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Asian Journal of Animal and Veterinary Advances

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS **Asian Journal of Animal and Veterinary Advances**

ISSN 1683-9919
DOI: 10.3923/ajava.2018.114.121

Research Article
A Serological Survey of Four Abortifacient Infectious Agents among Small Ruminant in Egypt

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Abstract
Background and Objective: Abortions in small ruminants constitute a key problem not only in Egypt but also in other European countries which have developed the livestock sector. Many pathogens causes abortion, sever economic losses and have ability to transmit to human. **Materials and Methods:** A serological investigation was performed to estimate the seroprevalence of four abortive infectious pathogens in small ruminants in five governorates of Egypt. A total of 284 serum samples were collected from sheep and goats and examined for *Brucella* sp., *Chlamydophila abortus* (*C. abortus*), *Coxiella burnetii* (*C. burnetii*) and *Toxoplasma gondii* (*T. gondii*). **Results:** The seroprevalence confirmed the presence of four investigated pathogens with non-significant difference ($p>0.05$) among sheep and goats in different localities. The most prevalence pathogen was *Brucella* sp. 27 (16.8%), 29 (23.6%) followed by *C. abortus* 24 (14.9%), 17 (13.8%) and *C. burnetii* 25 (15.5%), 16 (13%) in sheep and goats, respectively. *Toxoplasma gondii* showed the low detection rate in sheep 10 (6.2%) and goats 8 (6.5%). The results revealed that the single infection with *Brucella* sp., *C. abortus* and *C. burnetii* or mixed infection with *Brucella* sp. and *C. burnetii* had a highly significant effect on the occurrence of abortion in small ruminants. **Conclusion:** The seroprevalence of *Brucella* sp. is high and other pathogens (*C. abortus* and *C. burnetii*) can be found and should be diagnosed to develop an applicable control program.

Key words: Brucellosis, *Coxiella burnetii*, *Chlamydophila abortus*, ELISA, small ruminants, *Toxoplasma gondii*

Received: September 25, 2017 Accepted: December 19, 2017 Published: February 15, 2018

Citation: Abdelfattah Selim, Mahmoud Elhaig and Sherif Moawed, 2018. A serological survey of four abortifacient infectious agents among small ruminant in Egypt. Asian J. Anim. Vet. Adv., 13: 114-121.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Abortion in small ruminants may be due to infectious and non-infectious agents. Abortion had severe economic implications (e.g., loss of fetus and reduce in milk production). In addition, the causative agent of abortion may pose a threat to public health in general as may get transmitted to human[1-3] *Brucella* sp., *C. abortus*, *C. burnetii* and *T. gondii* were some of infectious causes of abortion in small ruminant and also are zoonotic to human[4,5]. Brucellosis causes abortion in the last third of pregnancy and infertility in small ruminant. It is shed in milk, urine, feces and vaginal discharge for 2-3 months. In Egypt, the *Brucella* sp. was the first suspected organism when the abortion was occurred either in animal or in contact workers[6,7].

The obligate intracellular pathogens such as *C. abortus* and *C. burnetii* were detected in domestic small ruminants and characterized by abortion, still birth or delivery of weak newborn[8,9]. Nahed and Khaled[10] reported seroprevalence of Q fever at 32.7 and 23.3% in sheep and goats, respectively, whilst seroprevalence of *C. abortus* was 25.7 and 16.2% in sheep and goats, respectively[11].

Toxoplasmosis in sheep caused some reproductive disorder as abortion, still birth and born weak lamb[12]. The seroprevalence rates of *T. gondii* in naturally exposed sheep worldwide, ranging from 7-50% as reported by Dubey[13] and Rizzo *et al.*[14].

Over the past years, farmers in several regions in Egypt had reported unusual abortion rates, causing significant economic losses in sheep and goats. The causes of these abortions remained undiagnosed. The present investigation attempted to explore, through a serological survey, the most important pathogens cause abortion in small ruminants (*Brucella* sp., *C. abortus*, *C. burnetii* and *T. gondii*) during spring, 2015-2016 in five governorates in Egypt. In addition to, determination the effect of location, species and age on the prevalence of the four investigated pathogens.

MATERIALS AND METHODS

Samples collection: The present study was conducted according to the principles of good clinical practice and was approved by the Ethical Committee for Animal Experiments of Benha University.

Animals were selected by a random sampling strategy. In the selected herds, a number of individuals sufficient to detect an intra-herd prevalence of 15-20% and will be chosen randomly for blood collection.

The investigation of four target pathogens in small ruminants was performed during spring, 2015-2016. A total 284 serum samples (161 ewes and 123 does) were collected randomly from eight flocks from five different governorates in Egypt showed in Fig. 1. The examined herds were movable and had not any history of previous vaccination for the examined pathogens. The collected samples were divided into 88 serum which were collected after 3 weeks from aborted animals (42 ewes and 46 does), these animals suffered from placentitis and prenatal deaths. In addition,196 serum samples from in contact animals without a history of abortion samples (119 ewes and 77 does) were collected.

Serological examination: All serum samples were examined serologically to detect antibodies against *Brucella* sp., *C burnetii*, *C. abortus* and *T. gondii* using ELISA followed by statistical analysis for the obtained data.

Antibodies against *Brucella* sp. were detected using Brucellosis ELISA kit (Bionoven, Beijing, China). Moreover, for *T. gondii* using Toxoplasmosis ELISA kit (Bionoven, Beijing, China). The test was performed following the manufacturers' instructions to samples with OD considered positive.

The CHEKIT Q-fever ELISA Test Kit (IDexx Laboratories, Westbrook, Maine, USA) was used for serological detection of *C. burnetii*. The test was performed according the manufacturers' instructions, a sample having an S/P% value equal or over 40% was considered positive.

The investigation of antibodies against *C. abortus* was performed using The CHEKIT Chlamydia ELISA Test Kit (IDexx Laboratories, Westbrook, Maine, USA) following the manufacturers' instructions. A sample having an S/P% value equal or over 40% was considered as positive.

Statistical analysis: The present study showed different applications of parametric and nonparametric tests for explaining potential risk factors and causes of abortion in sheep and goats. Nonparametric tests of categorical data were introduced for detection of a possible association between the outcome of abortion and pathogens *(Brucella* sp., *C. abortus*, *C. burnetii* and *T. gondii*). Also, the associations between both of abortion and pathogens and all studied potential risk factors (species, age and location) were tested. The parametric F-test of ANOVA was used to test the significance effect of

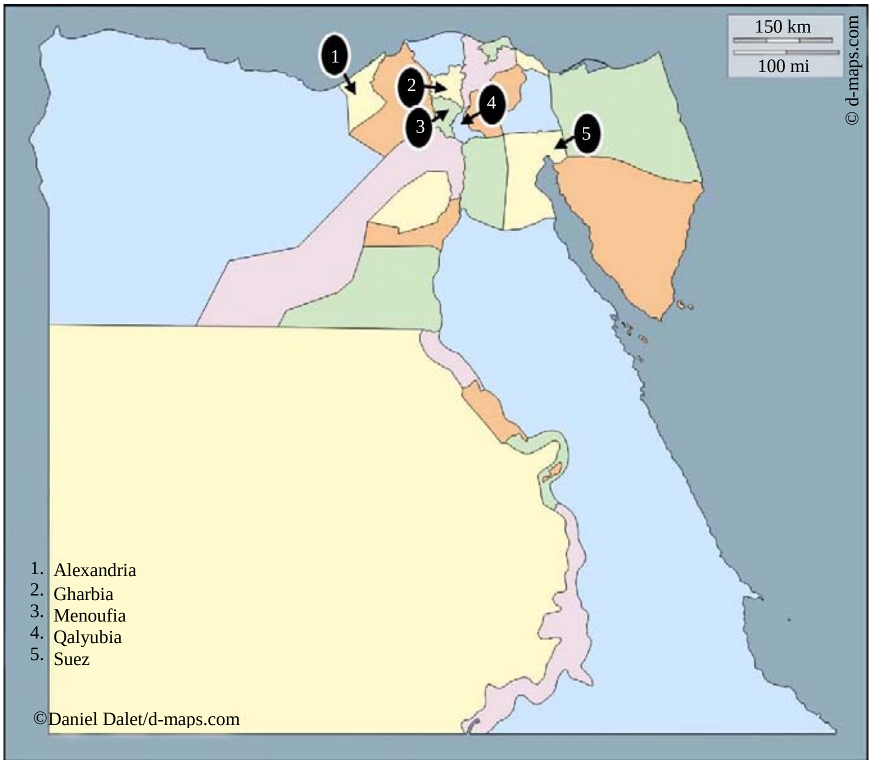

Fig. 1: Map of Egypt showing the governorates of study in relation to the rest of Egypt

mixed infection on abortion. Statistical analyses were done using SPSS version 20 for Windows. Results were considered significant at a probability level ≤ 0.05.

RESULTS

Prevalence of abortive infectious pathogens in different localities: The seropositivity of the four detected pathogens was differed according to locality as showed in Table 1. There was a highly significant difference ($p \leq 0.01$) in the prevalence of *Brucella* sp. and *T. gondii* between different governorates. The highest prevalence rate of brucellosis was observed in Suez 21 (46.7%) and Menoufia 20 (25.0%) but for *T. gondii* was in Alexandria 7 (13%) and Suez 6 (13.3%). In addition, there is a significant difference ($p \leq 0.01$) in the prevalence of *C. abortus* and *C. burnetii* at different localities. The highest prevalence rate for *C. abortus* was observed in Alexandria 16 (29.6%) and Menoufia 13 (16.2%), while for *C. burnetii* was observed in Menoufia 16 (20%) and Suez 12 (26.7%).

Seroprevalence of the four investigated infectious pathogens in association with species and age: There is a non-significant difference ($p > 0.05$) for the prevalence of the four investigated pathogens between sheep and goats as shown in Table 2. Moreover, the results revealed that the most common seropositive causes incriminated in abortion in sheep and goats after *Brucella* sp., were *C. abortus* 24 (14.9%), 17 (13.8%) and *C. burnetii* 25 (15.5%), 16 (13%), respectively.

The statistical analysis of the prevalence in relation to age showed increase the percentage of abortions with age but with no significant difference ($p > 0.05$) between different age groups as tabulated in Table 3.

Regarding the interaction effects of location, species and age on seroconversion of pathogens investigated indicated that the highest significant effect was for the interaction between species and location ($p \leq 0.01$) on seropositivity due to *Brucella* sp. and *C. burnetii*. While, there was no significant effect between other interactions on seroprevalence of examining abortive pathogens as shown in Table 4.

Incidence of single and co-infection with the investigated infectious pathogens: The obtained data in Table 5 revealed that the effect of single infection with *Brucella* sp., *C. abortus* and *C. burnetii* on the incidence of abortion was significant ($p \leq 0.05$). The effect of *T. gondii* on abortion was non-significant ($p > 0.05$).

Table 1: Seroprevalence of different abortion pathogens in different governorates

	Brucella sp.			*Chlamydophila abortus*			*Coxiella burnetii*			*Toxoplasma gondii*		
Governorates	Positive (%)	Negative (%)	Confidence interval (95%)	Positive (%)	Negative (%)	Confidence interval (95%)	Positive (%)	Negative (%)	Confidence interval (95%)	Positive (%)	Negative (%)	Confidence interval (95%)
Alexandria	10 (18.5)	44 (81.5)	8.14-28.85	16 (29.6)	38 (70.4)	17.42-41.77	7 (13.0)	47 (87.0)	4.03-21.96	7 (13.0)	47 (87.0)	4.03-21.96
Gharbia	4 (8.5)	43 (91.5)	0.53-16.47	2 (4.3)	45 (95.7)	1.49-10.09	3 (6.4)	44 (93.6)	0.59-13.39	0 (0.0)	47 (100.0)	0.00-0
Suez	21 (46.7)	24 (53.3)	32.12-61.27	7 (15.6)	38 (84.4)	4.99-26.20	12 (26.7)	33 (73.3)	13.77-39.62	6 (13.3)	39 (86.7)	3.37-23.22
Menoufia	20 (25.0)	60 (75.0)	15.51-34.48	13 (16.2)	67 (83.8)	8.12-24.27	16 (20.0)	64 (80.0)	11.23-28.76	5 (6.2)	75 (93.8)	0.91-11.48
Qalyubia	1 (1.70)	57 (98.3)	1.63-5.030	3 (5.2)	55 (94.8)	0.51-10.91	3 (5.2)	55 (94.8)	0.51-10.91	0 (0.0)	58 (100.0)	0.00-0
Total	56 (19.7)	228 (80.3)	15.07-24.33	41 (14.4)	243 (85.6)	15.07-24.33	41 (14.4)	243 (85.6)	15.07-24.33	18 (6.3)	266 (93.7)	15.07-24.33
p-value	0.001**			0.011*			0.007**			0.005**		

*Association is significant ($p \leq 0.01$), **Association is highly significant ($p \leq 0.01$)

Table 2: Seroprevalence of different abortion pathogens in sheep and goats

	Brucella sp.			*Chlamydophila abortus*			*Coxiella burnetii*			*Toxoplasma gondii*		
Species	Positive (%)	Negative (%)	Confidence interval (95%)	Positive (%)	Negative (%)	Confidence interval (95%)	Positive (%)	Negative (%)	Confidence interval (95%)	Positive (%)	Negative (%)	Confidence interval (95%)
Sheep	27 (16.8)	134 (83.2)	11.02-22.58	24 (14.9)	137 (85.1)	9.39-20.40	25 (15.5)	136 (84.5)	9.91-21.09	10 (6.2)	151 (93.8)	2.47-9.93
Goat	29 (23.6)	94 (76.4)	16.09-31.10	17 (13.8)	106 (86.2)	7.70-19.89	16 (13.0)	107 (87.0)	7.06-18.94	8 (6.5)	115 (93.5)	2.14-10.86
Total	56 (19.7)	228 (80.3)	15.07-24.33	41 (14.4)	243 (85.6)	15.07-24.33	41 (14.4)	243 (85.6)	15.07-24.33	18 (6.3)	266 (93.7)	15.07-24.33
p-value	0.153^{NS}			0.796^{NS}			0.549^{NS}			0.920^{NS}		

NS: Association is non-significant ($p > 0.05$)

Table 3: Relation between the abortive pathogens and age of animals

	Brucella sp.			*Chlamydophila abortus*			*Coxiella burnetii*			*Toxoplasma gondii*		
Species age	Positive (%)	Negative (%)	Confidence interval (95%)	Positive (%)	Negative (%)	Confidence interval (95%)	Positive (%)	Negative (%)	Confidence interval (95%)	Positive (%)	Negative (%)	Confidence interval (95%)
<2 years	11 (25.0)	33 (75.0)	12.21-37.79	6 (13.6)	38 (86.4)	347.00-23.72	11 (25.0)	33 (75.0)	12.21-37.79	1 (2.3)	43 (97.7)	2.12-6.73
2-3 years	14 (15.2)	78 (84.8)	7.87-22.54	17 (18.5)	75 (81.5)	10.56-26.43	9 (9.8)	83 (90.2)	3.72-15.87	6 (6.5)	86 (93.5)	1.46-11.53
>3 years	31 (20.9)	117 (79.1)	14.35-27.45	18 (12.2)	130 (87.8)	6.93-17.47	21 (14.2)	127 (85.8)	8.57-19.82	11 (7.4)	137 (92.6)	3.18-11.62
Total	56 (19.7)	228 (80.3)	15.07-24.33	41 (14.4)	243 (85.6)	15.07-24.33	41 (14.4)	243 (85.6)	15.07-24.33	18 (6.3)	266 (93.7)	15.07-24.33
p-value	0.351^{NS}			0.395^{NS}			0.061^{NS}			0.466^{NS}		

NS: Association is non-significant ($p > 0.05$)

Table 4: F-tests for the interaction effects of species, location and age on abortion with investigated pathogens

	Tests of between-subjects effects											
	Interaction effects of species location and age on pathogens											
	Brucella sp.			*Chlamydophila abortus*			*Coxiella burnetii*			*Toxoplasma gondii*		
Studied factors	Sum of squares	F-value	p-value	Sum of squares	F-value	p-value	Sum of squares	F-value	p-value	Sum of squares	F-value	p-value
Corrected model	11.403	3.222	0.0001	5.604	1.802	0.0110	5.541	1.780	0.012	1.607	0.999	0.471
Intercept	411.830	3141.900	0.0001	435.890	3785.800	0.0001	436.580	3783.600	0.0001	476.200	7992.9	0.0001
Species×age	0.106	0.403	0.668^{NS}	0.086	0.375	0.687^{NS}	0.180	0.780	0.460^{NS}	0.082	0.684	0.505^{NS}
Species×location	2.911	5.552	0.0001**	0.080	0.174	0.952^{NS}	1.871	4.053	0.003*	0.380	1.595	0.176^{NS}
Age×location	0.320	0.305	0.964^{NS}	1.704	1.850	0.068^{NS}	0.671	0.727	0.667^{NS}	0.063	0.133	0.998^{NS}
Species×age×location	0.508	0.646	0.693^{NS}	0.735	1.064	0.385^{NS}	0.213	0.308	0.932^{NS}	0.095	0.267	0.952^{NS}
Error	33.555			29.470			29.540			15.253		
Total	968.000			1013.000			1013.000			1082.000		
Corrected total	44.958			35.080			35.080			16.860		

^{NS}Non-significant at (p>0.05), *Significant at ($p \leq 0.05$), **Highly significant at ($p \leq 0.01$)

Table 5: F-tests for the effects of single and mixed infection on abortion

	Tests of between-subjects effects				
	Single and coinfection effects on abortion				
Effects	Sum of squares	Df	Mean square	F-value	Significant
Corrected model	29.462	11	2.678	23.020	0.0001
Intercept	39.177	1	39.177	336.717	0.0001
Brucella sp.	6.256	1	6.256	53.930	0.049*
C. abortus	0.696	1	0.696	5.981	0.015*
C. burnetii	1.024	1	1.024	8.805	0.003**
T. gondii	0.019	1	0.019	0.160	0.690^{NS}
Brucella sp.× *C. abortus*	0.653	1	0.653	5.610	0.019*
Brucella sp.× *C. burnetii*	0.337	1	0.337	2.897	0.090^{NS}
Brucella sp.× *T. gondii*	0.526	1	0.526	4.522	0.034*
Cp. abortus× *C. burnetii*	0.626	1	0.626	5.381	0.021*
Cp. abortus× *T. gondii*	0.368	1	0.368	3.166	0.038*
C. burnetii× *T. gondii*	0.326	1	0.326	2.800	0.095^{NS}
Brucella sp.× *C. abortus*× *C. burnetii*	0.004	1	0.004	0.031	0.859^{NS}
Error	31.65	272	0.116		
Total	859.0	284			
Corrected total	61.11	283			

*Significant at ($p \leq 0.05$), **Highly significant at ($p \leq 0.01$), ^{NS}Non-significant at (p>0.05)

Regarding the interaction effects of the four investigated pathogens on abortion, it was found that the highest significant effect was for the interaction between *Brucella* sp. and *C. abortus* ($p \leq 0.01$), followed by the mixed infection with both *C. abortus* and *T. gondii*. In addition, the coinfection with *Brucella* sp. and *T. gondii* was significant ($p \leq 0.05$). The other computed interactions were non-significant ($p > 0.05$).

The interactions (*Brucella* sp.×*C. abortus*×*T. gondii*), (*Brucella* sp.×*C. burnetii*×*T. gondii*), (*C. abortus*×*C. burnetii*×*T. gondii*) and (*Brucella* sp.×*C. abortus*×*C. burnetii*×*T. gondii*) could not be computed because of data restriction and very small degrees of freedom.

DISCUSSION

Abortion among small ruminants can be caused by several infectious agents. This results in severe economic losses for farmers, reproductive disorders among the animals and several zoonotic implications in humans. Monitoring the major causes such as *Brucella* sp., *C. abortus, C. burnetii* and *T. gondii* is economically crucial for farmers and doing so had also benefitted the citizens living near the farms. The most common identified cause of abortion in small ruminants in Egypt was brucellosis because there is no sufficient epidemiological data about *C. abortus* and *C. burnetii*, although these pathogens circulate among animals[6,10,15].

In this current study, a serosurvey showed that there was a wide seropositivity range for the four infectious agents among sheep and goats in the regions that were investigated.

In regard to *Brucella* sp., the prevalence rate was found to be in accordance with Abdel Hamid *et al.*[16] they reported the highest infection rate with brucellosis in (18.4%) in sheep. However, there was not enough epidemiological data from different governorates in Egypt to determine the prevalence of the other investigated pathogens.

In this current study, the seropositive results of the serosurvey showed that *Brucella* sp., had the highest rate of infection related to abortions in small ruminants in 56 of the animals (19.7%), followed by *C. abortus* in 41 of the animals (14.4%) and *C. burnetii* in 41 of the animals (14.4%). While, the *T. gondii* showed the lowest rate of infection in 18 of the small ruminants (6.3%).

A previous published investigation of abortion in small ruminants in Ontario by Hazlett *et al.*[17] listed the most common cause as *T. gondii* (33%), followed by *Campylobacter* sp. (22%), *C. abortus* (20%) and *C. burnetii* (12%) while Otlu *et al.*[18] detected *Brucella* sp. and *C. abortus* in sheep at 40.11 and 5.38%, respectively.

In addition, the study conducted by Chanton-Greutmann *et al.*[19] in Switzerland reported *C. abortus* (39%) was the most common cause of abortion in small ruminants, followed by *T. gondii* (19%) and *C. burnetii* (1%).

The results of the current study revealed a seroprevalence of *C. burnetii* among small ruminants especially sheep and goats (15.5 and 13%, respectively). These finding was lower than the prevalence rates previously obtained in Egypt by Nahed and Khaled[10], which were 32.7 and 23.3% for sheep and goats, respectively and by Mazyad and Hafez[20], which were 22.5 and 16.5% for sheep and goats, respectively.

The *Toxoplasma* prevalence rates found in this current study were higher in goats than in sheep (6.5 vs. 6.2%, respectively). This finding was in agreement with the results reported by Ahmed *et al.*[21], who concluded that goats were more susceptible to toxoplasmosis than sheep because they have higher levels of activity. These activities increase the probability of contact with contaminated sources. The prevalence rate of the four pathogens in increase with age but without a significant difference as previously reported by Benkirane *et al.*[4].

The interaction between species and location was found to have a significant effect on the prevalence of *Brucella* sp. and *C. burnetii.* This may be due to the failure of the vaccination and control program for *Brucella* sp., the free movement of animals between different localities and the lack of a specific diagnosis and a serosurvey especially for *C. burnetii*[6,22].

The current study of the prevalence of the four pathogens revealed that a single infection with *Brucella* sp., *C. abortus* or *C. burnetii* or a mixed infection with *Brucella* sp. and *C. burnetii* had a highly significant effect on the occurrence of abortion in small ruminants.

These findings were in accordance with a previous study that investigated the prevalence of abortion in small ruminants in Egypt, where *Brucella* sp., *C. abortus* and *C. burnetii* were the most common causes[11,23,24].

CONCLUSION

It is concluded that, brucellosis was the most common cause of abortion in small ruminant in Egypt. Moreover, other pathogens such as *C. abortus* and *C. burnetii* were detected in a large number of aborted animals. Consequently, veterinarians should identify diagnose the different pathogens when they make their diagnoses in order to reduce economic losses for farmers and implement effective control programs.

SIGNIFICANCE STATEMENT

This study discovers the prevalence rate of four pathogens which cause of abortion among small ruminants in Egypt that can be beneficial for identification of the most common cause and more epidemiological information t o help in control of the problem. This study will help researchers to uncover the critical area of the abortion causes in sheep and goats that many researchers were not able to explore. Thus a new findings on the most common cause of abortion in small ruminants in Egypt may be arrived at *C. abortus* and *C. burnetii* circulating among animals in Egypt and have risk problem to human.

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