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Original article

First prevalence report of contagious agalactia by *Mycoplasma agalactiae* in sheep in Jericho-Palestine

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ABSTRACT

Contagious agalactiae caused by *Mycoplasma agalctiae* (*M. agalctiae*) implicated for important losses in small ruminant due to mortality, decreased milk production, and cost of treatment and prevention. There is a lack of information about the disease status in Palestine. In this study, a survey of *M. agalctiae* antibodies in sheep blood from Jericho city near the Jordan River in the eastern Palestine was conducted. A total of 611 randomly selected sheep of different ages were investigated from different farms in two regions in Jericho city during the period from December 2016 to March 2017 to. Blood samples were collected and serum was subjected to serologic examination for detection of antibodies against *M. agalctiae* major membrane lipoprotein of (p48) using enzyme-linked immunosorbent assay (ELISA, CHECKIT, IDEXX). The overall *M. agalctiae* seroprevalence rate was 11.5%. Regarding the distribution of the disease in the investigated regions in Jericho city; the seroprevalence rate of *M. agalctiae* in sheep was 13.5% in Al-Jiftlik and 4.4% in Fasa'il. This is the first report of *M. agalactiae* infection in Palestine. Further studies are required to determine other causes of contagious agalactiae and to include other regions in Palestine.

1. Introduction

Mycoplasma agalctiae (*M. agalctiae*) is the main cause among five causing contagious agalactia in the small ruminant. Other important *Mycoplasma* affecting sheep and goats are *M. mycoides* subsp. mycoides large colony, *M. capricolum* subsp. *capricolum*, *M. mycoides* subsp. *capri* and *M. putrefaciens* (Madanat et al., 2001). The disease is characterized by polyarthritis, keratoconjunctivitis, pneumonia as well as mastitis and occasional abortion in the female. The morbidity range from 30% to 60% and the mortality rate in adult animals is not high. However, the mortality in offspring is 40% to 70% (Corrales et al., 2007). The decrease or loss of milk production and abortions in pregnant dams are the main economic impact of the disease. In Palestine, sheep industry has traditional relevant as well as important foods and commercial products. The milk, urine, placenta and fetal fluid of the infected animals are the main sources of the infection in sheep and other susceptible animals. The natural routes of infection are oral, respiratory, mammary routes and venereal transmission (Corrales et al., 2007). Incubation varies from one week to two months, infected animals develop bacteremia accompanied by fever (Madanat et al., 2001). The infectious agent is then transferred by circulating blood to the target organs, i.e., the mammary gland, eyes, lymph nodes, joints and tendons, in which inflammatory changes are produced. Affected animals show mastitis and drop of milk production (Nicholas et al., 2008). Pregnant animals may abort due to inflammation of the uterus or may give birth to non-viable offspring. Infected rams may develop epididymitis and infections of testes, leading to male sterility and infertility. The organism also excretes in semen that has a role in venereal transmission route of the disease (Prats-van der Ham et al., 2016).

The occurrence of contagious agalactia has been reported in different parts of the world (Al-Momani et al., 2008; Zendulkova et al., 2007). Considering the abundance of sheep in Palestine and the importance of contagious agalactia, and taking into the account the lack of data about the presence of the infection in sheep in our country, the aim of this study was to investigate the presence and the seroprevalence of contagious agalactia by *Mycoplasma agalactia* in sheep for the first time in Palestine.

2. Materials and methods

2.1. Area of the study, sample collection and preparation

This study was performed in the period from December 2016 to March 2017 to determine the seroprevalence rate of *M. agalctiae* in sheep. The study was carried out in Jericho (31°52'16"N 35°26'39"E) in Eastern Palestine. 611 animals were sampled from two regions; Al-Jiftlik (n = 475) and Fasa'il (n = 136) in Jericho city (Fig. 1). The samples were randomly collected from sheep in plain tubes containing clotting activator (Vacuette, Serum Clot activator, Greiner bio-one, Kremsmunster, Austria), and sent to the diagnostic laboratory at Palestinian Livestock Development Center (PLDC) for analysis.

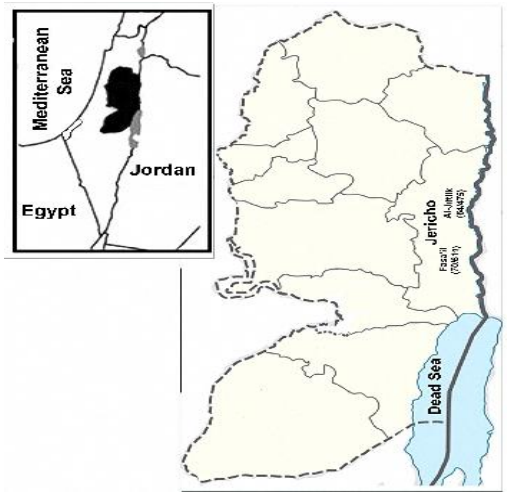

Fig. 1. Geographical distribution of seroprevalence of sheep *Mycoplasma agalactiae*. Numbers shown are positive animals from the tested animals in each region of Jericho city.

2.2. Laboratory examination

Antibodies to *M. agalactiae* were detected by IDEXX *M. agalactiae* Screening Ab Test (IDEXX Laboratories (Westbrook, ME). The enzyme-linked immunosorbent assay (ELISA) developed by IDEXX Laboratories (Westbrook, ME) uses microwells coated with major membrane lipoprotein of *M. agalactiae* (p48). Following the manufacturer instructions serum were considered positive when sample/positive control ration (S/P %) greater than or equal to 60%.

3. Results and discussion

Of the 611 blood samples serologically analyzed in this study, 70 (11.5 %) were *M. agalactiae* seropositive and 541 (88.5 %) were seronegative (Table 1). Out of 475 samples analyzed from Al-Jiftik region, 13.5% were seropositive and 411 were negative. The seroprevalence rate at Fasa'il region was 4.4% (6 out of 136).

M. agalactiae is implicated for important economic losses in small ruminants in numerous countries of Middle East (Al-Momani et al., 2011; Khezri et al., 2012; Zendulkova et al., 2007) and worldwide (Ariza-Miguel et al., 2012; Giangaspero et al., 2012). The economic loss is related to the clinical signs in the infected animals, decrease production and cost of treatment and prevention. *M. agalactiae* can infect both sex, transmitted venereally and cause infertility and abortion (Corrales et al., 2007; Prats-van der Ham et al., 2016). The occurrence of contagious agalactiae in Palestine has not been reported. This is the first epidemiological investigation of the disease in Palestine that report the presence and estimates prevalence rate of *M. agalactiae* antibodies of randomly selected sheep in two regions in Jericho city in Palestine. Currently, vaccination of sheep against *M. agalactiae* is not practiced in Palestine and the detected antibodies in this survey reflect a natural response to infection. At the animal level, our results revealed a seroprevalence rate of 11.5% among 611 animals analyzed. The seroprevalence rate of *M. agalactiae* in Palestinian sheep is within the range of seroprevalence rate in other reports from other countries. In Jordan, the seropositive rate in sheep in Jordan showing clinical signs of the disease was 3.8 % (3 out of 78) animals and 7.6 % had dubious results (6 out of 78) (Madanat et al., 2002). In Iran 32.5% (24 out of 69) were infected with *Mycoplasma* in culture, 15 isolates of them (32.6%) were positive for *M. agalactiae* with PCR (Khezri et al., 2012). In Spain, 36.8% (339 out of 922) of sample investigated with real time PCR and 9.2% (85 out of 922) by microbiological identification were positive for *M. agalactiae* (Ariza-Miguel et al., 2012).

Table 1
Seroprevalence of *M. agalactiae* in 611 sheep analyzed in two regions in Jericho city in Palestine.

Region	No. of animals	Positive	Negative	Seropositive (%)
Al-Jiftlik	475	64	411	13.5
Fasa'il	136	6	130	4.4
Total	611	70	541	11.5

The variation in the prevalence rate is mostly related to the sensitivity and specificity of the diagnostic test as well as the number of screened animals. The etiologic agent of contagious agalactiae is different in different geographical area. In Japan 26% (64 out of 246) of sheep investigated were seropositive to *M. ovipneumoniae* and none of the sera tested were serologically positive to *M. agalactiae* (Giangaspero et al., 2012). The seroprevalences of *M. mycoides* subsp. *capri* was 34% in Jordan (Al-Momani et al., 2011). The role of other *Mycoplasma* species in sheep contagious agalactiae in Palestine needs further investigation. In Palestine, it is common to rare sheep with other susceptible animals mainly goat in same herds for dairy production (statistics, 2014); contagious agalactiae is highly infectious disease of sheep and goats (Madanat et al., 2001). The disease is transmitted by direct contact as well as by milking the animals together (Al-Momani et al., 2008). The disease in goats is sporadic and the infection may persist for some time and be a source of infection for the other animals in a herd (Madanat et al., 2001). A number of risk factors for occurrence and dissemination contagious agalactiae have been previously studied; the presence of asymptomatic carriers in a herd appears to be a serious health risk. These animals carry and shed the infectious agent in their genital tracts and milk (Corrales et al., 2007). Improper discard the used water in the milking and cleaning area enhance disseminating the micro-organisms in the environment.

Uses of disinfectant and proper cleaning of the environment and milking utensils reduce the prevalence of the disease (Al-Momani et al., 2008).

Disease control options have been used in different countries in order to reduce incidence or prevalence of the disease. These measures include improvement the diagnosis and general awareness of the disease, especially animal shedders (Nicholas, 2002). Screening and eradication of carried and infected animals, but is not always practical in endemic and in developing countries (Nicholas, 2002). Perform epidemiological studies and actions to reduce dissemination of the infection between farm animals and humans. Using disinfectants as well as the availability of veterinary services reduce the risk for *M. agalactiae* infection (Al-Momani et al., 2008). Prophylactic treatment by antibacterial preparation reduces the severity of infection and limits the shedding of pathogens (Loria et al., 2003). Using of inactivated vaccine provides a good protection against the disease (Greco et al., 2002).

The current study covers only a small part of the issue concerned with contagious agalactia of sheep. Our further objectives are to using the most recent diagnostic methods and investigated other contagious agalactiae in other susceptible animals. The results presented here with more exact and comprehensive data on the isolation and identification of *M. agalactiae* in Jordan animals and to provide more details on the epidemiology of the disease.

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