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# Detection of *Chlamydomphila abortus* antibody in active reproductive rams in sheep herds in northern Palestine

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## ABSTRACT

*Chlamydomphila abortus* is an important pathogen in human and a variety of animals. Ovine chlamydiosis (also known as Enzootic Abortion of Ewes (EAE)) is a venereal transmitted disease associated with late abortion and economic impact in sheep production. There is a lack of information about its status in Palestine, in which sheep are the most abundant farm animal that has a traditional and economic importance. Particularly, the infection rate in male is not well documented. In the present study, 2608 blood samples were collected from active reproductive rams between April and June 2016 in five Palestinian cities (Jenin, Nablus, Jericho, Tulkarm, and Tubas). Sera were tested by ID Screen® *Chlamydomphila abortus* Indirect Multi-species enzyme-linked immunosorbent assay (ELISA) kit for detection of *C. abortus* immunoglobulin G (IgG) (IDvet, France). At the individual level, among 2608 samples analyzed, 385 (13.7%) were seropositive for *C. abortus*. The chi-square analysis was used to evaluate the risk factors associated with regions, which could provide the foundation to understand the occurrence of the diseases. There was a significant difference between the regions studied ( $P < 0.05$ ). Our study revealed *C. abortus* seroprevalence rate of 17.7% in Nablus and Tubas cities, followed by Jericho (14.2%), Jenin (9.6%) and Tubas (3.8%).

At the herd level, among the 353 farms studied, 188 (53.3 %) had at least one seropositive animal. The highest rate was found in the herds of Tubas city (90.9%), followed by Nablus (78.3%), Jenin (56.9%), Jericho (44.9%) and Tulkarm (36.4%). Most of the seropositive cases were observed in cities with large herd size. In conclusion, detection of *C. abortus* antibody in rams serum suggests a potential for venereal transmission that further attention should be paid to this disease in this region of the country.

**Keywords:** Seroprevalence, *Chlamydomphila abortus*, Rams, Sheep, Palestine.

## RÉSUMÉ

**Détection d'anticorps de *Chlamydomphila abortus* chez les béliers de reproduction dans le nord de la Palestine**

*Chlamydomphila abortus* est un agent pathogène important chez l'homme et l'animal. La chlamydiose ovine est une maladie transmissible vénérienne associée à des avortements tardifs et des impacts économiques dans la production de moutons. Il y a un manque d'information sur le statut de cette affection en Palestine, notamment chez les béliers. Dans cette étude, 2608 échantillons de sang ont été prélevés sur des bêtes à la reproduction entre avril et juin 2016 dans cinq villes palestiniennes (Jenin, Naplouse, Jéricho, Tulkarm et Tubas). Les sérums ont été testés par méthode ELISA quant à la présence d'immunoglobuline G (IgG) de *C. abortus* (ID Screen® *Chlamydomphila abortus*, IDvet, France). Au niveau individuel, parmi les 2608 échantillons analysés, 385 (13,7%) étaient positifs pour *C. abortus*. Une différence significative entre les régions étudiées ( $P < 0,05$ ) a été notée avec un taux de séroprévalence de 17,7% dans les villes de Naplouse et Tubas, suivi de Jéricho (14,2%), Jenin (9,6%) et Tubas (3,8%). Au niveau du troupeau, parmi les 353 exploitations étudiées, 188 (53,3%) avaient au moins un animal séropositif. Le taux le plus élevé a été trouvé dans les troupeaux de Tubas (90,9%), puis Naplouse (78,3%), Jenin (56,9%), Jéricho (44,9%) et Tulkarm (36,4%). La plupart des cas séropositifs ont été observés dans les troupeaux de grande taille. En conclusion, la détection de l'anticorps de *C. abortus* dans le sérum des béliers suggère un potentiel de transmission vénérienne important dans cette région du pays.

**Mots-clés:** Séroprévalence, *Chlamydomphila abortus*, bélier, mouton, Palestine.

## Introduction

The family *Chlamydiaceae* are obligate intracellular gram-negative bacteria, known to cause a variety of diseases in animals and humans [19]. The family consists of two genera: *Chlamydomphila* and *Chlamydia*. *Chlamydomphila* (*C.*) *abortus* (formerly *Chlamydia psittaci* serotype 1) and *C. pecorum* in the genus *Chlamydomphila* are known to infect sheep [18]. The disease was first described in sheep in 1936, named enzootic abortion of ewes (EAE)[7]. Nowadays *C. abortus* is one of the most important causes of reproductive failure in sheep, especially in intensively managed flocks [23]. The disease is usually manifested as subclinical infections. However, the infection can cause important economic losses in sheep due to reduced fertility, spontaneous abortion in the last 2 to 3

weeks of gestation, perinatal death, and stillbirth. Ewes that abort are resistant to future reproductive failure due to *C. abortus*, but they become silent carriers and persistently shed the organism from their reproductive tracts during estrus [13]. *Chlamydomphila pecorum* is the other member of the genus that affects small ruminants, and it is recognized as a primary cause of keratoconjunctivitis and of polyarthritis in sheep [27].

Sheep are of importance significant for economic in Palestine. With approximately 670,000 heads, sheep is considered as the most abundant farm animal [20]. Several studies in different countries demonstrated that *C. abortus* is widely spread in sheep [2, 8]. The incidence of sheep's abortion is very high in Palestine, without specific clinical signs and

known etiology. A significant number of these abortions occurring in mid to late gestation [12]. The placentas and fetal fluid of the infected animals are the main sources of infection in sheep and other susceptible animals. In addition, infected animals shed the infectious agent in feces and urine, as well as in the milk [18]. Ingestion is considered to be the main route of infection, although inhalation may also be a route of transmission [11]. Infected ewes develop late abortion due to placentitis which is the main clinical presentation. Infected rams may develop epididymitis, seminal vesiculitis and infections of testes, leading to male sterility and infertility [18]. The organism is also excreted in semen that has a role in venereal transmission route of the disease [22]. Finally, due to carrier and shedding, and the pathogeny of the disease, the incidence of abortions increases in the following year and becomes enzootic in the next reproductive seasons [18].

Risk factors for *C. abortus* infection in people include direct contact with infected animals or reproductive products contaminated with, as well as consumption of contaminated milk [19]. The subclinical state of the disease and the long period shedding of untreated infected animals enhance the risk of human infection. Late term abortion is the major zoonotic risk in pregnant women [16]. Other clinical presentations varies from asymptomatic self-limiting disease, or mildly symptomatic influenza-like illness, or may be fatal disease due to septic shock, acute renal failure, or respiratory distress [3, 25].

Considering the abundance of sheep in Palestine and the importance of Chlamydiosis for both animal and public health, 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 *C. abortus* in sheep and sheep herds in Palestine. Prevalence data of *C. abortus* are important to support risk assessments or decisions on preventive measures regarding public and animal health.

## Materials and methods.

### AREA OF THE STUDY, SAMPLE COLLECTION AND PREPARATION

This study was performed in 2016 to determine the seroprevalence rate of *C. abortus* in the Palestinian sheep population. The study was carried out in Jenin, Jericho, Nablus, Tubas and Tulkarm cities in Northern Palestine (Figure 1). The number of farms sampled on each city was 47, 238, 46, 11 and 11 respectively; in every farm, all the rams were sampled. All the rams in the farms were tested. The numbers vary according to farm size from 5-15 ram. The targeted regions were chosen as they were the highest sheep population. The samples were collected from active reproductive rams during reproductive season (April to June 2016). All samples were collected 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.

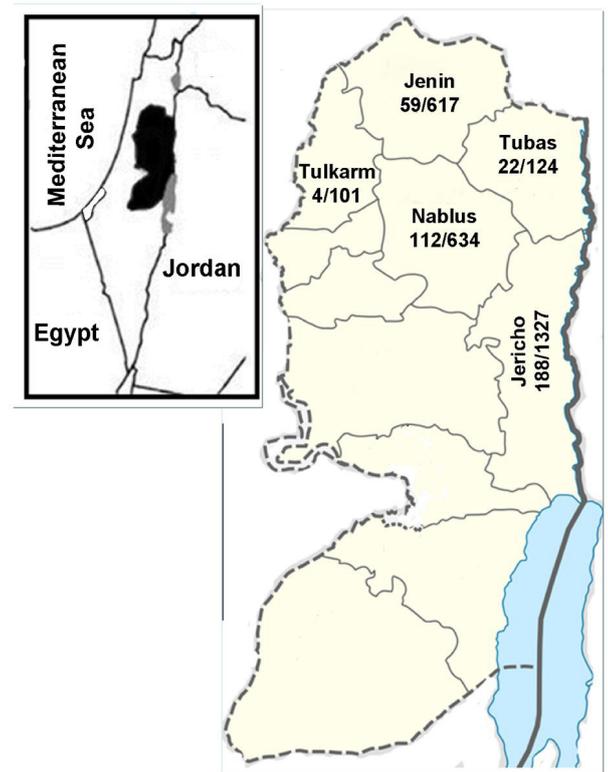


FIGURE 1: Geographical distribution of seroprevalence at animal level. Numbers shown are positive animals from the tested animals in each city.

### LABORATORY EXAMINATION

Antibodies to *C. abortus* antigens were detected by ID Screen *Chlamydophila abortus* indirect multi-species ELISA kit, purchased from IDvet (IDvet Innovative Diagnostics, Montpellier, France). The kit is a commercially available uses microwells coated with a *C. abortus*-specific synthetic peptide from the major outer membrane protein (MOMP). Following the manufacturer instructions serum were considered positive when sample/positive control ratio (S/P %) greater than or equal to 60%.

### STATISTICAL ANALYSIS

Chi-square test of statistical software SPSS version 20<sup>th</sup> (SPSS Inc, USA) was used to determine the association between prevalence of *C. abortus* antibodies and the location. *P* value < 0.05 was considered statistically significant.

## Results

Of the 2806 blood samples serologically investigated in this study, 385 (13.7%) were *C. abortus* seropositive and 2421 (86.3 %) were seronegative. The chi-square test showed statistical significant differences between the studied cities (*P* > 0.05); the prevalence rates were the same in the cities of Nablus and Tubas (17.7%) with no statistically differences were observed in Jericho (14.2%) (*P* < 0.05). However, the

prevalence rate in Jenin (9.6%) and Tulkarm (3.8%) was significantly lower than other cities ( $P < 0.05$ ). No significant difference was observed between Jenin and Tubas ( $P < 0.05$ ). Table 1.

At the herd level (Table 2), among the 353 farms studied, 188 (53.3 %) had at least one seropositive animal. The highest rate was found in the herds of Tubas city (90.9%), followed by Nablus (78.3%), Jenin (65.9%), Jericho (44.9%) and Tulkarm (36.4%).

## Discussion

*C. abortus*, the causative agent of enzootic abortion of ewes is a main cause of abortions in small ruminants in numerous countries of Middle East [1, 2, 14] and worldwide [4, 6, 8, 26]. The disease is characterized by abortion in late pregnancy stage implicated for important economic losses. Ingestion and inhalation of contaminated materials are the major sources of infection in animals and human. *C. abortus* can infect male and causes epididymitis and infertility. The infectious agent can also be excreted in the semen in the absence of genital symptoms and contributed in the venereal transmission of the disease [22]. The infection rate in male is not well documented; screening the rams during the reproductive season could provide estimation about the occurrence of the disease in the studied area. In addition, this approach is useful as a screening of venereal transmitted diseases in subclinically infected animals.

This is the first epidemiological investigation of ovine chlamydiosis in Palestine that report the presence and

estimates prevalence rate occurrence of *C. abortus* antibodies in a representative number of randomly selected rams. Currently, vaccination against ruminant chlamydiosis is not practiced in Palestine and the detected antibodies in this survey reflect a natural response to infection. At the individual level, our results revealed a seroprevalence rate of 13.7% for the Palestinian rams population. The seropositive rate in Palestinian rams was lower than those detected in ewes in Jordan (21.8%) using complement fixation test (CFT) [2]. Nevertheless, this variation may in part be related to differences in sensitivity and/or specificity of serological tests used. CFT has lower specificity compared to ELISA and can cross-react with other *Chlamydiaceae* members [24]. Hireche et. al., reported 24.5% of screened sheep were seropositive to *C. abortus* in Algeria [8]. The infection rate was 46.6% in Turkey [6]. A study conducted in Egypt using PCR from conjunctival swabs revealed 68% of asymptomatic and 80% diseased sheep were positive to *Chlamydomphila psittaci* (The former name of *C. abortus*) [15]. Previous studies include both genders showed no differences between infection rate between male and female [9, 17]. However, other studies in camel and cattle revealed that the infection rate in male is less than female [5]. Therefore, the infection rate in Palestinian sheep could be underestimated. In addition, previous vaccination campaign for *Chlamydomphila* in sheep was conducted in Palestine, However, this campaign was terminated in 2013 (Ministry of Agriculture, Dr. Mohammad Manasrah, personal communication). This could explain the low prevalence rate of *C. abortus* in Palestinian rams.

Significant differences have been observed between studied areas (Table 1). Nablus and Tubas cities were

City	Animal tested (N)	Infected animals (N)	Seroprevalence (%)
Jenin	617	59	9.6 <sup>ab</sup>
Jericho	1327	188	14.2 <sup>c</sup>
Nablus	634	112	17.7 <sup>c</sup>
Tubas	124	22	17.7 <sup>ac</sup>
Tulkarm	104	4	3.8 <sup>b</sup>
Total	2608	385	13.7

Pearson Chi-Square = 27.830. Each subscript letter denotes a subset of state categories whose column proportions do not differ significantly from each other at the .05 level.

TABLE I: Seroprevalence of *C. abortus* in 2806 ram surveyed in five cities in Northern Palestine

City	Farm tested (N)	Infected flocks (N)	Seroprevalence (%)
Jenin	47	31	65.9 <sup>b</sup>
Jericho	238	107	44.9 <sup>b</sup>
Nablus	46	36	78.3 <sup>b</sup>
Tubas	11	10	90.9 <sup>b</sup>
Tulkarm	11	4	36.4 <sup>a</sup>
Total	353	188	53.3

Pearson Chi-Square = 140.0. Each subscript letter denotes a subset of state categories whose column proportions do not differ significantly from each other at the .05 level.

TABLE II: Seroprevalence of *C. abortus* in 353 sheep herds surveyed in five cities in Northern Palestine.

the highest seroprevalence rate with 17.7%. The lowest seroprevalence rate was observed in Tulkarm city (3.8%). This variation mostly related to factors such as sheep population density, herd size, management practices, and livestock trade. Cities with higher prevalence (Tubas, Nablus) have higher sheep population density and are characterized by larger herd size. Tubas is close to Jenin where animal roaming between these cities is frequent, and the management in these two cities is almost identical which may explain the no significant differences between the cities. Herd density in Tulkarm city is relatively smaller compared to other investigated cities. These findings were in agreement with a report in Jordan demonstrated the effect of population density and management practices on the prevalence of ovine chlamydiosis [2].

At herd level, from a total of 353 investigated herds, 188 (53.3 %) has at least one seropositive animal, ranged from 36.4 % to 90.9 %. The wide range of herds seropositive in Palestinian herds can be explained by same risk factors mentioned in the individual infection rate. Cities with higher prevalence (Tubas, Nablus and Jericho) have higher sheep population density and are characterized by larger herd size[20]. The seroprevalence rate in Palestinian sheep is in comparison to most other reports in other countries. The infection rate at herd level in Turkey was 46.6% [6], and 70.4% in Algeria [8].

A number of disease control options have been used in different countries in order to reduce emergence or re-emergence of the disease. These measures include improvement the diagnosis and general awareness of the disease, especially animal shedders[21]. 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 *Chlamydia* infection [8]. Prophylactic treatment by oxytetracycline preparation reduces the severity of infection and limits the shedding of pathogens [21]. Using of live attenuated vaccine provides a good protection against the disease[10].

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