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Survey for ovine and caprine chlamydiosis by ELISA in AL-Fallujah city/Iraq

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Abstract
Our study was aimed to detect the prevalence of ovine and caprine chlamydiosis in Al-Fallujah city-Iraq by using ELISA, samples were randomly collected from sheep and goats in herds with a history of abortion during the period between October 2016 to March 2017, from out of 184 serum samples only 21 (11.41%) serum showed positive result to ELISA test, these positivity results are 12/124 (9.67%) ewes, 1/30(33.33%) rams, 6/21(28.57%) does and 2/9(22.22%) bucks. The highest prevalence of chlamydiosis in ovine and caprine was in Albu thaher (%16) while the lowest prevalence was in Alshehaa and Alsecher (%0). The age group (1-3 years) was recorded the highest prevalence of chlamydiosis (%17.54) in sheep, and (%13.88) in goat. For sheep the highest prevalence of chlamydiosis was recorded in December (20%), and lowest prevalence was recorded in October (%0), while in goats, the highest prevalence of chlamydiosis was recorded in January (20%), and the lowest prevalence was recorded in October and March (%0) with a significant difference ($P<0.05$). The study concluded that Chlamydiosis is endemic and a serious cause of abortion in sheep and goat.

Keywords: chlamydiosis; ELISA; sheep: goats; AL-Fallujah

1. Introduction
Chlamydophila abortus is an obligate intracellular, Gram-negative bacterium that belongs to the family Chlamydiaceae [1], it is considered as an agent of causing 20 to 50% of abortions and stillbirths in sheep [2]. Most infections in sheep and goats are asymptomatic apart from abortion or stillbirth at late pregnancy. Infection caused by *Cp. abortus* is manifested by placentitis, accumulation of reddish brown exudate in intercoty-ledonary areas and necrotic changes in the cotyledons [3].In addition to the economic importance in the sheep and goat industry, *Cp. abortus* induces abortions in humans as a result of contact with aborted sheep or goats [4]. Aborted infected fetal tissues, placentas and uterine discharges are the main sources of contamination and the abortion typically occurs in the last 2 to 3 weeks of pregnancy. That affects sheep, goats, and cattle, causing abortions during the final trimester of gestation or the birth of weak offspring that generally die during the first days of life [5]. Epidemiology and clinical features of the disease are not specific to *Cp. abortus* and are concomitant to infection with other abortifacient agents [6]. The clinical diagnosis of chlamydiosis is often difficult, serological detection of *Cp. abortus* using the complement fixation test (CFT), immunofluorescence test, or enzyme-linked immunosorbent assay (ELISA) are often based on the use of preparations whose main components are lipopolysaccharide (LPS) and major outer membrane protein (MOMP) which are common to all members of the Chlamydiacae family [7].
In Iraq, (Dhahir, 1990)[8] was the first researcher who isolated and discovered the organism in the specimens taken from aborted and non-aborted sheep and goat in different parts of Iraq, and has detected chlamydiosis by CFT, and this revealed (55.9%) positive reactors among aborted sheep and (52.1%) positive among aborted goats.
(Cati *et. al*. 2008) [9] detected *Cp. abortus* antibodies in cattle and sheep in south of Iraq by using of iELISA and passive Hemagglutination tests, 700 sera from aborted ewes were submitted to iELISA test, Chlamydial antibodies were detected from sera of (3.41%).All positive samples (22) which tested by PHA test, 18 from 22 samples were positive for PHA test.
(Al-Dabagh *et. al*. 2014) [10] in Nineveh governorate-Iraq, used ELISA for detection antibodies of toxoplasmosis, brucellosis and chlamydiosis in sera of aborted sheep and to recorded the prevalence of these diseases, showed that 32.8%, 56% and 11.2% were positive for toxoplasmosis, brucellosis and chlamydiosis respectively.

2. Materials and Methods

2.1 Study area and design

During the period October 2016 to March 2017 a survey was conducted in ten villages around Al-Fallujah city-Iraq, one hundred and eighty four sheep and goats were sampled randomly to determine of chlamydiosis in the herds.

2.2 Serum Samples

Ten ml of blood samples were collected from jugular vein and put into sterile tubes and allowed them to stand at ambient temperature for 30 minutes to separate serum, then transported on ice pack to laboratory, then were centrifuged at 2000 rpm for 10 minutes to obtain serum which was stored at (-20ºC) in Eppendorf tubes until use.

2.3 Indirect ELISA procedure for the detection antibodies against *Cp. abortus*

Procedure was followed the instructions of the manufacturer of the kit ID Screen® *Chlamydophila abortus* Indirect Multi-species kit product by Innovative Diagnostics (ID.vet) France origin.

2.4 Statistical analysis

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. Chi-square test was used to compare between percentages in this study.

3. Results

Out of a total *184* sera samples from sheep and goats tested by iELISA assay to detect *chlamydiophilla abortus*, a total of 21(11.413%) gave positive results, while 20 (10.86%) were suspected and 143(77.71%) were negative, also 124 ewes tested with iELISA 12(9.67%) were positive, While out of 30 rams tested only 1(3.33%) gave positive results and out of 21 does tested there were 6 (28.57%) positive to iELISA and out of 9 bucks only 2(22.22%) were positive as in table (1). The prevalence of both gender in sheep and goats in our study revealed to significant differences $P< 0.05$, in positive percentages of males which were less than the prevalence in females. All tested animals in the study were divided into age groups which were less than one year, 1-3 years and above 3 years. The results in sheep revealed that a higher chlamydiosis prevalence in age group 1-3 years(17.54%)with a significant differences ($P<0.05$) than the low prevalence in less than 1 year old group(5.26%) and above 3 years old group(6.25%) as shown in figure (1). This study in goat showed a higher chlamydial prevalence in age groups 1-3 years old (13.88%) and above 3 years old The results in sheep revealed that a higher chlamydiosis prevalence in age group 1-3 years (17.54%) with a significant differences ($P<0.05$) than the low prevalence in less than 1 year old group (5.26%) and above 3 years old group (6.25%) as shown in figure (1). This study in goat showed a higher chlamydial prevalence in age groups 1-3 years old (13.88%) and above 3 years old (10.52%) than the less than 1 year's old group (0%). The highest prevalence of ovine chlamydiosis was in December 5 (20%) and January 4 (16%), while the lowest prevalence was in October 0(0%) and November 1(4.76%) as shown in table (2), the highest prevalence of caprine chlamydiosis was found in January 3 (20%) and December 2 (13.33%), while the lowest prevalence was in October 0(0%) and March 0 (0%). All the positive samples that gave positive results by iELISA were distributed in (10) administrative regions around Al Fallujah city. The higher seroprevalence of chlamydiosis in sheep was in Abu thaher (4/25) 16%; Al abeyaar (3/19) 15.78% and Alsaqlaweyh (2/14) 14.28% and all of them were significant at ($p <0.05$), while the lower prevalence was recorded in Alshehaa, Albu shegel and Alsecher which were 0(0%) as shown in figure (2). While the higher seroprevalence of chlamydiosis in goats was in Albu farrag (2/11) 18.18%; Albu shegel (1/7) 14.28% and Alsaqlaweyh (1/9) 11.11% and all of them were significant at ($p <0.05$), while the lower prevalence was recorded in Alshehaa, Al abeyaar, ALFalahaat and Alsecher which were 0(0%) as shown in figure (2).

Table 1: seroprevalence of ovine and caprine chlamydiosis according to gender**.**

Gender	No. of tested animals	positive cases	Percentage (%)	Chi-Square
Ewes	124	12	9.67%	4.15
Rams	30	1	3.33%	3.42
Does	21	6	28.57%	2.39
Bucks	9	2	22.22%	1.02
Total	184	21	11.41%	10.98

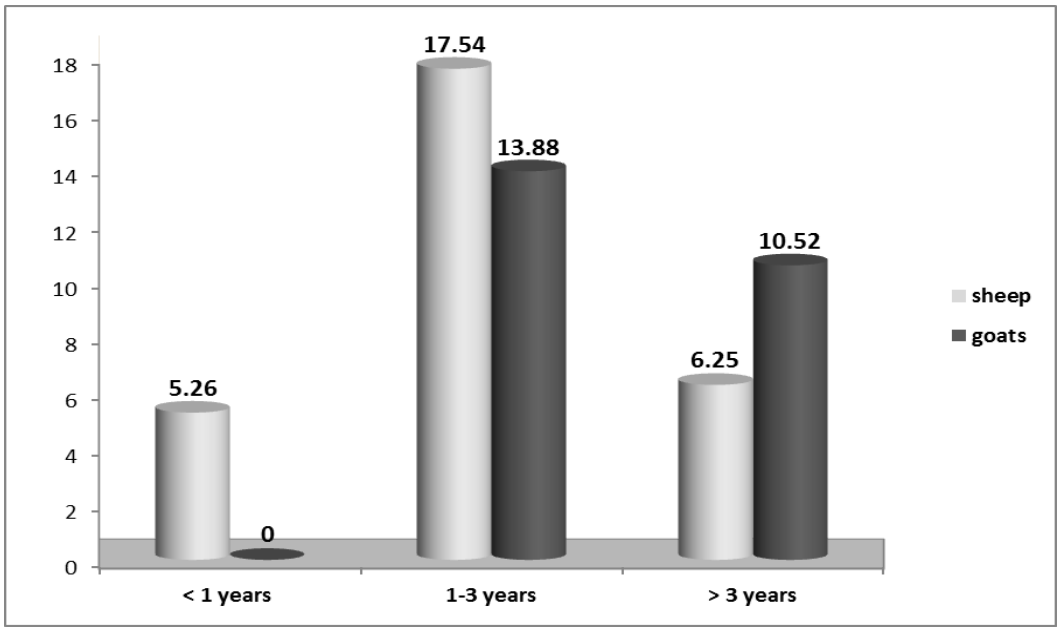

Fig 1: seroprevalance of ovine and caprine chlamydiosis according to age group

Table 2: Seroprevalence of ovine and caprine chlamydiosis according to months.

Month	SHEEP			GOATS		
	No. of tested sera	positive sera	Percentage (%)	No. of tested sera	sera	Percentage (%)
October	15	0	0%	5	0	0%
November	21	1	4.76%	10	1	10.0%
December	25	5	20.0%	15	2	13.33%
January	25	4	16.0%	15	3	20.0%
February	23	3	13.04%	10	1	10.0%
March	15	1	6.66%	5	0	0%
Total	124	14	11.29%	6	7	11.66%

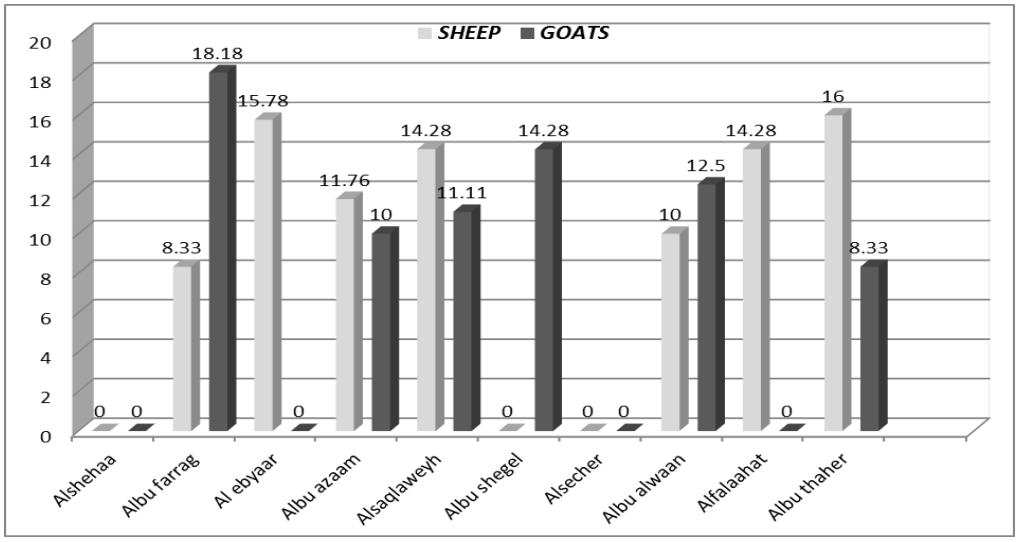

Fig 2: Seroprevalence of chlamydiosis in sheep and goat according to area.

4. Discussion

The results are comparable with Abd-El-Razik *et al*. (2011) [11]; Aljumaah and Hussein, (2012) [12]; Al- Dabagh *et al*.(2014) [10]; Campos-Hernández *et al*. (2014) [13]; Špičić *et al*. (2015) [14]; Chahota *et al*. (2015) [15]; Mora Díaz *et al*. (2015) [16]; Roukbi, *et al*. (2016) [17] and Bhardwaj *et al*. (2017) [18] with a percentage of 9.07%, 7.5%, 11.2%, 13.51%, 11.4%, 19.33%, 9.60%, 13.5% and 16.3% respectively. But our present study disagreed with Rekiki *et al*. (2002) [19]; Stuen and Longbottom, (2011) [20]; Yin *et al*. (2014) [21]; Merdja *et al*. (2015) [22]; Villagra-Blanco *et al*. (2015) [23]; and Krkalić *et al*. (2015) [24] with a percentage of 58%, 44%, 2.74%, (35 ± 08)%, 35%, 5.29% and 91.7% respectively. The variations in the results between our study and other studies may be due to many factors such as the geographical location of the study; type of the serological test used and its efficacy; size and type of sample taken; Breed of animal; grazing strategies ; population density; bad management; nutritional deficiency; uncontrolled restriction of diseased animal movement from infected area; faulty disposal of infected animals and aborted fetus and placental membrane; ignorance of zoonotic importance of chlamydiosis and its economic losses and the type of the study performed on aborted or healthy animals.

Our study was in agreement with (Aljumaah and Hussein, 2012) [12] who noticed in the prevalence of chlamydophilosis for sheep and goats, that the females were around 6-fold that in males (17.24% versus 2.83%, respectively, in sheep and 41.0% versus 6.7%, respectively, in goats), (Esmaeili *et al*, 2015) [25] found *Cp. abortus* antibodies among females (26.8%) was significantly (P<0.01) higher than males (21.2%), (Zenebe *et al*, 2015)[26] found 2000 (70.27%) of the cases observed were females, higher than 846 (29.73%) males.

On the other hand, our result disagreed with Cubero-Pablo *et al*. (2000) [27] who noticed that the differences in prevalence between the sexes (male versus female) were insignificant, Salinas *et al*. (2008) [28] recorded that no effect of age and sex will be observed, Zhao *et al*. (2012) [29] found the seroprevalence in male goats (4.48%) was higher than that in females (2.72%), but the difference was not statistically significant (P>0.05). The higher odds ratio of the infection in males, compared to females is in concordance with Qin *et al*. (2014) [30] who obtained higher seroprevalence of males (21.5%) against females (17.4%) in Tibetan sheep, Roukbi *et al*. (2016) [17] noticed in a serological analysis performed in rams, there were 8/49 (16.32%) and in ewes showed 57/617 (9.23%) positive samples.

Our study showed a higher chlamydial prevalence in age group 1-3 years old with significant differences (P<0.05) than the low prevalence in <1 year old group and this agreed with

Entrican *et al*. (2001)[31] who noticed since in endemic areas animals of any age are likely to become infected, it is expected that most abortions occur in the first pregnancy, animals that have aborted due to *Cp. abortus*, develop protective immunity for about 3 years; therefore, in this situation next abortion will happen in the third or fourth pregnancy. It also agreed with Zhao *et al*.(2012) [29] who found that the highest prevalence (10.17%) of chlamydial infection was found in the goats between 1 and 2 years old, and Qin *et al*.(2014) [30] who considered season and age as a major risk factors associated with *Cp. abortus* infection in Tibetan sheep, and the ages of the tested Tibetan sheep varied from 0 years to 3 years or greater and seroprevalence in different age groups ranged from 7.46% to 24.41%. Esmaeili, *et al* (2015) [25] recorded that the seropositivity in 2 years old animals was higher than 4 years old ($P<0.05$). While our study disagreed with Cubero-Pablo *et al*. (2000) [27] who found that the differences in prevalence between different ages were insignificant, Salinas *et al*. (2008) [28] noticed that no effect of age and sex will be observed, McCauley *et al*. (2010) [32] found no differ between sex, age, breed or state of origin, but differed greatly between properties in Australian sheep.

In our study the highest prevalence of ovine chlamydiosis was found in December (5/25) 20% and January (4/25) 16%, while the lowest prevalence was in October 0(0%) and November (1/21) 4.76%. The highest prevalence of caprine chlamydiosis was found in January (3/15) 20% and December (2/13) 13.33%, while the lowest prevalence was in October 0(0%) and March (0) 0%. Qin *et al*. (2014) [30] found that the risk of *C. abortus* infection in summer was more than 2.0-fold increase compared to *Cp. abortus* infection in winter indicting that season and age were considered as major risk factors associated with *Cp. abortus* infection, with higher exposure risk in different seasons and distinct geographical distribution. Roukbi, *et al*. (2016) [17] noticed that the season has also a significant risk factor for *Cp. abortus* prevalence, and the *Cp. abotus* elementary bodies can remain infective in the environment for only several days in typical spring.

5. Conclusions

Chlamydiosis in sheep and goats is relatively a prevalent disease around AL-Fallujah city and may considered one of the main causes of abortion. Seroprevalence of chlamydiosis in females was higher than males in both sheep and goats. Most susceptible age for chlamydial infection is the first three years of life. The higher prevalence rate occurred in the December/ January while the lowest prevalence rate was in the October/March. ELISA is a suitable and useful technique for the diagnosis of chlamydiosis.

6. References

1. Sachse K, Bavoil PM, Kaltenboeck B, Stephens RS, Kuo CC, Rossello-mora R *et al*. Emendation of the family Chlamydiaceae: Proposal of a single genus, Chlamydia to include all currently recognized species. Systemic Application Microbiology. 2015; 38:99-103.
2. Aitken I. Clamydial abortion, Diseases of sheep. 3rd Ed. Oxford, UK: John Wiley & Sons. 2008, 81-86.
3. OIE. Enzootic abortion in ewes (Ovine Chlamidydiosis) OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, World Organization for Animal Health. 2012; 6(2):1013-1020.
4. Aitken I, Longbottom D. Chlamydial abortion, Diseases of Sheep, 4th Ed. Blackwell Science, Edinburg. 2007, 105-112.
5. Kuo CC, Stephens RS, Bavoil PM, Kaltenboeck B. In: Krieg NR, Staley JT, Brown DR, Hedlund BP, Paster BJ, Ward NL, Ludwig W, Whitman WB. (editors) Genus Chlamydia, in Bergey's manual of systematic bacteriology, 2nd Ed. New York, Springer: Heidelberg. the actinobacteria, part A, 2011; 5:845.
6. Ababneh HS, Ababneh MMK, Hananeh WM, Alsheyab FM, Jawasreh KI, Al-Gharaibeh MA. *et al*. Molecular identification of chlamydial cause of abortion in small ruminants in Jordan. Tropical Animal Health and Production. 2014; 46(8):1407-1412.
7. Masala G, Porcu R, Daga C, Denti S, Canu G, Patta C *et al*. Detection of pathogens in ovine and caprine abortion samples from Sardinia, Italy, by PCR. Journal of Veterinary Diagnostic Investigation. 2007; 19:96-98.
8. Dhahir SH. Bacteriological and serological study on some infectious causes of ovine and caprine abortion. Ph. D. College of Veterinary Medicine - University of Baghdad, 1990.
9. Dhahir JA, Hasso SA, Abbod BC. Detection of Chlamydophila abortus antibodies in cattle and sheep in south of Iraq by using of iELISA and passive Hemagglutination tests. Al-qadesia for vetereinary Scinces. 2008; 7(2):66-72.
10. Al-Dabagh II, Jasim BM, Jarjees MT. Seroprevalence of antibodies to toxoplasmosis, brucellosis and chlamydiosis in abortive sheep in Nineveh governorate, Iraq. Iraqi Journal of Veterinary Sciences. 2014; 28(1):21-25.
11. Abd-El-Razik KA, Al-Humiany AA, Ahmed WM, Barakat AMA, El-Fadaly HA. Investigations on non Brucella abortifacients in small ruminants in Saudi Arabia with emphasis on zoonotic causes. Global Veterinaria. 2011; 6(1):25-32.
12. Aljumaah RS, Hussein MF. Serological prevalence of ovine and caprine chlamydophilosis in Riyadh region, Saudi Arabia. African Journal of Microbiology Research. 2012; 6(11):2654-2658.
13. Campos-Hernández E, Ju Q, Vázquez-Chagoyán C, Abdelfattah Z, Salem M, Saltijeral-Oaxaca JA *et al*. Prevalence and molecular identification of Chlamydia abortus in commercial dairy goat farms in a hot region in Mexico. Tropical Animal Health Production. 2014; 46:919-924.
14. Špičić S, Račić L, Andrijanić M, Duvnjak S, Zdelar-Tuk, M, Stepanić M *et al*. Emerging cases of chlamydial abortion in sheep and goats in Croatia and Bosnia and Herzegovina. Berliner und Munchener Tierarztliche Wochenschrift. 2015; 128, Heft 5/6, Seiten. 180-187.
15. Chahota MR, Gupta S, Bhardwaj B, Malik P, Verma S, Sharma M. Seroprevalence studies on animal chlamydiosis amongst ruminants in five states of India. Veterinary World. 2015, 72-75.
16. Mora Díaza JC, Apariciob ED, Lópezb EH, Güemesa FS, Ochoac CE, Villarreald SJ *et al*. Isolation of Chlamydia abortus in dairy goat herds and its relation to abortion in Guanajuato, Mexico, Veterinaria-Mexico, Publicación Digital de la Facultad de Medicina Veterinaria Zootecnia. 2015; 2(1-10).
17. Roukbi M, Al-omar A, Al-najjar K, Salam Z, Al-suleiman H, Mourii M *et al*. Seroprevalence of antibodies to *Chlamydophila abortus* in small ruminants in some provinces in Syria. Net Journal of Agricultural Science. 2016; 4(2):29-34.
18. Bhardwaj B, Chahota R, Gupta S, Malik P, Sharm M.

Identification of chlamydial strains causing abortions and pneumonia in sheep and goat flocks during trans Himalayan seasonal migration in the northern region of India. Veterinary Archive. 2017; 87:157-170.
19. Rekiki A, Sidi-Boumendine K, Souriau A, Jemli J, Hammami S, Rodolokis A. Isolation and characterization of local strains of *Chlamydophila abortus* (*Chlamydia psittaci* serotype1) from Tunisia. Veterinary Research. 2002; 33:215-222.
20. Stuen S, Longbottom D. Treatment and control of chlamydial and rickettsial infections in sheep and goats. Veterinary Clinics of North America Food Animal Practice. 2011; 27:213-233.
21. Yin L, Schautteet K, Kalmar ID, Bertels G, Van Driessche E, Czaplicki G *et al*. Prevalence of *Chlamydia abortus* in Belgian ruminants. Vlaams Diergeneeskundig Tijdschrift. 2014, 83.
22. Merdja SE, Khaled H, Dahmani A, Bouyoucef A. Chlamydial abortion in Algerian small ruminants. Bulletin UASVM Veterinary Medicine. 2015; 72(1):23-26.
23. Villagra-Blanco R, Dolz G, Montero-Caballero D, Romero-Zúñiga JJ. Detection of antibodies against *Chlamydophila abortus* in Costa Rican sheep flocks. Open Veterinary Journal. 2015; 5(2):122-126.
24. Krkalić L, Šatrović E, Goletić T, Džaja P, Severin K. *Chlamydophila abortus* infection in a flock of goats in Bosnia and Herzegovina - a case report. Veterinarski Archive. 2015; 85(3):359-368.
25. Esmaeili H, Bolourchi M, Mokhber-Dezfouli MR. Seroprevalence of *Chlamydia abortus* infection in sheep and goats in Iran. Iranian Journal of Veterinary Medicine. 2015; 9(2):73-77.
26. Zenebe T, Kebede G, Gerbi F, Fikirte L. Retrospective Study (2010- 2014) of disease conditions among reproductive system of ruminants at Guto-Gida Veterinary Clinic, East Wollega Zone, Ethiopia. Nature and Science. 2015; (9):13.
27. Cubero-Pablo MJ, Plaza M, Pérez L, González M, León-Vizcaíno L. Sero-epidemiology of chlamydial infections of wild ruminants in Spain. Journal of Wildlife Diseases. 2000; 36(1):35-47.
28. Salinas J, Caro MR, Vicente J, Cuello F, Reyes-Garcia AR, Buendia AJ *et al*. High prevalence of antibodies against Chlamydiaceae and *Chlamydophila abortus* in wild ungulates using two in house blocking ELISA tests. Veterinary Microbiology. 2008; 135:46-53.
29. Zhao GH, Shang CC, Zhao YQ, Gao M, Fan GY, Tian TT *et al*. Seroprevalence of chlamydial infection in dairy goats in Shaanxi Province, Northwestern China. African Journal of Biotechnology. 2012; 11(7):1796-1799.
30. Qin SY, Yin MY, Cong W, Zhou DH, Zhang XX, Zhao Q *et al*. Seroprevalence and Risk Factors of *Chlamydia abortus* Infection in Tibetan Sheep in Gansu Province, Northwest China. The Scientific World Journal. 2014, 1-6.
31. Entrican G, Buxton D, Longbottom D. Chlamydial infection in sheep: immune control versus fetal pathology. Journal of the Royal Society of Medicine. 2001; 94:273-277.
32. McCauley LM, Lancaster MJ, Butler KL, Ainsworth CG. Serological analysis of *Chlamydophila abortus* in Australian sheep and implications for the rejection of breeder sheep for export. Australian Veterinary Journal. 2010; 88(1-2):32-38.