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# Analysis of Risk Factors Associated with Seroprevalence of *Toxoplasma gondii* in Dairy Animals from Khartoum State, Sudan

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**ABSTRACT**

A cross-sectional study was conducted between October 2012 and April 2014 to determine the sero-prevalence and the main risk factors associated with the *T. gondii* infection in dairy cattle and co-herded camels, sheep and goats in dairy farms from Khartoum State. A total of 1477 serum samples from 1216 dairy cows, 61 camels, 100 sheep and 100 goats were examined for specific *T. gondii* IgG antibodies using Latex Agglutination Test (LAT). The overall seroprevalence determined were 92.7% and 45.3% at herd and individual level, respectively. The antibody levels ranged from 1:2 to 1:32 in cattle and camels and up to 1:128 in sheep and goats. Possible association of the infection with the investigated risk factors was analyzed by univariate analysis and variables significant at $p \leq 0.25$ were included in multivariate logistic regression models. The results showed that, the differences between the seven localities of the State and the four animal's species were statistically highly significant ($p<0.01$). The univariate analysis included herd type, source of fodder, source of water, neosporosis, keeping cats, stray cats, keeping both dogs and cats and presence of both stray dogs and cats as risk factors associate with LAT positive status of *T. gondii* infection. Districts, production system, herd size, source of concentrate, brucellosis and presence of stray cats were not significant risk factors. The multivariate analysis indicated region (Omdurman and Bahri), Animal species (sheep), source of water (common canal) and source of fodder (prepared in the farm) as the significant ($p<0.05$) risk factors of *T. gondii* LAT seropositivity. The study also reported occurrence of abortion (72.4%), repeat breeding (76.3%), repeated abortion (18.2%) and stillbirth (18.4%) during interview with the owners of the investigated dairy herds. Interestingly, 61.8% of the interviewed farmers send their dairy animals with reproduction problems to slaughter houses. Generally, this is the first comprehensive report on risk factors associated with seropositivity of *T. gondii* infection in dairy animals in Sudan. The study concluded that dairy animals in the Sudan are widely exposed to *T. gondii* infection and Sudanese people are at risk of toxoplasmosis.

## INTRODUCTION

*Toxoplasma gondii* (*T. gondii*) is one of the most important causes of protozoal parasitic infectious abortion in humans and livestock in all continents (Huong *et al.,* 1998, Dubey 2004; Taylor *et al.,* 2007; Weiss and Kim, 2007; Innes, 2011). Toxoplasmosis is an important globally distributed zoonotic parasitic disease resulting in a significant impact in foetuses and the immunosuppressed individuals. In the Sudan, data on *T. gondii* infection in humans and domestic animals mostly used for human consumption are scarce. Moreover, studies on risk factors for *T. gondii* infection in humans and animals are not available. Studies of risk factors for human toxoplasmosis cases were relatively related to food borne infection (Slifko *et al.,* 2000; Tenter *et al.,* 2000; Ogendi *et al.,* 2013) during consumption of raw or undercooked meat. Intake of milk or milk by-products containing tachyzoites may cause the infection in animals and humans (Dubey and Beattie 1988; Ataseven *et al.* 2006). A few studies were made to identify risk factors that may be associated with acquiring *T. gondii* infection postnatally (Gilot-Fromont *et al.,* 2009; Clun *et al.,* 2006; Figliuolo *et al.,* 2004). The prevalence of *T. gondii* infection in humans in the Sudan is not continuously monitored and the information on the infection in domestic animals is scarce (Seri *et al.,* 2003, Khalil and Elrayah, 2011; Elfahal *et al.,* 2013, Awadia, 2013).

The public health importance of *T. gondii* infection and the lack of its epidemiological data in the Sudan, prompted us to conduct this cross-sectional study in dairy farms. This is to investigate the possible main risk factors associated with the *T. gondii* seroprevalence in animal species mostly used for human consumption in the Sudanese food culture.

## MATERIALS AND METHODS

**The Study Area:** The Khartoum State is one of the eighteen states of the Sudan (Figure 1). The State has seven localities, two (Khartoum and Jabal Aolya) in Khartoum district, two (Bahri and eastern Nile) in Khartoum North and three (Karary, Ombadda and Omdurman) in Omdurman district (MLFR 2014).

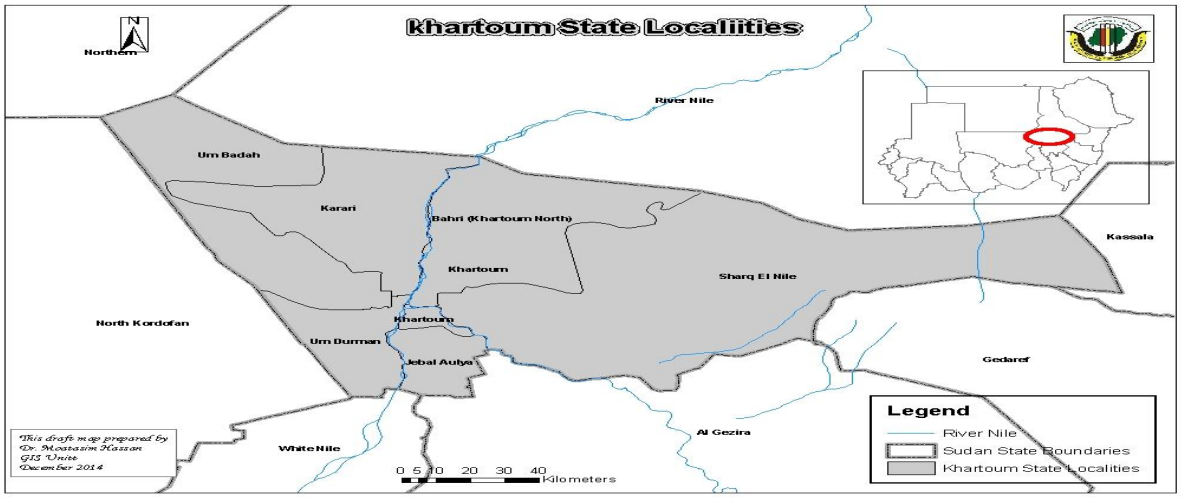


**Figure 1: The Sudan map showing the Khartoum State (the area of the study in red colour).**

**Study Population:** the study involved a total of 1477 adult animals, including 1216 heads of dairy cows, 61 she-camels, 100 sheep and 100 goats from dairy farms (177 herds) selected at random from various dairy clusters in the different regions of the Khartoum State.

**Samples:** Blood for serum was collected from the jugular vein of the above mentioned animals during October 2012 to April 2014. Their sera were separated and cryo-preserved in -20ºC until needed.

**Serological examination:** The presence of *T. gondii* specific IgG antibodies was examined by Latex Agglutination Test as described in our recent report (Ibrahim *et al.* 2014). Generally, serum with antibody titer of 1:2 was considered positive and a herd considered positive when at least one serum sample from the herd reacted positive.

**The Results of other diseases:** data on Brucella and Neospora seropositivity in these farms were available since these animals were included in the national brucellosis and neosporosis research projects. The details of which were reported elsewhere (Ibrahim *et al.* 2014).

**Collection of Epizootiological data:** Questionnaire was filled in by farm personnel (owner, farm manager or worker) at the time of blood collection. The questionnaire included data on farm type, herd size (small≤100, Large>100 heads), production system, feeds and water, reproductive problems and intervention as well as presence of dogs and cats in the farm.

**Statistical analysis:** Differences in the seroprevalence among the investigated areas and animal species were analyzed by Chi-square test. The differences were considered statistically significant when ($p \leq 0.05$). The same test was used to analyze the influence of all examined factors on *T. gondii* seroprevalence (Univariate analysis). Variables significant at $p \leq 0.25$ at 95% confidence Intervals (95% CI) level were selected for inclusion in multivariate analysis. The overall fit of the logistic regression models was assessed using Hosmer-Lemeshow goodness of fit statistics. Results are presented as odds ratios (OR) with 95% CI. The level of significance was 5% ($p \leq 0.05$). All statistics were performed using SPSS version 17.0 statistical package.

**RESULTS**

Zoographic and farm management characteristics of the investigated population are presented in Tables (1 and 2). Antibodies were detected in 669 out of 1477 (45.3%) dairy animals examined distributed in 164 out of 177 (92.7%) dairy herds. The seropositive rates of sheep, goats, camels and cattle were 75%, 64%, 54.1% and 40.9% respectively, with highly statistically significant ($p<0.01$) differences among localities and animal's species (Table 1). The levels of antibody titrations were presented in Figure (2). Univariate analysis of risk factors showed that region, animal species, herd type, source of fodder, source of water, neosporosis, keeping cats, keeping both dogs and cats, and presence of both stray dogs and cats were significantly ($p<0.05$) associated with the seropositivity of *T. gondii*. Districts, production system, herd size, source of concentrate, brucellosis and presence of stray cats were not significant risk factors (Tables 1 and 2). Results of multivariate analysis revealed more statistically significant likelihood of infection in Bahri ($p=0.000$) and Omdurman ($p=0.044$) than in Ombadda locality and in sheep ($p=0.006$) than in cattle. The effect of source of water (common canals) and source of fodder were significantly ($p=0.027$ and $p=0.004$) associated with *T. gondii* infection (Table 3). Localities (Eastern Nile), keeping cats, keeping both dogs and cats, and

presence of both stray dogs and cats were the factors found not to be significantly associated with *T. gondii* infection (p>0.05) but with increasing odds of being LAT positive (Table 3).

**Table 1: Estimated Seroprevalence of *T. gondii* infection in Dairy animals in Khartoum State and Univariate Analysis for the associated Risk factors using Chi square (October 2012 to April 2014)**

| Risk Factors | | No of Animals examined | No of P+ve | Seroprevalence (%) | 95% CI | | *p-value* |
|---|---|---|---|---|---|---|---|
| | | | | | Lower | Upper | |
| **Districts** | Khartoum | 474 | 226 | 47.7 | | | |
| | Kh. North | 629 | 282 | 44.8 | | | 0.386 |
| | Omdurman | 374 | 161 | 43.0 | | | |
| **Localities** | Khartoum | 96 | 34 | 35.4 | | | |
| | Jabal Aolia | 378 | 192 | 50.8 | | | |
| | Eastern Nile | 301 | 123 | 40.9 | | | |
| | Bahri | 328 | 159 | 48.5 | | | 0.002 |
| | Omdurman | 186 | 93 | 50.0 | | | |
| | Karary | 83 | 32 | 38.6 | | | |
| | Ombadda | 105 | 36 | 34.3 | | | |
| **Animal sp.** | Cattle | 1216 | 497 | 40.9 | | | |
| | Camels | 61 | 33 | 54.1 | | | 0.000 |
| | Sheep | 100 | 75 | 75.0 | | | |
| | Goats | 100 | 64 | 64.0 | | | |
| **Prod. system** | Intensive | 161 | 78 | 48.4 | | | 0.395 |
| | Semi-intensive | 1316 | 591 | 44.9 | | | |
| **Total** | | **1477** | **669** | **45.3** | | | |

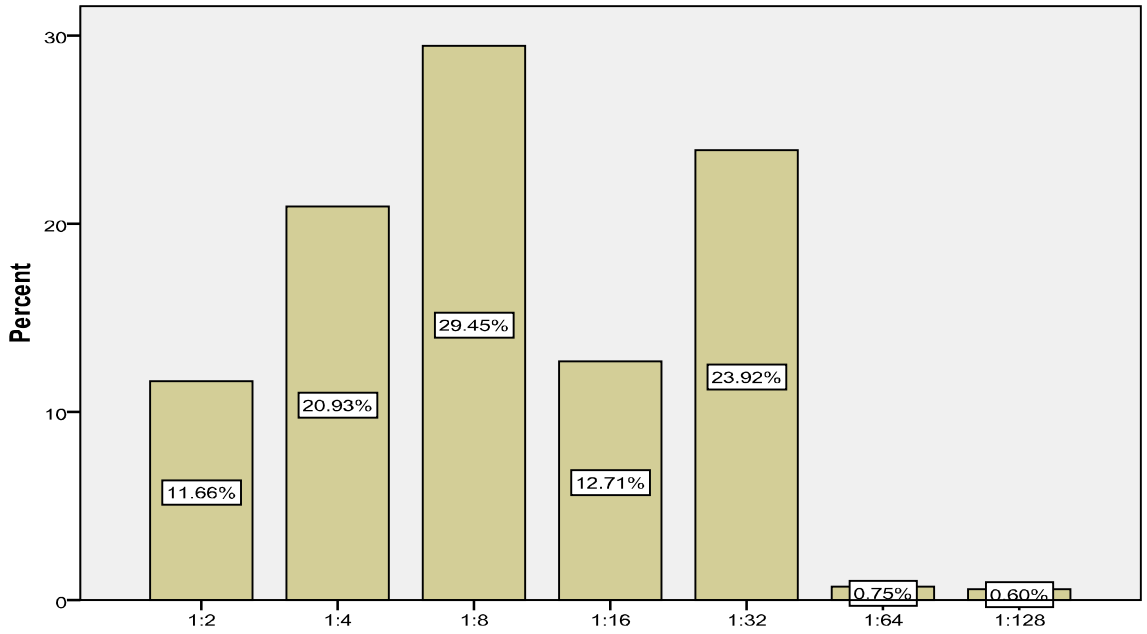


**Fig. 2. Anti-T. gondii Antibody titration in dairy farm animals from Khartoum State using LAT.**

**Table 2: Results of univariate association of Risk factor with LAT toxoplasma seropositivity in dairy animals from Khartoum state using Chi square (October 2012 to April 2014)**

| Risk factors | | No of Animals examined | N of P+ve | P+ve % | p-value |
|---|---|---|---|---|---|
| Herd size | Large | 939 | 425 | 45.3 | 0.973 |
| | Small | 538 | 244 | 45.4 | |
| Herd type | One species | 1176 | 512 | 43.5 | 0.007 |
| | Multi-species | 301 | 157 | 52.2 | |
| Source of concentrate | Ready made | 547 | 236 | 43.1 | 0.203 |
| | Prepared in farm | 930 | 433 | 46.6 | |
| Source of fodder | Buy from market | 848 | 349 | 41.2 | 0.000 |
| | Cut from farm | 629 | 320 | 50.9 | |
| Source of water | Tap water/well | 1225 | 511 | 41.7 | 0.000 |
| | Common canals | 252 | 158 | 62.7 | |
| *Other Diseases | Brucella P+ve | 311 | 129 | 41.5 | 0.260 |
| | Brucella N-ve | 1087 | 490 | 45.1 | |
| | Neospora P+ve | 80 | 36 | 45.0 | 0.041 |
| | Neospora N-ve | 826 | 470 | 56.9 | |
| Keep cats | Yes | 285 | 151 | 53.0 | 0.004 |
| | No | 1192 | 518 | 43.5 | |
| Stray cats | Yes | 712 | 340 | 47.8 | 0.067 |
| | No | 765 | 329 | 43.0 | |
| Keep dogs and cats | Yes | 188 | 121 | 64.4 | 0.000 |
| | No | 1289 | 584 | 42.5 | |
| Stray dogs and cats | Yes | 607 | 294 | 48.4 | 0.043 |
| | No | 870 | 375 | 43.1 | |
| **Total** | | **1477** | **669** | **45.3** | |

*Total animals examined for Brucella and Neospora were 1398 and 906 respectively.

The summary of response of the farm owners was presented in Tables (4 and 5). Only two respondents (Veterinarians) know *Toxoplasma gondii*. The majority of farm managers (61.8%) send cows with reproductive problems (failure) to slaughter houses. The occurrence of reproductive problems such as abortion (72.4%), repeat breeding (76.3%), still-birth (18.4%) and repeated abortion (18.2%) were recorded. Most of the aborted calves (75.7%) ages were less than 7 months, and 45.9% of the aborted cows ages were less than 6 years old while the rest (54.1%) were older (more than 6 years old) when they aborted. A number of 24 owners out of 76 (31.6%) know causes of abortion. Out of these 24 owners, 10 (41.7%) mentioned Brucella as the cause of abortion while the majority 14(58.3%) mentioned other causes including FMD, young age of pregnant cow or young bull, concentrates and out grazing. Almost, all of the visited dairy farms suffer from neonatal deaths.

**Table 3: Results of multivariate association of Risk factor with LAT toxoplasma seropositivity in dairy animals from Khartoum state using Chi square (October 2012 to April 2014)**

| Risk factors | | No of Animals examined | No of P+ve (%) | Wald (L.R) | p-value | Exp(B) | 95% CI for Exp(B) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower | Upper |
| Localities | Ombadda | 105 | 36(34.3) | | | Ref. | | |
| | Khartoum | 96 | 34(35.4) | 3.772 | 0.052 | 0.318 | 0.100 | 1.010 |
| | Jabal Aolia | 378 | 192(50.8) | 0.700 | 0.403 | 0.669 | 0.261 | 1.714 |
| | E. Nile | 301 | 123(40.9) | 2.090 | 0.148 | 1.883 | 0.798 | 4.440 |
| | Bahri | 328 | 159(48.5) | 15.707 | 0.000 | 0.076 | 0.021 | 0.272 |
| | Omdurman | 186 | 93(50.0) | 4.067 | 0.044 | 0.347 | 0.124 | 0.971 |
| | Karary | 83 | 32(38.6) | 2.478 | 0.115 | 0.383 | 0.116 | 1.265 |
| Animal sp. | Cattle | 1216 | 497(40.9) | | | Ref. | | |
| | Camels | 61 | 33(54.1) | 1.167 | 0.280 | 0.359 | 0.056 | 2.303 |
| | Goats | 100 | 64(64.0) | 3.336 | 0.068 | 0.255 | 0.059 | 1.105 |
| | Sheep | 100 | 75(75.0) | 7.499 | 0.006 | 0.111 | 0.023 | 0.536 |
| Herd type | One species | 1176 | 512(43.5) | | | Ref. | | |
| | Multi-species | 301 | 157(52.2) | 0.008 | 0.927 | 1.024 | 0.615 | 1.705 |
| Other diseases | Brucella P+ve | 311 | 129(41.5) | | | Ref. | | |
| | Brucella N-ve | 1087 | 490(45.1) | 3.713 | 0.054 | 1.480 | 0.993 | 2.204 |
| | Neospora P+ve | 80 | 36(45.0) | | | Ref. | | |
| | Neospora N-ve | 826 | 470(56.9) | 0.750 | 0.387 | 0.791 | 0.466 | 1.344 |
| Keep cats | No | 1192 | 518(43.5) | | | Ref. | | |
| | Yes | 285 | 151(53.0) | 0.047 | 0.829 | 1.075 | 0.558 | 2.070 |
| Keep dogs and cats | No | 1289 | 584(42.5) | | | Ref | | |
| | Yes | 188 | 121(64.4) | 1.588 | 0.208 | 2.829 | 0.561 | 14.258 |
| Stray cats | No | 765 | 329(43.0) | | | Ref. | | |
| | Yes | 712 | 340(47.8) | 1.577 | 0.209 | 0.638 | 0.317 | 1.286 |
| Stray dogs and cats | No | 870 | 375(43.1) | | | Ref. | | |
| | Yes | 607 | 294(48.4) | 0.464 | 0.496 | 1.272 | 0.637 | 2.541 |
| Source of concentrate | Ready made | 547 | 236(43.1) | | | Ref. | | |
| | Prepared in farm | 930 | 433(46.6) | 3.087 | 0.079 | 0.552 | 0.285 | 1.071 |
| Source of water | Tap water/well | 1225 | 511(41.7) | | | Ref. | | |
| | Common canals | 252 | 158(62.7) | 4.879 | 0.027 | 0.421 | 0.195 | 0.907 |
| Source of fodder | By from market | 848 | 349(41.2) | | | Ref. | | |
| | Cut from farm | 629 | 320(50.9) | 8.121 | 0.004 | 2.652 | 1.356 | 5.186 |

**Table 4: Results of owner's interview on occurrence of abortion, repeat breeding and still-birth among the dairy herds in the Khartoum State.**

| Reproductive problem | No of Herds | No of Yes | Percent |
|---|---|---|---|
| Occurrence of abortion | 76 | 55 | 72.4 |
| Occurrence of repeated abortion | 55 | 10 | 18.2 |
| Occurrence of repeat breeding | 76 | 58 | 76.3 |
| Occurrence of still-birth | 76 | 14 | 18.4 |

**Table 5: Results of owner's awareness on causes of abortion and toxoplasmosis**

| Awareness | N of Owner's interviewed | Yes (%) | No (%) |
|---|---|---|---|
| Know cause of abortion | 76 | 24(31.6) | 52(68.4) |
| Know Toxoplasmosis | 76 | 4(5.3) | 72(94.7) |
| Know Neospora | 76 | 3(3.9) | 73(96.1) |
| Intervention | 65 | 15(23.1) | 50(76.9) |
| Culling (Slaughtering) | 76 | 47(61.8) | 29(38.2) |

## DISCUSSION

The overall seroprevalence of *T. gondii* (45.3%) and the seroprevalence in the different animal species reported in the present study were higher than that (38%) reported by Khalil and Elrayah (2011). The later authors examined limited number of sera (200 samples) from a limited area in Khartoum State (Kadaro) and they did not include goats in their study. Our results in cattle, camel and goats were relatively in consistent only with that reported by Zein Eldin *et al.,* (1985). These authors reported lower seroprevalence (34%) in sheep compared to our results (75%). That may be just because their samples were collected from (Abattoirs) where usually young males are slaughtered. So as to consider the economical value of the infection in farm industry, our data was collected from only adult females. Also because several references stated that age and breed have no effect on the seroprevalence of toxoplasmosis, but adult animals are more significantly seroposistive than the young ones (Gebremedhin *et al.,* 2013; Bahrieni *et al.,* 2008; Clun *et al.,* 2006; Dubey and Kirkbride 1989; Dubey *et al.,* 1992). According to Alexander and Stimson (1988), Van der Puije *et al.* (2000) and Gebremedhin *et al.,* (2013a&b), females are generally more susceptible to protozoan infection than males. Internationally, the different animal species examined in the present study revealed higher seroprevalence than the world average (Fayer 1981; Dubey 2004; Clun *et al.,* 2006; Dubey *et al.,* 2008). Based on these results, *T. gondii* infection in food animals in the Sudan is dramatically increasing. It is worth mentioning that Tenter *et al.,* 2000) stated that, the infection rate in livestock is an important predictor of human toxoplasmosis risk. In the present study, the statistically significant ($p<0.05$) risk factors for dairy animals included farm location and animal species. Similar observations were reported by Clun *et al.,* (2006) on the farm location factor. The effect of source of water (common canals) and source of fodder were also significantly ($p=0.027$ and $p=0.004$) associated with *T. gondii* infection. Vesco *et al.,* (2007) stated that using of surface water for drinking and farm size were factors associated with *T. gondii* seropositivity in Italy. *T. gondii* was demonstrated in water surface and underground water by DNA amplification in France (Villena *et al.,* 2004). An increasing odds of infection without statistically significant association ($p>0.05$) was found with risk factors like Localities (Eastern Nile), keeping cats, keeping both dogs and cats, and presence of both stray dogs and cats. Interestingly and in agreement with Santos *et al.*. 2009) and in contrast with several authors (Dubey 1980; Dubey and Bettie 1988; Vesco *et al.,* 2007), presence of cats was not significantly associated with Toxoplasma seropositivity in dairy farms. That is may be because stray cats were observed on or near of all farm premises, even when farm personnel/owners denied their presence. Based on the results of this

study, the contaminative factors such as source of water and feeds played an effective role on *T. gondii* infection in dairy animals. Similar observation was reported by Villena *et al.,* (2004) and Vesco *et al.* (2007). On the other hand, this study stated that causes of abortion other than *Brucella* in the Sudan are neglected, although 75.7% of the abortions occur in the first or the second trimester of the pregnancy. The study revealed high prevalence of reproductive failure problems and most of these dairy cows (61.8%) with reproductive problems were (culled) sent to slaughter houses. Hereby, the public health importance of the seropositive cattle needs to be reconsidered as suggested by Clun *et al.,* (2006). The slaughter house survey revealed that 56.3% of the culled dairy cows were infertile and metritis and pyometra were the main signs observed (Elfadel 2014). Economic-wise, most of these culled (Slaughtered) dairy cows due to reproductive problems were in their peak of milk production ($2^{nd}$ or $3^{rd}$ production period, i.e. less than 6 years old). In conclusion, in addition to providing large scale data on *T. gondii* infection in dairy animals and its public health importance as well as its possible economical impact in the Sudan, this is the first report on analysis of possible potential risk factors associated with *T. gondii* infection in the Sudan. Raising the awareness of farmers on toxoplasmosis may serve in control measures. Additionally, aetiological laboratory diagnosis of animal abortions should not be restricted to common bacterial abortifacients only.

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