Global Veterinaria 6 (1): 25-32, 2011
ISSN 1992-6197

Investigations on non Brucella Abortifacients in Small Ruminants in Saudi Arabia with Emphasis on Zoonotic Causes

[1]K.A. Abd El-Razik, [2]A.A. AL-Humiany, [1]W.M. Ahmed,
[3]A.M.A. Barakat and [3]H.A. ELfadaly

[1]Department of Animal Reproduction and A.I. National Research Center, Dokki, Giza, Egypt
[2]Faculty of Applied Science, Al-Taeef University, KSA
[3]Department of Zoonotic Diseases, National Research Center , Dokki, Giza, Egypt

Abstract: A total of 3996 serum samples was collected from aborted ewes and does in 11 flocks at five governorates of Al-Kaseem area, KSA during 2006-2007. Only 2411/3996 (60.33%) were brucella seropositive, while 1585/3996 (39.7%) animals were brucella sero-negative. 278 out of the 1585 animals (17.53%) were positive for one or both of *Toxoplasma gondii* and *Chlamydophila abortus* by (ELISA on blood samples) and confirmed by Polymerase Chain Reaction technique(placenta and fetal tissues). *T. gondii* and *C. abortus* played definite important role in ovine and caprine abortion, with an overall respective incidence of 12.48 and 9.07% with high possibilities for animal's reproductive disorders and zoonotic hazards. Mixed infection with both diseases was noticed in some animals (4.03%). It was concluded that although Brucella is the major abortifacient agent in small ruminants in KSA, also other pathogens were of great importance and should be followed in diagnosis and control.

Key words: Chlamydiasis · Toxoplasmosis · Small ruminants · Abortion · ELISA · PCR

INTRODUCTION

Most abortifacient animal pathogens are of zoonotic impact, affecting both public and animal health, with extensive animal's economic losses due to abortion and new born mortalities. Brucellosis is the most common and prevalent contagious disease, but others significant abortifacient zoonoses must be taken in consideration. Usually personnel in contact with aborted small ruminants are exposed to occupational zoonotic hazards during handling aborted or sometimes during helping of normal labored witch harboring abortifacient agents. Also, healthy animals usually catch infection through licking fetal fluids, with subsequent fetus or new born deaths [1]. Al-kaseem area (73,000Km^2) is located at the northern middle part of Saudi Arabia (KSA). It is considered as an important area for animal production and markets. It contains about one million heads of sheep and about 600,000 heads of goats. In KSA, sheep constitute an integral part of the animal population and are raised principally by private breeders mainly for meat production [2]. The main recorded infectious diseases that cause abortion in sheep and goats are *Brucella melitensis* and *B. ovis,* with high incidence of human infection. Others abortifacient zoonotic pathogens must be identified such as , *Chlamyophila abortus* [3], *Salmonella abortus ovis*, *Champylobacter foetus*, *Listeria monocytogenes* and *Toxoplasma gondii* infection [4].

Chlamydophila abortus (formally called *Chlamydia psittaci*), is an obligate intracellular parasite causing enzootic abortion of ewes (EAE) or ovine enzootic abortion (OEA) and is one of the most important causes of reproductive failure in many small ruminant-rearing countries causing great economic losses [3]. It was found that infected and latently infected sheep and goats may shed *C. abortus* in their reproductive tract for up to 3 years post infection. Moreover, lambs and kids born to infected animals are usually weak and die within a few days after birth. Besides, Chlamydiasis is a zoonotic disease as the pregnant women can develop severe, life threatening illness and abortion, pneumonia and urogenital signs [5].

Corresponding Author: Khaled A. Abd El-Razik, Department of Animal Reproduction, National Research Center, Postal code 12622, Giza, Egypt.
Tel.: +20123670455, E-mail: khaledemara707@yahoo.com.

Listeriosis is an important zoonotic infectious disease with world-wide prevalence and it affects many species of animal as well as humans. In most cases, the disease is caused by *Listeria monocytogenes.* The infection may be manifested by three distinct clinical syndromes, encephalitis, septicaemia and abortion. An outbreak of septicaemic listeriosis has been reported in Saudi Arabia in sheep during winter. Adult animals and pregnant ewes were principally affected, with a morbidity rate of 7.1% and a mortality rate of 2.4%. However; no abortions were recorded during the outbreak as reported by Al-Dughaym *et al*. [5].

Toxoplasma gondii is an obligate intracellular tissue cyst forming coccidian protozoan that can determine serious widespread zoonoses in humans, small ruminants and many other warm-blooded mammals [6]. Toxoplasmosis causes serious economic losses in the sheep industry all over the world, especially at the time of lambing, fetal resorption, abortion, fetal mummification, stillbirth, or birth depletation [7]. The incidence of abortion is very high in KSA with inadequate diagnostic data and prophylaxis [2]. *Toxoplasma gondii* infection is a common cause of abortion in pregnant women and can cause mental retardation in children. Humans become infected mainly by ingesting uncooked meat containing viable tissue cysts or by ingesting food or water contaminated with oocysts from the feces of infected cats. Occupational zoonotic hazards via skin abrasions, occurs during manipulating aborted feti or fluids that harboring tachyzoites [8].

The present study was carried out to investigate the role of some abortifacient zoonoses other than brucella in abortion of small ruminants at KSA using immunological and molecular identification. Special emphases were given to the possibilities of occupational human bio-hazards and reproductive drawbacks in small ruminants.

MATERIALS AND METHODS

This study was done in KSA, to throw light on the other zoonoses inducing abortion in small ruminants rather than brucellosis. The immunological part of this work was done at Al-Kaseem Laboratories in KSA, while the molecular work was done at the National Research Center and the Veterinary Serum and Vaccine Research Institute (VSVRI) -Egypt, during the period of 2006-2007.

Sampling: A total number of 3996 blood samples was collected from 11 flocks, known to be suffered from abortion. Ewes and Does, from five governorates of Al-Kaseem, KSA (Breedah, Al-Badee, Al-Moznib, Al-Asiah, Eneeza) during 2006-2007.

Samples were collected by jugular venipuncture without anticoagulant and sera samples were separated and stored at-20°C until examination by ELISA for detection of antibodies against *B.melitensis, B. ovis, M. agalactiae, C. abortus, L. monocytogenes* and *T. gondii*.

Tissue Specimens: During 2006-2007, aborted fetuses and stillbirths with signs of intrauterine death (i.e. autolysis) or malformation were submitted for laboratory examination from same 11 flocks. Breeding data on each ewe from which materials were available. These animals were not vaccinated against any ovine abortifacients and proved to be free of infection with *B. mellitensis, B. abortus* and *B.ovis* by ELISA testing.

Serological Diagnosis

All Animals Were Serologically Diagnosed Using:

- Pourquier®Elisa kits (Institut Pourquier, Montpellier, France) targeting B. abortus, C. abortus (80-90 kDa protein fragment), Mycoplasma agalactiae and T. gondii (Toxoplasma-specific IgG-antibodies). Reading the optical densities at 450nm (O.D. 450).
- Ceditest® Brucella (Cedi-Diagnostics B.V, the Netherlands). ELISA test targeting B.abortus and B. mellitensis and the O.D. were red at 450nm.
- Ingezim B. ovis (Ingenasa, Spain). ELISA test for detection of antibodies to B. ovis in ram serum and the O.D. were red at 450nm.
- Serology for L. monocytogenes: by agglutination tests according to the method described by Osebold and Aalund [9]. Samples with a titer =1/100 were considered positive.

Polymerase Chain Reaction (PCR): PCR assay was applied for routine detection of seropositive (*C. abortus* and *T. gondii*) aborted ewes and does. DNA was extracted from placenta and Foetal internal organs (brain, liver, spleen, abomasum) using the DNeasy Blood & Tissue Kit (Qiagen Co. Cat. no. 69504) according to the manufacturers instruction. However, in some cases placental tissue was unavailable and only fetal lung and abomasum were submitted and the condition of some of the fetuses was such that not all tissues could be collected.

For *T. gondii*: amplification was performed in 25µl samples using Taq PCR Master mix Kit (Qiagen Co. Cat. no. 201443). In each reaction 12.5 µl of Master mix was added for each sample, 3µl of DNA extracted from each sample, 7.5µl ultra-pure water and 1µl of each primer (100pM) of the primers targeting B1 gene as described by Burg *et al*. [10].°C for 60s and 72°C for 90s.The expected product (300bp) was detected by 2 % agarose gel electrophoresis. *T. gondii* RH strain was used as a positive control.

For *C. abortus*: amplification was performed in 25µl samples using Taq PCR Master mix Kit (Qiagen Co. Cat. no. 201443) .In each reaction 12.5 µl of Master mix was added for each sample, 3µl of DNA extracted from each sample, 8µl ultra-pure water and 0.75µl of each primer (100pM) of the primers targeting 16S rRNA gene as described by Thiele *et al.* [11]. A sequence of 40 amplification cycles was performed with initial denaturation at 95°C for 10 min, then 94°C for 2min, 50°C for 1min and 72°C for 75s with final extension at 72°C for 10 min. The expected product (119bp) was detected by 2 % agarose gel electrophoresis. *C. abortus* B577 culture was used as a positive control.

Data were computed and statistically analysed.

RESULTS

To investigate the other causes of abortion rather than brucellosis in small ruminants in Saudi Arabia, 278 brucella free aborted ewes and does (out of 1585 sheep and goats) were serologically examined against *L. monocytogenes*, *M. agalactiae* and *C. abortus* using ELISA.

Among the total collected serum samples (3996) collected from aborted ewes and does in 11 flocks located at five governorates of Al-Kaseem area, KSA during 2006-2007 (Tables 1 and 2) ,only 2411 (60.33%) were brucella seropositive. Results confirmed that 278 out of the remaining 1585 animals (17.53%) were positive for *T. gondii (*12.48%) and *C. abortus* (*9.07%*) by using ELISA on blood samples and confirmed by PCR on placenta and fetal tissues. Mixed infection with both diseases was noticed in some animals (4.03%).

The seroprevalence of single infection was 8.45% (134/1585) while it was 4.03% (64/1585) in mixed infection with *C. abortus* with an overall seroprevalence of 12.48% (198/1585) as shown in table 3.The seroprevalence of single *C. abortus* infection was 5.04% (80/1585) while it was 4.03% (64/1585) in mixed infection with an overall

Table 1: Serological classification of animal's sera.

Total serum samples from aborted ewes and does	NO of Sero-positive brucella & %	NO of Sero-negative brucella & %	positive for one or both of *Toxoplasma gondii* and *Chlamydophila abortus*	NO of non-diagnostic causes
3996	2411/3996 (60.3%)	1585/3996 (39.7%)	278/1585 (17.53%)	1307/1585 (82.5%)

Table 2: Aborted brucella sero-negative ewes collected from different areas of Al-Kassim governorate.

Code No	Spec.	Age	Area	No of +cases	No of animal / Flock	Case History
1	Goat	Diff. Age	Al-Badaee	18	150	Abortion at last stages
2	Sheep	Diff. Age	Al-Asiaah	16	40	Abortion
3	Sheep	Over 3 years	Al-Moznib	32	146	Abortion at different stages of pregnancy with post-partum fetal death.
4	Goat	2-3 years	Breedah	6	8	Continuous abortion
5	Goat	1 year	Breedah	90	520	Continuous abortion
6	Goat	1 Year	Al-Moznib	8	28	Late abortion (15d-1m before labor) and premature deaths.
7	Sheep	2 years	Eneeza	16	100	Abortion at the late stages of pregnancy
8	Sheep	-----	Al-Badaee	32	192	Abortion at last stages
9	Sheep	2 years	Eneeza	16	100	Abortion
10	Sheep	-----	Al-Badaee	16	184	Abortion at the late stages of pregnancy (Animals was not vaccinated against brucellosis)
11	sheep	Diff. age	Breedah	28	117	Abortion
TOTAL				278 (17.53%)	1585	

Table 3: Sero-prevalence of abortifacient agents in small ruminants using ELISA technique

Code No	Flock No	No of +cases	*M. agalactiae*	*C. abortus*	*T. gondii*	*Mixed Infection (Chl+Toxo)*	*L.mono- cytogenes*
1	150	18	0	18	0	0	0
2	40	16	0	0	0	16	0
3	146	32	0	0	32	0	0
4	8	6	0	0	0	6	0
5	520	90	0	22	48	20	0
6	28	8	0	0	8	0	0
7	100	16	0	0	16	0	0
8	192	32	0	14	0	18	0
9	100	16	0	0	12	4	0
10	184	16	0	16	0	0	0
11	117	28	0	10	18	0	0
TOTAL	1585	278	0	80	134	64	0
+ve%		17.53	0	5.04	8.45	4.03	0

Table 4: Seroprevalence of abortifacient agents in different areas of Al-Kassim governorate.

Area	Flock no	Abortion cases %	*C. abortus*	*T. gondii*	Mixed C&T
Breedah	645	124 (19.22%)	32 (4.96%)	66 (10.23%)	26 (4.03%)
Al-Badaee	526	66 (12.54%)	48 (9.12%)	-	18 (3.42%)
Al-Asiaah	40	16 (40%)	-	-	16 (40%)
Al-Moznib	174	40 (22.98%)	-	40 (22.98%)	-
Eneeza	200	32 (16%)	-	28 (14%)	4 (2%)
Total	1585	278 (17.53%)	80 (5.04%)	134 (8.45%)	64 4.03%)

Table 5: Seroprevalence of abortifacient agents in sheep and goats.

Species	Flock No	No of +cases	C. abortus	T. gondii	Miced infection (Chl+Toxo)
Sheep	879	156(17.74%)	40(4.55%)	78(8.87%)	38(4.32%)
Gost	706	122(17.28%)	40(5.66%)	56(7.93%)	26(3.68%)
Total	1585	278(17.53%)	80(5.04%)	134(8.45%)	64(4.03%)

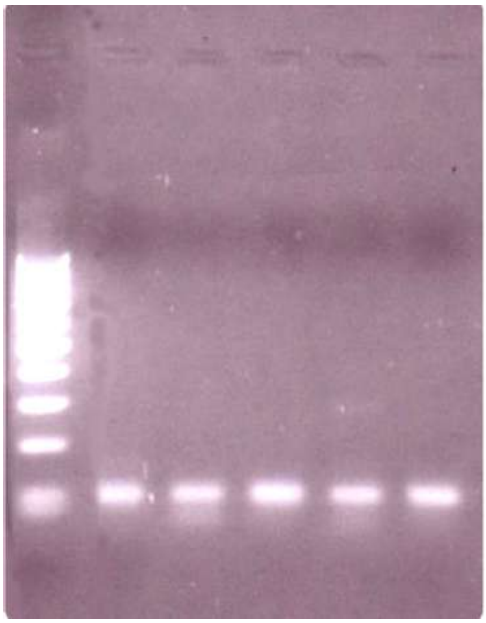

Fig. 1: Showing electrophoretic pattern of *C. abortus* specific PCR product (119 bp) in 2% agarose gel stained with ethedium bromide. Lane 1: 100bp DNA marker.Lane2: Positive control (extracted DNA of *C. abortus* DNA), Lane 3, 4, 5 and 6: specific *C. abortus* PCR product (119 bp) from placenta and fetal tissues.

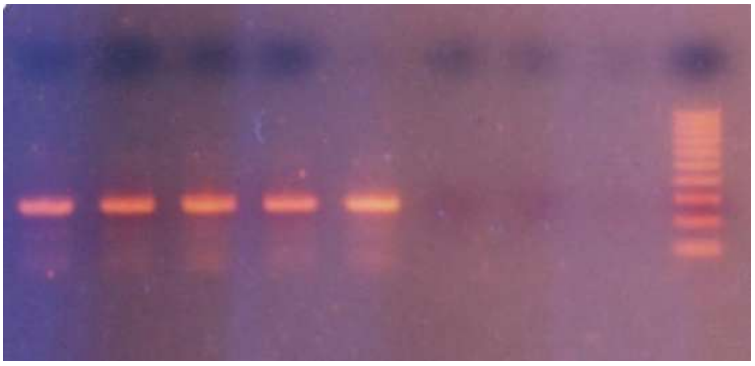

Fig. 2: Showing electrophoretic pattern of specific PCR product (300 bp) in 2% agarose gel stained with ethedium bromide. Lane 9: 100bp DNA marker.Lane1: Positive control (Extracted DNA of DNA), Lane 2, 3, 4 and 5: specific PCR product (300 bp) from placenta and fetal tissues.

C. abortus seroprevalence of 9.07% (144/1585) as shown in table 3. All animals were serologically free of Listeriosis and mycoplasmosis (Table 3).

The total abortion rate was high in sheep (17.74%) than goat as shown in table 5. Goats showed higher *C. abortus* infection while sheep was higher in single and mixed infection with *C. abortus*.

In the present study, PCR assay was applied as a confirmatory test for *C. abortus* and seropositive ewes and does.All the examined samples of seropositive aborted ewes and does (placenta and fetal tissues) were either positive for *C. abortus* (5.04%) or (8.45%) or both of them (4.03%) as shown in figures 1 and 2. Positive controls of *C. abortus* and DNA gave the expected products (119bp and 300bp respectively).

DISCUSSION

Although brucellosis is the most prevalent zoonoses inducing abortion in small ruminants (11.6%) in the Arabian zone [12], the current work focused on the other abortifacient zoonoses (*T. gondii*, *C. abortus, L. monocytogenes* and *M. agalactiae*), which reflect the need of public health worry by KSA veterinary authorities for controlling reproductive drawbacks in small ruminants.

This study revealed high incidence of abortion of non-brucella origin in the examined small ruminants (17.53%) which imposes the application of new schedule for the epidemiology of abortifacient agents. As a result, the diagnosis and screening of *C. abortus* infection is achieved mainly by serological detection of anti-chlamydia antibodies. Clamydial infections from birds are among the longest recognized zoonotic infectious but recently several publications that suggest that the spectrum of diseases caused by such Chlamydia infection may be much wider that has been realized before [13].

Indirect enzyme-linked immunosorbent assays (IELISA) based on chlamydial LPS [14], the major outer membrane protein (MOMP) [15] and the polymorphic outer membrane proteins (POMPs) [16], as well as an indirect immunofluorescence assay have been developed in an attempt to improve the detection of *C. abortus* antibodies, with varying degrees of sensitivity and specificity. Here we used Pourquier®Elisa kit that based on a recombinant 80-90 kDa protein fragment from *C. abortus*. From our results (9.07%),Pourquier ELISAs proved to be one of the most specific methods as its results came in agreement with the results of PCR method, this was parallel to the results of Wilson *et al.* [17]. This proved that the ELISA-*C. abortus*is a suitable method for diagnosis of chlamydiasis in order to implement early prophylactic measures from the first cases of abortion [18].

The seropositivity of *C. abortus* in goats was higher than that of sheep and comes in agreement with that of Masala *et al.* [19]. PCR has become a useful tool for the detection of Chlamydophila in biological samples. The isolation of these pathogens from aborted samples (fetuses and placentae) represents the gold standard for definitive diagnosis. However, isolation requires obtaining samples in optimal conditions (they must be fresh, with little or no contamination and free of toxic factors) that contain a threshold number of live and viable microorganisms. In fact, contamination with other bacteria, inadequate transport conditions, autolysis and other factors may all adversely affect isolation.DNA detection is more rapid than isolation and can be considered a useful technique for diagnosing pathogens [20 - 22].

Different diagnostic methods for assaying toxoplasmosis sero-prevalence in aborted sheep and goats provided variable figures in aborted sheep in KSA

with an incidence of 41.8 (IHA), 23.4(LAT) and 52.2(IFA) as reported by Sanad and Al-Ghabban [23]. The high incidence of toxoplasmic abortion in sheep and goats may be related to their ability to graze closer to the ground than can cattle; therefore they could have more possibilities to ingest oocysts contaminating soil. Gamarra *et al.* [24] showed that the likelihood of being sero-positive in sheep was more than 90 times higher than in cattle. Also, most sheep acquire infection after birth and less than 4% of persistently infected sheep transmit the parasite vertically to the next generation [25]. Prenatal mortality rates (including ovine abortion and neonatal mortality due to *T. gondii*) in affected flocks can be as high as 50%. The prevalence usually higher in herds that suffers toxoplasmic abortions due to placental transmission or postnatal infection via licking aborted foeti and amniotic fluids containing tachyzoites [25]. Where, ewe's congenital transmission may occur as many as 66% of pregnancies [26].

The higher prevalence in KSA could be attributed to the high temperature and humid condition favorable for longer viability of oocyts spourulation and survival. Confirming our results, small ruminants sero-prevalence were found to be country dependent and increase in endemic communities, where the hot and humid environments found to create higher incidence of toxoplasmosis than the cold and dry ones [25]. like Egypt 48%,Paraná 52%, Canada 57 %, Bangladesh 64%, USA 66%,France 68%, Côte d'Ivoire -Ivory (coast) 69%, Ahuaz (Iran) 70%, Israel 72% and Serbia 84.5%,While sheep sero-prevalence was lowered in other countries, where the environmental conditions unfavorable and hindrance oocysts sporulation and survival, like Chile 27%, Jordan 20% and Italy 14% [27].

Also, insufficient cooked mutton connect vital source for food-borne human toxoplasmosis: Recently *T.gondii* identified as the third leading cause of death among food-borne immunocompromised individuals due to latency [28].Cook *et al*. [28] identified eating uncooked lamb as a risk factor for infection in pregnant women in Europe. In a retrospective study of 131 mothers in the USA who had given birth to children infected with *T. gondii*, 50% recalled having eaten uncooked meat [29].

In this study, ELISA techniques showed mixed infection with *C. abortus* and *T.gondii* of 4.03% (64/1585).This came in agreement with Brodie *et al*. [30] described the simultaneous detection of and *C. abortus* in an abortion outbreak in a Scottish sheep flock, while Szeredi and Bacsadi [31]observed simultaneous infection with *C. abortus* and in 4 ovine placentae; even though they caused lesions in separate locations of the cotyledons. Occasionally *T.gondii* and *C. abortus* can be detected simultaneously from the same outbreak of abortion [32].

Therefore it is advised to administrate two intramuscular injections of high-dose, long-acting oxytetracycline (20 mg/kg) during the last month of gestation can reduce the number of abortions and the quantity of *C. abortus* shed at parturition [33] and for toxoplasmosis, two months post treatment with sulfademidine 33.3 %, for 5 successive days at a dose of 200 mg/kg [34].

1307/1585 (82.5%) are sero-negative brucella, found not containing antibodies to any of tested pathogens. Those aborted animals may be due to other infectious pathogens that not were tested in this study, or related to non- infectious causes (feeding, traumatic, toxic grassland, Alfa toxin …). Further studies are needed to determine the possible role of other bacterial and viral agents in ovine and caprine abortion.

It was concluded that although brucella is the major abortifacient agent in small ruminants in KSA, also other pathogens were of great importance and should be followed in diagnosis and control. Also, this study revealed that in KSA, aborted animals were primary exposed to *T. gondii* and *C. abortus* through direct contact with oocysts shedder cats' or carrier wild birds or via contaminating ration. But, the higher incidence of abortion may be maintained through materno-foetal diffusion.

REFERENCES

1. Glenda Dvorak, 2009. Health Protection Agency Chlamydophila zoonotic infections. National standard methods QSOP47 Issue, 1, 1:13.
2. Al-Dughaym, A.M., A. Fadl El mula, G.E. Mohamed, A.A. Hegazy, Y.A. Radwan, F.T.M. Housawi and A.A. Gameel, 2001. First report of an outbreak of ovine septicaemic listeriosis in Saudi Arabia .Rev. sci. tech. Offint. Epiz, 20: 777-783.
3. Longbottom, D. and L.J. Coulter, 2003. Animal chlamydioses and zoonotic implications. J. Comp. Pathol., 128: 217-244.
4. Darwish, M. and A. Benkirane, 2001. Field investigations of brucellosis in cattle and small ruminants in Syria, 1990 to 1996. Rev. Sci. Tech., 20: 769-775.
5. Aitken, I.D., 2000. Chlamydia abortion. In: Martin, W.B. and I.D. Aitken, (eds.): Diseases of Sheep. 3rd ed. Blackwell Science, Oxford.

6. Tenter, A.M., A.R. Heckeroth and L.M. Weiss, 2000. *Toxoplasma gondii*: from animals to humans. Int. J. Parasitol., 30: 1217-1258. Al-Qudah, K.M. L.A. Sharif, R.Y. Raouf, N.Q. Hailat and F.M. Al-Domy, 2004. Sero-prevalence of antibodies to *Chlamydophila abortus* shown in Awassi sheep and local goats in Jordan. Vet. Med. Czech, 49(12): 460-466.
7. Hassig, M., H. Sager, K. Reitt, D. Ziegler, D. Strabel and B. Gottstein, 2000. *Neosporacaninum* in sheep: a herd case report. Vet. Parasitol., 117: 213-220.
8. SYROCOT (Systematic Review on Congenital Toxoplasmosis) Study Group, Thiebaut, R. S. Leproust, G. Chene and R. Gilbert, 2007. Effectiveness of prenatal treatment for congenital Toxoplasmosis: a meta-analysis of individual patients' data. Lancet, 369(9556): 115-122.
9. Osebold, J.W.V. and O. Aalund, 1968. Interpretation of serum agglutinating antibodies to *Listeria monocytogenes* by immunoglobulin differentiation. J. Infect. Dis., 118(2): 139-48.
10. Burg, J.L., C.M. Grover, P. Poletty and J.C. Boothroyd, 1989. Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase chain reaction. J. Clin. Microbiol., 27: 1787-1792.
11. Thiele, D., M.M. Wittenbrink, D. Fisher and H. Krauss, 1992. Evaluation of the polymerase chain reaction (PCR) for detection of *Chlamydia psittaci* in abortion material from ewes. Zbl. Bakteriol. Int. J. Med. Microbiol., 277: 446-453.
12. Al-Sekait, M.A,. 1999. Seroepidemiological survey of brucellosis antibodies in Saudi Arabia. Ann. Saudi. Med., 19: 219- 222.
13. Walder, G., H. Hotzel, C. Brezinka, W. Gritsch, R. Tauber, R. Wurzner and F. Ploner, 2005. An unusual cause of sepsis during pregnancy: recognising infection with *Chlamydophila abortus*. Obstet Gynecol, 106: 1215-1217.
14. Griffiths, P.C., J.M. Plater, M.W. Horigan, M.P.M. Rose, C. Venables and M. Dawson, 1996. Serological diagnosis of ovine enzootic abortion by comparative inclusion immune-fluorescence assay, recombinant lipopolysaccharide enzyme-linked-immunosorbent-assay and complement fixation test. J. Clin. Microbiol., 34: 1512-1518.
15. Hoelzle, L., E. K. Hoelzle and M.M. Wittenbrink, 2004. Recombinant major outer membrane protein (MOMP) of *Chlamydophila abortus*, *Chlamydophila pecorum* and *Chlamydia suis* as antigens to distinguish chlamydial species-specific antibodies in animal sera. Vet. Microbiol., 103: 85-90.
16. Buendia, A.J., F. Cuello, L. Del Rio, M.C. Gallego, M.R. Caro and J. Salinas, 2001. Field evaluation of a new commercially available ELISA based on a recombinant antigen for diagnosing *Chlamydophila abortus* (*Chlamydia psittaci* serotype 1) infection. Vet. Microbiol., 78: 229-239.
17. Wilson, K., M. Livingstone and D. Longbottom, 2009. Comparative evaluation of eight serological assays for diagnosing *Chlamydophila abortus* infection in sheep. Veterinary Microbiology, 135: 38-45. Aspinall, T.V. D. Marlee, J.E. Hyde and P.F. Sims, 2002. Prevalence of in commercial meat products as monitored by polymerase chain reaction: food for thought. Int. J. Parasitol., 32: 1193-1199.
18. Rekiki, A., S. Hammamia and A. Rodolakis, 2006. Comparative evaluation of a new commercial recombinant ELISA and the complement fixation test for the diagnosis of *Chlamydophila abortus* infection in naturally infected flocks in Tunisia. Small Ruminant Res., 66: 58-63.
19. Masala, G., R. Porcu, G. Sanna, A. Tanda and S. Tola, 2005. Role of *Chlamydophila abortus* in ovine and caprine abortion in Sardinia, Italy. Vet. Res. Commun., 29: 117-123.
20. Hartley, J.C., S. Kaye, S, Stevenson, J. Bennett and G. Ridgway, 2001. PCR detection and molecular identification of Chlamydiaceae species. J. Clin. Microbiol., 39: 3072-3079.
21. Hurtado, A., G. Aduriz, B. Moreno, J. Barandika and A.L. García-Pérez, 2001. Single tube nested PCR for the detection of *Toxo-plasma gondii* in fetal tissues from naturally aborted ewes. Vet. Parasitol., 102: 17-27.
22. Masala, G., R. Porcu, C. Daga, S. Denti, G. Canu, C. Patta, C. and S. Tola, 2007. Detection of pathogens in ovine and caprine abortion samples from Sardinia, Italy, by PCR. J. Vet. Diagn. Invest, 19: 96-98.Borde, G. G. Lowhar and A. Adesiyun, 2006. *Toxoplasma gondii* and *Chlamydophila abortus* in Caprine Abortions in Tobago: a Sero-Epidemiological Study. J. Vet. Med. B., 53: 188-193.
23. Sanad, M.M. and A.J. Al-Ghabban, 2007. Serological survey on toxoplasmosis among slaughtered sheep and goats in Tabouk, Saudi Arabia. J. Egypt. Soc. Parasitol., 37: 329-340.
24. Gamarra, J.A., O. Cabezon, M. Pabon, M.C. Arnal, D.F. Luco, J.P Dubey, C. Cortzar and S. Almera, 2008. Prevalence of antibodies against *Toxoplasma gondii* in roe deer from Spain. Vet. Parasitol., 153: 152-156.

25. Aspinall, T.V., D. Marlee, J.E. Hyde and P.F. Sims, 2002. Prevalence of in commercial meat products as monitored by polymerase chain reaction: food for thought. Int. J. Parasitol., 32: 1193-1199.
26. Pereira-Bueno, J., A. Quintanilla-Gozalo, V. Perez-Perez, G. Alvarez-Garcia, E. Collantes-Fernandez and L.M. Ortega-Mora, 2004. Evaluation of ovine abortion associated with *Toxoplasma gondii* in Spain by different diagnostic techniques. Vet. Parasitol., 121: 33-43.
27. Buxton, D., S.W. Maley, S.E. Wright, S. Rodger, P. Bartley and E.A.Innes, 2007. *Toxoplasma gondii* and ovine toxoplasmosis: new aspects of an old story. Vet. Parasitol., 149: 25-28.
28. Cook, A.J., R.E. Gilbert, W. Buffolano, J. Zufferey, E. Petersen, P.A. Jenum, W. Foulon, A.E. Semprini and D.T. Dunn, 2000. Sources of toxoplasma infection in pregnant women: European multicentre case-control study. European Research Network on Congenital Toxoplasmosis. BMJ., 15,321(7254): 142-7.
29. Boyer, K.M., E. Holfels, N. Roizen, C. Swisher, D. Mack, J. Remington, S. Withers, P. Meier and R. McLeod, 2005. Risk factors for *Toxoplasma gondii* infection in mothers of infants with congenital toxoplasmosis: Implications for prenatal management and screening. Am. J. Obstet. Gynecol., 192(2): 564-71.
30. Brodie, T.A., J.L. Duncan and M.J.A. Harvey, 1983. Role of enzootic abortion and toxoplasmosis in an outbreak of abortion in a Scottish sheep flock. Vet. Rec., 5: 437-440.
31. Szeredi, L. and A. Bacsadi, 2002. Detection of *Chlamydophila* (*Chlamydia*) *abortus* and *Toxoplasma gondii* in Smears from Cases of Ovine and Caprine Abortion by the Streptavidin-Biotin Method. J. Comp. Path., 127: 257-263.
32. Steuber, S., A. Niu, C. Bauer, J. Reetz, A. Roth and K. Janitschke, 1995. Detection of *Toxoplasma gondii* in tissues of ovine abortions using the polymerase chain reaction. Deutsche Tiera Èrztliche Wochenschrift, 102: 91-93.
33. Al-Qudah, K.M., L.A. Sharif, R.Y. Raouf, N.Q. Hailat and F.M. Al-Domy. 2004. Sero-prevalence of antibodies to *Chlamydophila abortus* shown in Awassi sheep and local goats in Jordan. Vet. Med. Czech., 49(12): 460-466.
34. Ramadan, M.Y., A.D. Abdel- Mageed and H.F. Khater, 2007. Seroprevalence and preliminary treatment of toxoplasmosis of pregnant goats in Kalubyia Governorate, Egypt. Acta Scientiae Veterinariae. 35(3): 295-301.