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SEROPREVALENCE AND RISK FACTORS OF CHLAMYDIA ABORTUS INFECTION IN AWASI SHEEP IN HAMA GOVERNORATE IN THE MIDDLE REGION OF SYRIA

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ABSTRACT

Chlamydia abortus is an obligate intracellular Gram-negative and an important pathogenic bacteria, which can infect animals and humans. 312 blood samples were collected from 17 flocks of Awasi sheep in the down-town and province in Hama governorate between February 2019 and January 2020. The total number of sheep in the study flocks were 3715 including 2972 pregnant sheep. Seropositive cases of *C. abortus* antibodies were found in 5 of 17 study flocks with a percent of 29.41%. The overall prevalence was (36/312) 11.53% as positive to specific antibodies of *C. abortus* using ELISA test, which ranged between (0-100%) in tested samples from evry flock in Hama governorate and the seroprevalence in downtown was 14.285%, 16.666 in Koumhana, 33.333% in Almbarkat and 33.333% in Tal Altut, and 100% in Maar Shoor. The higher prevalence of OEA was reported in the winter season comparing with other Seasons (OR= 2.52) and the riskiest ages in OEA were ages between 2 – 4 years.

Keywords: *Chlamydia abortus*, Ovine Enzootic Abortion, Seroprevalence, Risk Factors.

INTRODUCTION

Chlamydial abortion in ewes or ovine enzootic abortion [OEA]) is an important disease in sheep caused by *Chlamydia abortus*. Ovine Chlamydiosis causes abortion in the last period of pregnancy and may cause serious economic loss in sheep-breeding areas and threaten sheep industry development worldwide, particularly where herds are closely congregated during the last 2-3 weeks of gestation (Aitken and Longbottom, 2007).

Infected ewes may show no clinical symptoms before abortion, but behavioral changes, a vulval and vaginal secrations may be observed in ewes within the last 48 hours of gestation. Pathogenesis sgins occur usually at the end of three months of pregnancy period coincident with a phase of rapid fetal growth when the *C. abortus* invasion of placentomes produces placental damge with progressively diffuse inflammatory response and tissue necrosis. Milder Pathogenesis sgins may occur in the fetal liver and lung and sometimes

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hypoxic brain damage in cases with severe placental damage (Buxton *et al*., 2002; Longbottom *et al*., 2013 and OIE, 2018).

Chlamydia abortus (*C. abortus*) is causing ovine enzootic abortion (OEA), and recognized as main cause of abortion in sheep and lamb loss throughout the world. *C. abortus* manifests an assortment of symptoms which mainly minify the economic feasibility of flocks such as endometritis, premature of weakly lambs, stillborn lambs, orchitis, epididymitis, and seminal vesiculitis. Pneumonia, encephalomyelitis, arthritis, and conjunctivitis have been other illness sgins of OEA. In some regions in the world like united kingdom *C. abortus* is considered as the principal agent of death in lambs and leading to losses rated about 20 million dollars yearly (Longbottom *et al*., 2013).

The family Chlamydiaceae is an obligate intracellular gram-negative and pathogen bacteria which can infect animals and humans. It has a single genus chlamydia that comprises eleven species which include: *C. trachomatis* and *C. pneumonia* (humans pathogen), and some of them can infect animals such as *C. suis* (swine), *C. muridarum* (mouse), *C. psittaci* (avian), *C. felis* (cat), C. *caviae* (guinea-pig), *C. pecorum* (sheep, cattle and, koala), *C. avium* and *C. gallinaceae* (both infect birds), and especially *C. abortus* which can infect sheep, goat, cattle and humans (Sachse *et al*., 2015).

The serological diagnostic and identifaction of *C. abortus* antibodies in sheep sera can be detected through using serological tests such as immunofluorescence test, complement fixation test (CFT), or enzyme-linked immunosorbent assay (ELISA) as lacks specificity test (Griffiths *et al*., 2006). *C. abortus* can be detected the positive sera-samples using a complement fixation test or enzyme-linked immunosorbent assay (Masala *et al*., 2007).

Recently, a new indirect ELISA based on POMP90 has been commercialized and shown to be both sensitive and specific for *C. abortus*, in particular in differentiating animals infected with *C. pecorum* (Anon, 2015; Essig and Longbottom, 2015).

Abortion probably results from a combination of impairment of materno-fetal nutrient and gaseous exchange, disruption of hormonal regulation of pregnancy, and induced cytokine aggression (Entrican, 2002).

MATERIALS AND METHODS

Serological diagnostic of Ovin enzootic Abortion:

The current study was carried out within the period of February 2019 - January 2020 on 312 samples taken from 17 sheep herds, in the Hama governorate in the middle region of Syria. Samples of blood were taken from sheep between 1 - 5 years using a random technique. In the epidemiological term, the overall number of herds was counted by dividing the overall individual sample volume by the numeral of sheep to be sampled from every herd. The sera were obtained by leaving blood specimens at room temperature for separating of serum which stocked in Eppendorf tubes at −20 °C till further tested. Specimens were examined for the detection of specific antibodies to C. abortus employing the indirect ELISA kit (ID-Vet, Innovative Diagnostics Vet, Grabels, France).

Method of testing:

ELISA test:

The serum was examined for the detection of IgG antibodies for C. abortus employing the ID-Screen C. abortus multispecies

indirect ELISA kit (ID-Vet, Innovative Diagnostics Vet, Grabels, France). The microtiter plates (96 wells) were provided plated with the recombinant protein. Results optical density (OD) was measured at 450 nm employing a microtiter plate reader. The final values were expressed as sample/positive control percentage (S/P %). Sera with S/P % equal to or lower than 50 % were considered negative, sera with an S/P % between 50 and 60 % were doubtful and sera with an S/P % >60 % were considered positive. This ELISA assay uses a synthetic antigen from a major outer-membrane protein (Momp) specific to C. abortus which reduces the frequency of non-specific reaction.

Epidemiological data collection:

The main data which depended on flock size, giographical regions of the animals, age, year seasons, stage of abortion, stillborn, death in young lambs, born of weakly lambs, therapy of newly purchased sheep, cleaning methods and use of disinfection materials, presence of other animals in the farm such as cats, dogs, birds, and farmworkers with contact to other farms were collected.

Potential Risk factors analysis:

Differences in the Seroprevalence of *C. abortus* among Awase sheep of different geographical regions, season, and age was analyzed, and the Potential relationship and risk factors of *C. abortus* infection in sheep were discussed by statistical significance analysis. P< 0.5 was considered statistical significant. Each factor was analyzed with odds ratios (ORs) and 95% confidence intervals (Cls) in the study. All statistical significant analyses were performed by SAS (Statistical Analysis Sestem, Version 9.1) (Zhang *et al*., 2016 and Zheng *et al*., 2016).

RESULTS

In this study, 36 (11.53%) out of 312 tested serum samples from Awasi Sheep in the Hama governorate were seropositive for *C. abortus* antibodies and 276 (88.46%) samples were seronegative by ELISA-Test (Figure 1).

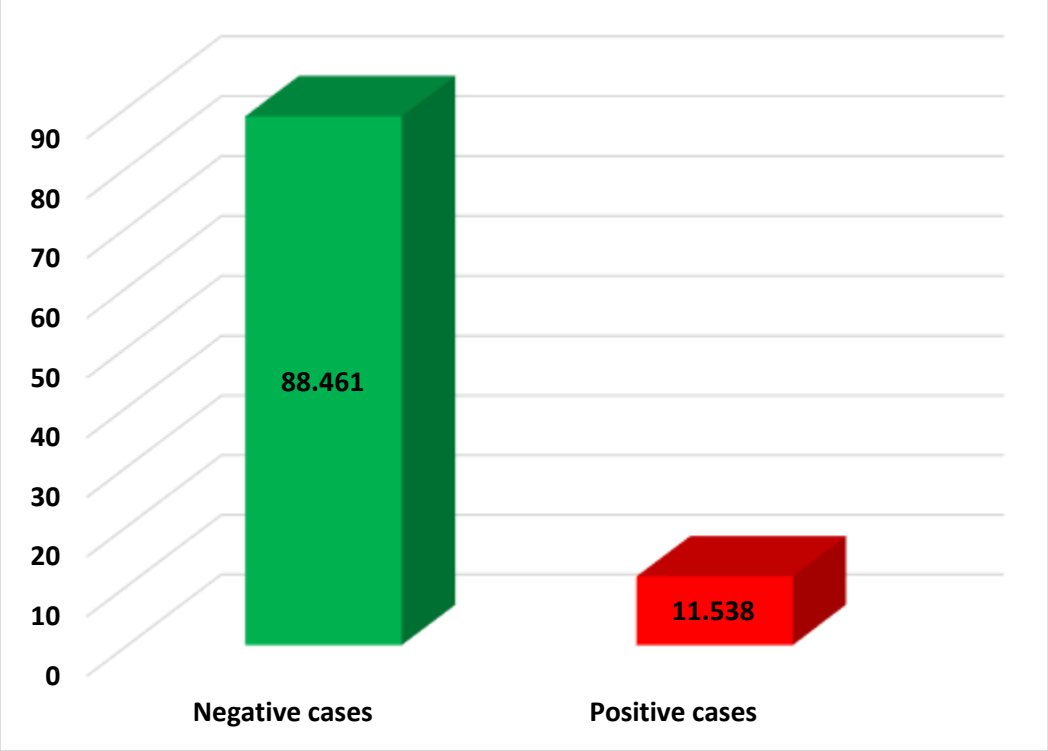

Figure 1: Seroprevalence of *C. abortus* antibodies in tested sheep in the Hama governorate.

Seropositive sheep were found in 5 out of 17 (29.41%) flocks with Seroprevalence of *C. abortus* antibodies ranging from 0% to 100% in individual flocks (Figure 2).

The seroprevalence was different among regions. It was 14.285% in downtown, 16.666 in Koumhana, 33.333% in Almbarkat, and 33.333% in Tal Altut, 100% in Maar shoor (Table 1 & Figure 3).

Table 1: Seroprevalence of *C. abortus* antibodies in study regions in Hama governorate.

Regions	Tested blood sample	Positive cases	Negative Cases	Prevalence %
Town Center	**42**	**6**	**36**	**14.285**
Gpren	18	0	18	0
Koumhana	**36**	**6**	**30**	**16.666**
Kana Hasna	18	0	18	0
ALmbarkat	**18**	**6**	**12**	**33.333**
AenAlbad	18	0	18	0
Altaeba	18	0	18	0
Morek	18	0	18	0
Maar Shoor	**12**	**12**	**0**	**100**
Alswaek	12	0	12	0
Kasone	12	0	12	0
Tal Aldraah	12	0	12	0
Alsapora	6	0	6	0
Akerbat	18	0	18	0
ImAlamad	18	0	18	0
Alsaan	18	0	18	0
Tal Altut	**18**	**6**	**12**	**33.333**
TOTAL	**312**	**36**	**276**	**11.538**

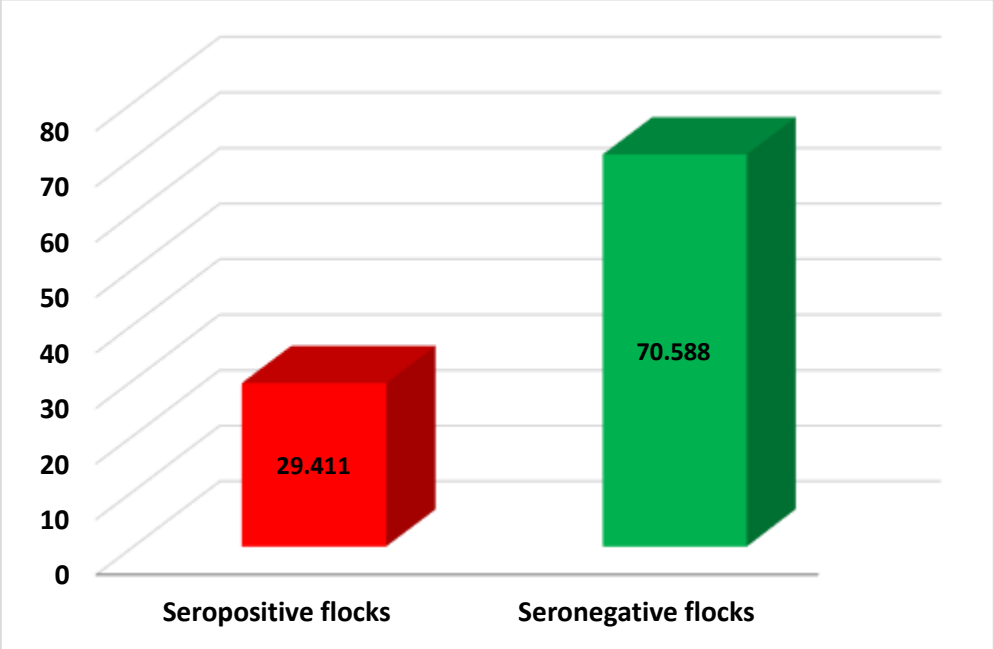

Figure 2: Seroprevalence of *C. abortus* antibodies in study flocks in Hama governorate.

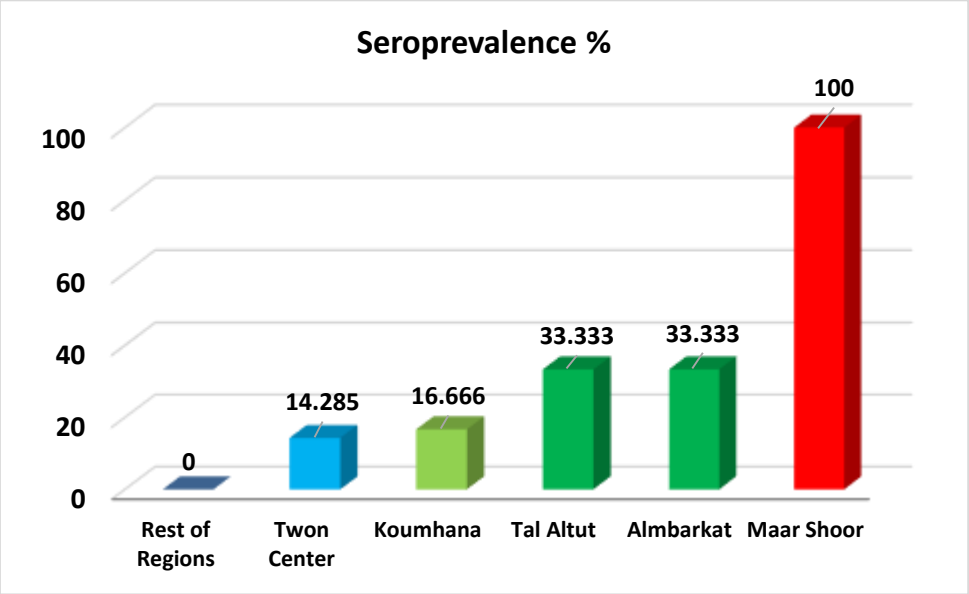

Figure 3: Seroprevalence results of *C. abortus* antibodies of the 312 tested serum samples.

A highly significant correlation has been detected between the seroprevalence of infection and the geographic location ($p<0.0001$). Prevalence proportions varied between 0 and 1.51% (Table 2).

Table 2: Odds ration of the impact of the region according to Prevalence of the disease.

Regions	Lower limit of OR 95%	OR	Upper limit of OR 95%
Town Center	**0.67**	**0.013**	**1.00**
Gpren	0.00	0.00	0.00
Koumhana	**0.08**	**0.012**	**1.01**
Kana Hasna	0.00	0.00	0.00
ALmbarkat	**0.12**	**1.51**	**0.21**
AenAlbad	0.00	0.00	0.00
Altaeba	0.00	0.00	0.00
Morek	0.00	0.00	0.00
Maar Shoor	**0.052**	**0.073**	**2.05**
Alswaek	0.00	0.00	0.00
Kasone	0.00	0.00	0.00
Tal Aldraah	0.00	0.00	0.00
Alsapora	0.00	0.00	0.00
Akerbat	0.00	0.00	0.00
ImAlamad	0.00	0.00	0.00
Alsaan	0.00	0.00	0.00
Tal Altut	**1.00**	**1.51**	**2.061**

No significant difference has been observed between the seroprevalence of OEA and different age groups (p>0.05). The highest prevalence rate has been recorded in ewes aged between 2 years and 35 months. The riskiest ages in OEA were ages between 2 – 4 years (Table 3).

Table 3: Odds ration of the effect of some Risk factors season and age associated with disease occurrence.

Category	No. of ovine examined	No. of ovine positive	Prev. % (95% CI)	Upper limit of OR 95%	Odds Ratio OR	Lower limit of OR 95%
Season summer	**77**	**14**	**18.18 13.82-22.54**	**2.011**	**0.4631**	**0.325**
Season spring	**85**	**6**	**7.05 4.15-9.95**	**1.01**	**0.172**	**0.143**
Season winter	**75**	**16**	**21.33 16.69-25.97**	**2.52**	**1.87**	**1.00**
Season autumn	**75**	**0**	**0**	**0.00**	**0.00**	**0.00**
Age (yr)≤2	**100**	**12**	**12 8.32-15.68**	**2.51**	**1.01**	**0.991**
Age (yr)>2≤4	**100**	**14**	**14 10.07-17.93**	**3.44**	**3.011**	**2.51**
Age (yr)>4	**112**	**10**	**8.92 5.69-12.15**	**1.00**	**0.883**	**0.51**
Total	**312**	**36**	**11.53 7.92-15.14**	**-**	**-**	**-**

From the above table, it was reported the higher prevalence of OEA in the winter season comparing with other Seasons (OR= 2.52). There were no reported cases in the autumn season. The riskiest ages in OEA were ages between 2 – 4 years.

DISCUSSION

Enzootic abortion in sheep is considered under the list of legally reported cases in Syria. This study reported that rate of abortion resulted from *C. abortus* was 11.538%. Positive antibodies were

present in 5 Flocks of 17 Flocks as a percent 29.41% of study Flocks.

Many studies were confirmed positive Cases of *C.abortus* in sheep and small livestock in the middle east and Asia. The Figures results of the prevalence reported in the present study were fitting with reported results of (Al-Dabagh *et al*., 2014) in Iraqi sheep (11.2%) using ELISA test. Also, current results were fitting with the results of (Mikaeel *et al*., 2016) in goat Flocks in Iraqi regions.

The same figures points were reported in a sheep flock in the northern and eastern regions of Turkey (13.98%) using indirect ELISA test (Gokce *et al*., 2007).

Similar results were reported in northern Palestine, where reported positive cases in Awasi sheep as 13.7% seroprevalence (Jalboush *et al*., 2017).

lower figures of seroprevalence of *C. abortus* were reported in north of Iraq (3.33%) (Arif *et al*., 2020), and in the Riyadh region (7.52%) (Al-Jumaah and Hussein, 2012) and (0.75%) in Madina (Shabana and Krimly, 2020) both in Saudi Arabia.

Lower prevalence level reported in regional regions like Turkey, using indirect ELISA test in Turkish sheep as a percentage (5.38%) (Otlu *et al*., 2007).

However, a higher level of prevalence was reported in Awasi sheep and Baldy goat in Jordan in the Al-Mafrak region as (21.8%) (AL-Qudah *et al*., 2004).

Wide world, prevalence level was higher in the western region of China (18.65%) using indirect hemagglutination test (Qin *et al*., 2014).

The various between proportions results may be justified by the variety of diagnostic test techniques (Wilson *et al*., 2009).

Furthermore, the Variant between figures among different scientific studies may be returned to the type of pastures, water sources, and contacts factor between infection and free disease animals (Rodolakis and Laroucau, 2015).

As long as, many filed cases of *C.abortus* are not diagnosed, as the main cause of abortion resulted from *C.abortus* may estimate in 10% in the USA, this because the filed diagnosis can be facing many difficulties, as long as clinical signs and lesion linked to *C. abortus* are not defined yet (Essig and Longbottom, 2015).

The present study considered the first study in Syria that determined risk factors associated with c. abortus in sheep flocks in Syria.

The most popular risk factors reported in the study were seasonal variation and age which corresponding with the results of (Hu *et al*., 2018) in China.

There was a correlation between infection and data of abortion in infected sheep and entry male sheep in the flock as likelihood risk factors (Hu *et al*., 2018).

In the study reported in Brazil, the geography position was a risk factor, and

infection in males was more affected compared with females, with no significant correlation between each other p>0.01 (Pinheiro *et al*., 2010).

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الانتشار المصلي وعوامل الخطورة لعدوى المتدثرة المجهضة في أغنام العواس في محافظة حماة في المنطقة الوسطى في سوريا

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قسم أمراض الحيوان في كلية الطب البيطري بجامعة حماة، حماة - سورية

المتدثرة المجهضة جراثيم خلوية مجبرة سالبة الغرام ومن العوامل المسببة الهامة للعدوى في الحيوان والانسان. تم جمع ٣١٢ عينة دم من ١٧ قطيع من أغنام العواس في المنطقة الوسطى في محافظة حماه مدينة وريفاً في الفترة ما بين شباط ٢٠١٩ وكانون الأول ٢٠٢٠، حيث بلغت أعداد الأغنام في القطعان التي تم دراستها ٣٧١٥ رأساً من الغنم وعدد الأغنام الحوامل منها ٢٩٧٢.

تم الكشف عن أضداد المتدثرة المجهضة مصلياً في ٥ من قطعان الأغنام المختبرة البالغة ١٧ قطيعاً وبنسبة ٢٩,٤١%. بلغت نسبة الانتشار المصلي لمرض الاجهاض المستوطن عند الأغنام في محافظة حماة مدينة وريفاً ١١,٥٣٨%، حيث كانت (٣١٢/٣٦) عينة إيجابية للأضداد النوعية للمتدثرة المجهضة باختبار الاليزا. وتراوحت نسب الانتشار المصلي للمتدثرة المجهضة في الأغنام في محافظة حماة بين (0 – ١٠٠%) في العينات المختبرة من قطعان الدراسة، حيث كانت نسب الانتشار المصلي في مركز المدينة ١٤,٢٨٥%، وفي قمحانة ١٦,٦٦٦%، وفي المباركات ٣٣,٣٣٣%، وفي تل التوت ٣٣,٣٣٣%، وفي معرشحور ١٠٠%.

وكانت أعلى نسبة لانتشار الاجهاض المستوطن عند الأغنام في فصل الشتاء (OR=٢,٥٢)، وأخطر مرحلة عمرية ما بين ٢ - ٤ سنوات من العمر.

الكلمات المفتاحية: المتدثرة المجهضة، الاجهاض المستوطن عند الأغنام، الانتشار المصلي.