test (14,15). It's performance is similar in cattle, sheep, and goats (16,17) and can be used with comparable sensitivity for either milk or serum samples (18). AGID test is considered less sensitive than ELISA and CFT (19). Since a strong humoral response does not occur until the later stage of Johne's disease, the sensitivity of these 3 tests is the highest for animals with lepromatous lesions (8,20,21), those with clinical symptoms (20,22,23), or those that shed large numbers of bacteria (18,24). Therefore, the main limitation of these antibody tests is their inability to identify animals in early infection (25,26). Conversely, all of these tests are highly specific, with false-positive results occurring at low frequency (25). The ELISA couldn't detect all animals with clinical signs. Comparative studies of the CFT, AGID test and ELISA repeatedly show discrepancies in the ability to identify all infected animals (27,28). This may be due to genetic variation of the individual animal or the lack of representation of the entire range of immunodominant antigens for Mycobacterium avium subsp. paratuberculosis within a given test (29). There are few studies about ovine Johne's disease in the Arab countries, so there is no

sufficient data to compare with the results of this study. The total positivity % (7.6%) is considered high as the disease now has a virtually worldwide distribution in farmed ruminants as well as many species of wild ruminants (1, 11), also the high S/P% for the all 7 positive sera indicate the high levels of antibodies against (Map) in the affected sheep (20, 22, 23), this mean that we need more investigations to confirm Johne's disease in sheep and also in other susceptible ruminants. In this study few numbers of male samples were included, only 3 samples were tested and all gives negative results 0.0%, because of the breeding style in keeping few numbers of rams for each flock. This study has resulted in detection of antibodies against *Mycobacterium avium* subsp. paratuberculosis in sheep. The study was done for the first time in Iraq to provide information about this disease for subsequent studies.

Acknowledgments

This study was supported by College of Veterinary Medicine, University of Mosul. Mosul, Iraq.

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