Review article

Neospora caninum in aborted bovine fetuses in Iran: a systematic review and meta-analysis

Maryam ANSARI-LARI

Department of Food Hygiene and Public Health, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

E-mail: ansari@shirazu.ac.ir; maryamansarilari47@gmail.com

ABSTRACT. The aim of the present study is to systematically review the studies on *Neospora caninum* infection in aborted bovine fetuses in Iran. Search for all published reports on *N. caninum* in Iran was performed from inception until January 2020. Major English (PubMed, Scopus and Science Direct) and Persian (SID, Iranmedex, and Magiran) electronic database sources were used. Finally, 16 articles were assessed for eligibility and 12 articles comprising 20 independent studies were selected for inclusion in the meta-analysis. Due to the statistically significant heterogeneity (Q=149.12, df=19, P<0.001 and I^2=87.26), the random effect model was used for meta-analysis. Prevalence of *N. caninum* infection in 1239 aborted bovine fetuses with 351 positive ones was 34.8% (95% CI: 26.5–44.0%). Sample size was categorized as < 50 and ≥ 50 and subgroup meta-analysis was performed to compensate for the loss of precision due to inclusion of small size studies. The result showed that overall prevalence of infection in the latter group (23.1%) is significantly lower than in the first category (45.7%). It may be suggested that the subgroup with large sample size would be more precise and conservative approximation of the infection at the country level. The present meta-analysis confirms the previous findings regarding the importance of *N. caninum* as a major abortion agent in cattle industry in Iran and the need for appropriate action toward prevention and control of this parasite.

Keywords: aborted fetus, cattle, Iran, meta-analysis, *Neospora caninum*

Introduction

Neosporosis is a disease of primarily dogs and cattle with worldwide distribution caused by an apicomplexan parasite, *Neospora caninum* (*N. caninum*). In cattle, the two major routes of infection are transplacental transmission from mother to fetus, and ingestion of oocyts shed by infected canids via contaminated feed and water [1]. It is one of the most efficiently transplacentally transmitted parasites among all known microbes in cattle [2]. Currently, *Neospora caninum* is a major cause of bovine abortion worldwide and is accompanied with considerable negative economic and reproductive impacts in the cattle industry [1,3]. Abortions may occur at any time, however, most abortions due to neosporosis occur at 5–6 months of gestation [4]. In cattle, neosporosis-induced abortions may be epidemic, following a primary (exogenous) maternal infection; or endemic, subsequent to recrudescence of a persistent

(endogenous) infection during pregnancy [5]. Abortion could be seen repeatedly in consecutive pregnancies in a small proportion (< 5%) of cows, and calves may be born infected in normal pregnancies [4].

In a recent study during 2005–2014, based on data of six large dairy herds (> 600 milking cows), located at three provinces of Iran, the overall rate of abortion was 15.4% [6]. Hence, abortion and reproductive failure are major limitations for dairy producers in Iran, like most other countries in the world. The first report of *N. caninum* in aborted fetuses was from Mashhad, northeast of the country which showed the presence of *N. caninum* in the brains of 13 aborted bovine fetuses by PCR [7]. Since then, many observational and experimental studies about *N. caninum* from different parts of the country have been published, and risk of abortion in *N. caninum* seropositive cows in Iran has been estimated to be nearly two folds higher than their seronegative herd mates [8–9]. Nevertheless, it

should be noted that examination of the serum from an aborting cow is only indicative of exposure to *N. caninum*. It is necessary to examine the fetus for a definitive diagnosis of abortion due to neosporosis.

The aim of the present study is to systematically review the observational studies that have investigated the role of *N. caninum* in aborted bovine fetuses in Iran, and estimate the pooled prevalence of infection in this population by meta-analysis. In addition, the sources of variation between different studies are investigated and the spatial distribution of the infection in aborted bovine fetuses in Iran is illustrated. This work demonstrates a country level picture of the *N. caninum* in bovine aborted fetuses, and estimate the role of this protozoan parasite as an abortion agent in cattle industry in Iran.

Database searches

Search for all published studies on *N. caninum* in Iran was conducted from inception (2007) until January 2020. Major English (PubMed, Scopus and Science Direct) and Persian (SID, Iranmedex, and Magiran) electronic database sources were used for the present study. The keywords used were: *Neospora*, neosporosis, *N. caninum*, abortion, cattle, fetus and Iran. Due to low number of studies, a conservative search term combination in most databases was selected to exclude the possibility of missing any eligible study. As an example, *Neospora* AND Iran was used to search PubMed after examining various combinations of search terms. List of the references of the relevant articles were also searched to find additional studies which were missed in database searches.

Eligibility criteria

Duplicate publications were removed and the author screened the other studies primarily based on the title, and then the abstract. All observational studies, which had been conducted to detect the *N. caninum* in aborted bovine fetuses and stillbirth calves in Iran, were selected, and unrelated studies were excluded. After screening, descriptions of the methods and results sections were evaluated by examining the full text of the selected studies. When publications with overlap information were found, only the data from the one with the best match to the eligibility criteria or with larger sample size was selected. All of these stages were conducted by the

author at least twice, carefully.

Data extraction and collection

Title, authors name, journal title and publication date of the selected articles were recorded. In addition, main study attributes including study design, year of study, diagnostic test, sample size, sampling procedure, geographical location and finally number of positive and negative samples were extracted by the author. Data regarding host characteristics such as age and breed were also collected, when available. When more than one diagnostic test was used in a study, the result for PCR method was extracted for the number of positive and negative samples. Thereafter, each article was reviewed again independently and the above-mentioned data were extracted and recorded again. Finally, two databases were compared, and all disagreements were checked against the original data. In one case, the author contacted the study investigator to obtain detailed information which was missing in the published paper. Selected studies were evaluated for risk of bias by considering clear statement of the following items: objective of the study, type of diagnostic test, study design, method of sampling, geographical area, sample size and thorough numerical results.

Statistical analysis

Extracted data were entered into excel spreadsheet and imported to Comprehensive Meta-Analysis software (Ver. 2.2, BioStat, USA) for further analysis. Cochran's Q statistic was determined for evaluation of heterogeneity between studies. Also, Higgins statistic (I^2) was calculated for assessment of true variation due to hetero-geneity. DerSimonian and Laird (1986) random effect model was used to estimate the pooled weighted infection rate (effect size) of *N. caninum* in aborted bovine fetuses [10].

Sample size was categorized into two groups, < 50 and ≥ 50 fetuses including 12 and 8 studies, respectively. A subgroup meta-analysis was performed for this categorical variable to find its potential role as a source of the heterogeneity in the selected studies. For further assessment of the heterogeneity and to assess if there is a linear trend in the infection, the year of the study was evaluated as continuous covariates using meta-regression analysis. Using visual inspection of the funnel plot
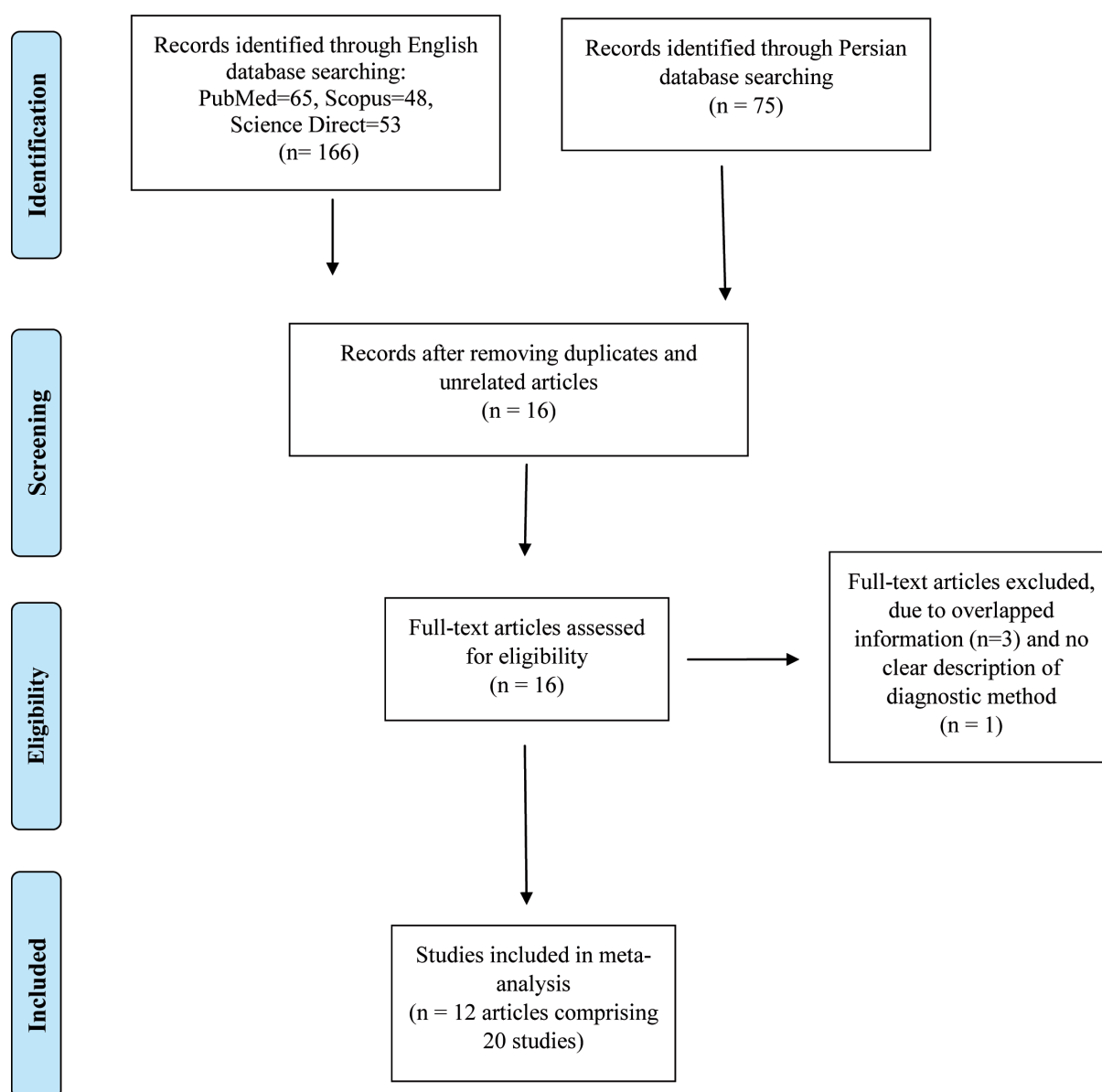
Figure 1. Flow diagram showing selection of studies for meta-analysis of *Neospora caninum* in aborted bovine fetuses in Iran

asymmetry and considering the Egger's regression method, the possibility of publication bias was determined. To assess the stability of the pooled estimate, sensitivity analysis was performed by excluding one study each time from the analysis. An additional sensitivity analysis was conducted to evaluate the effect of studies which were different with the other studies regarding type of study population (aborted fetuses from exclusively seropositive dams) on the pooled estimate. A choropleth map showing the spatial distribution of *N. caninum* in aborted bovine fetuses based on the apparent mean prevalence in each province was generated using Epi Info software (version 7.2 CDC). In all analyses, a P-value less than 0.05 was

considered as statistically significant.

Results

Description of included studies

After searching the English and Persian databases, and after removing duplicated and unrelated studies, 16 articles were selected for full text evaluation. Four additional articles were excluded after reading the full text, due to unclear presentation of diagnostic test or overlapped data. Finally, 12 articles were recognized as eligible for meta-analysis (Fig. 1). One of the selected articles comprised samples from nine different provinces [11]. Details about total number of samples and

Table 1. Studies included in the meta-analysis for *Neospora caninum* infection by PCR in bovine aborted fetuses in Iran

Authors, Year	Province	Total No.	Positive No.	%
Sadrebazzaz et al. 2007 [16]	Khorasan Razavi	6	4	0.67
Razmi et al. 2007 [7]	Khorasan Razavi	100	13	0.13
Razmi et al. 2010 [18]	Khorasan Razavi	151	18	0.12
Nematollahi et al. 2013 [15]	East Azarbaijan	14	6	0.43
Razmi et al. 2013 [21]	Khorasan Razavi	200	23	0.12
Kamali et al. 2014 [11]*	Nine provinces	395	179	0.45
1	Khorasan Razavi	69	27	0.39
2	North Khorasan	41	17	0.42
3	South Khorasan	34	19	0.56
4	Chahar Mahall & Bakhtiari	30	16	0.53
5	Kermanshah	36	13	0.36
6	Esfahan	65	31	0.48
7	West Azarbaijan	33	12	0.36
8	Golestan	44	26	0.59
9	Mazandaran	43	18	0.42
Rafati & Jaafarian 2014 [13]	Chahar Mahall & Bakhtiari	100	11	0.11
Salehi et al. 2015 [14]	Tehran	16	12	0.75
Kaveh et al. 2017 [20]	Qazvin	128	39	0.31
Hoseini et al. 2018 [12]	East Azarbaijan	82	34	0.41
Khani et al. 2018 [19]	Markazi	38	10	0.26
Amouei et al. 2019 [17]	Mazandaran	9	2	0.22

* This article comprised samples from nine different provinces. Data for each province was considered as an independent study.

number of positives in each province was missing in the article (but published as PhD dissertation) and were obtained through contact with the first author. Assuming the data from each province as an independent study, a total of 20 studies from 12 (out of total 31) provinces in the country were included in the meta-analysis (Table 1).

The sample sizes ranged from 6 to 200 aborted fetuses. In two articles, authors stated that they selected the aborted fetuses from a larger number of available samples, randomly [12–13]. Three of the selected articles reported that they examined fetuses exclusively from *N. caninum* seropositive mothers [14–16]. In two other articles, the aborted fetuses were referred cases to referral centers [11,17]. In the remaining [17–21], no obvious method for collection of samples was specified. In nearly all studies the dominant breed for aborted fetuses was referred to directly or could be presumed indirectly

to be Holstein.

While all studies used PCR on brain tissue of aborted fetuses for detection of *N. caninum*, some of studies used one or more of other diagnostic methods such as enzyme linked immunosorbent assay (ELISA), histopathology and immuno-histochemistry (IHC), as well [7,11, 15,16,18]. One study compared the results of PCR with IHC and fetal fluid ELISA and determined the agreement between tests [18]. Authors reported a good agreement between ELISA and PCR (κ values 0.69) and a poor agreement between IHC and PCR (κ values, 0.184). However, it should be mentioned that only 52 brains and not all samples were suitable for IHC [18]. In histopathological examination, various lesions including the characteristic lesions of *N. caninum* infection such as non-suppurative encephalitis as well as other non-specific findings were observed and some authors reported that they

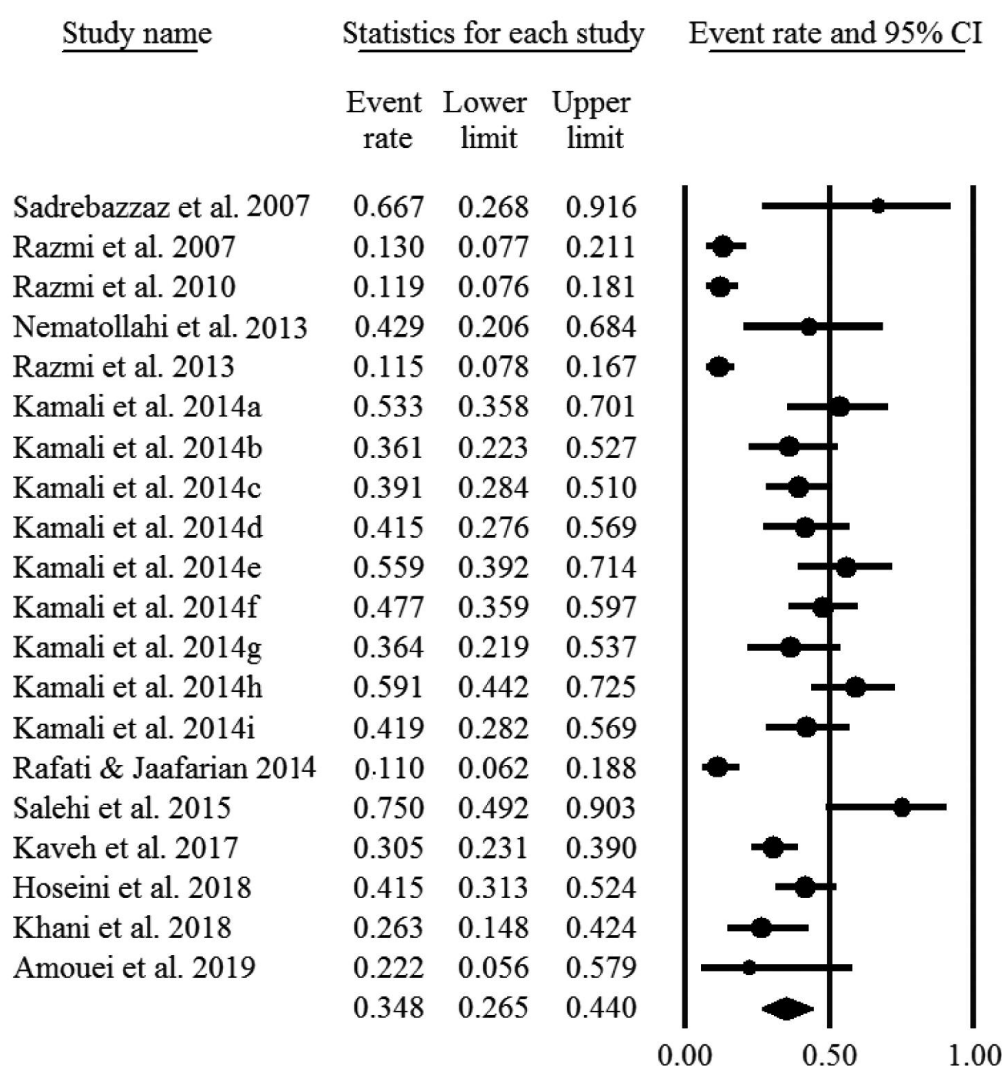| Study name	Statistics for each study			Event rate and 95% CI
	Event rate	Lower limit	Upper limit	
Sadrebazzaz et al. 2007	0.667	0.268	0.916	
Razmi et al. 2007	0.130	0.077	0.211	
Razmi et al. 2010	0.119	0.076	0.181	
Nematollahi et al. 2013	0.429	0.206	0.684	
Razmi et al. 2013	0.115	0.078	0.167	
Kamali et al. 2014a	0.533	0.358	0.701	
Kamali et al. 2014b	0.361	0.223	0.527	
Kamali et al. 2014c	0.391	0.284	0.510	
Kamali et al. 2014d	0.415	0.276	0.569	
Kamali et al. 2014e	0.559	0.392	0.714	
Kamali et al. 2014f	0.477	0.359	0.597	
Kamali et al. 2014g	0.364	0.219	0.537	
Kamali et al. 2014h	0.591	0.442	0.725	
Kamali et al. 2014i	0.419	0.282	0.569	
Rafati & Jaafarian 2014	0.110	0.062	0.188	
Salehi et al. 2015	0.750	0.492	0.903	
Kaveh et al. 2017	0.305	0.231	0.390	
Hoseini et al. 2018	0.415	0.313	0.524	
Khani et al. 2018	0.263	0.148	0.424	
Amouei et al. 2019	0.222	0.056	0.579	
	0.348	0.265	0.440	

0.00   0.50   1.00

Figure 2. Forest plot showing meta-analysis for *Neospora caninum* infection with 95% confidence intervals (CI) in aborted bovine fetuses in Iran

found *Neospora*-like tissue cysts in histo-pathological examination [7,11,15]. In another study, results for PCR of different fetal tissues including brain, liver, lungs, spleen, kidneys and rennet fluids were compared and the authors concluded that brain tissue is the most appropriate sample for detecting *N. caninum* infection in aborted fetuses [12].

In two articles, a number of stillbirths' calves were also included [11,18]. In four selected articles, data about age of all aborted fetuses or *N. caninum* positive ones have been presented [11]. According Kamali et al. [11], 43.3% of all aborted fetuses were between 3–6 months and 44.3% were older than 6 months; *Neospora caninum* was detected in 57.3% and 34.3% of the first and the second groups, respectively. Razmi et al. [18] reported that 98% of aborted fetuses were older than 5 months with a

mean of 7.5 months. Of aborted fetuses which were positive for *N. caninum*, 83% were older than 6 months. Sadrebazzaz et al. [16] reported that all examined fetuses were between 4–9 months old. Finally, in a study by Khani et al. [19], 50% of *N. caninum* infected fetuses were in the second trimester and the other 50% were in the third trimester of gestation.

Risk factors for infection with *N. caninum* such as herd size, parity [11], and abortion history of dam [19], as well as concomitant infection of fetuses with other pathogens such as bovine viral diarrhea virus, bovine herpes virus and *Leptospira* species, *Toxoplasma gondii* and *Brucella* were investigated in some of the selected studies [16,17,20]. No data regarding management system were presented in the selected studies.

*N. caninum infection in aborted fetuses
and meta-analysis*

Data for 20 studies based on province were extracted from the 12 selected articles and were included in the meta-analysis. The minimum *N. caninum* infection prevalence was 11% in the Chahar Mahall and Bakhtiari province [13]. The maximum prevalence was 75% from Tehran province [14] followed by 66% from Khorasan Razavi [16] (Table 1). Pooled estimate for the prevalence of *N. caninum* based on a total number of 1239 of aborted fetuses and 351 positive samples was 34.8% with 95% CI: 26.5–44 (Fig. 2). The Cochran Q value was 149.120, df=19 and P<0.001, and the Higgins index was 87.26%.

Results for the subgroup analyses of sample size categories showed significant difference between two groups (Q=8.77, df=1, P=0.003). Higher prevalence of *N. caninum* in aborted fetuses was observed in studies with <50 sample (45.7%) and the corresponding measures for ≥ 50 sample group was 23.1% (Fig. 3). Meta-regression for evaluation of the effect of year of the study and detection of linear trend was performed and showed no significant result (slope=0.028, SE=0.06, P=0.66).

Sensitivity analyses revealed no considerable change in the overall estimate for any individual study. Minimum and maximum pooled estimates in the sensitivity analysis were 33.1% (95% CI: 25.2–42.1) after removing Salehi et al. [14] and 36.6 (95% CI: 28.1–45.9) after excluding Rafati and Jaafarian [13]. Another sensitivity analysis was performed to investigate the effect of three studies which were on aborted fetuses from seropositive mothers. The results showed that pooled estimate declined from 34.8% to 31.7%, 95% CI: 23.7–41.0.

Publication bias was investigated by funnel plot analysis (Fig. 4) and Egger's regression method.
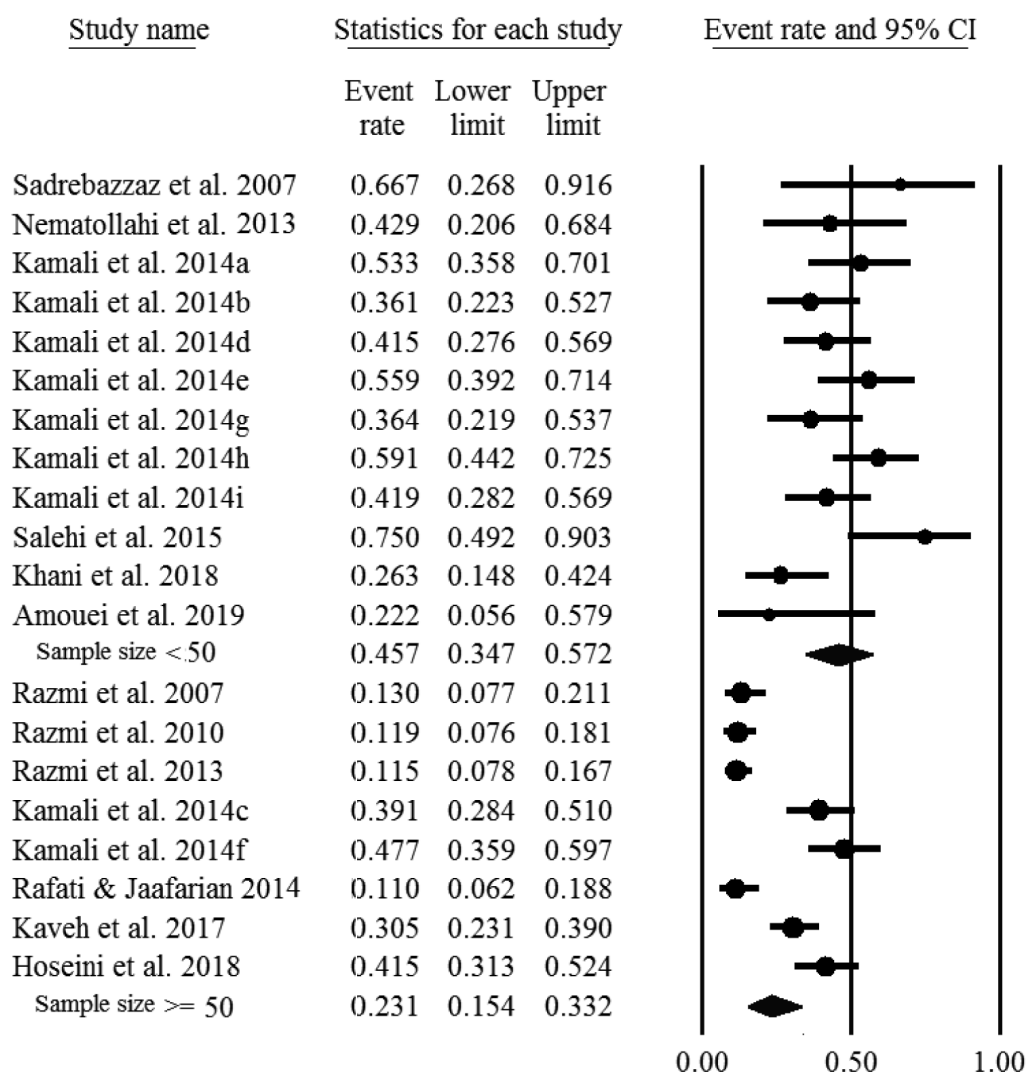
Study name	Statistics for each study			Event rate and 95% CI
	Event rate	Lower limit	Upper limit	
Sadrebazzaz et al. 2007	0.667	0.268	0.916	
Nematollahi et al. 2013	0.429	0.206	0.684	
Kamali et al. 2014a	0.533	0.358	0.701	
Kamali et al. 2014b	0.361	0.223	0.527	
Kamali et al. 2014d	0.415	0.276	0.569	
Kamali et al. 2014e	0.559	0.392	0.714	
Kamali et al. 2014g	0.364	0.219	0.537	
Kamali et al. 2014h	0.591	0.442	0.725	
Kamali et al. 2014i	0.419	0.282	0.569	
Salehi et al. 2015	0.750	0.492	0.903	
Khani et al. 2018	0.263	0.148	0.424	
Amouei et al. 2019	0.222	0.056	0.579	
Sample size < 50	0.457	0.347	0.572	
Razmi et al. 2007	0.130	0.077	0.211	
Razmi et al. 2010	0.119	0.076	0.181	
Razmi et al. 2013	0.115	0.078	0.167	
Kamali et al. 2014c	0.391	0.284	0.510	
Kamali et al. 2014f	0.477	0.359	0.597	
Rafati & Jaafarian 2014	0.110	0.062	0.188	
Kaveh et al. 2017	0.305	0.231	0.390	
Hoseini et al. 2018	0.415	0.313	0.524	
Sample size >= 50	0.231	0.154	0.332	

Figure 3. Forest plot showing subgroup meta-analysis based on sample size (<50 and ≥50) for *Neospora caninum* infection with 95% confidence intervals (CI) in aborted bovine fetuses in Iran
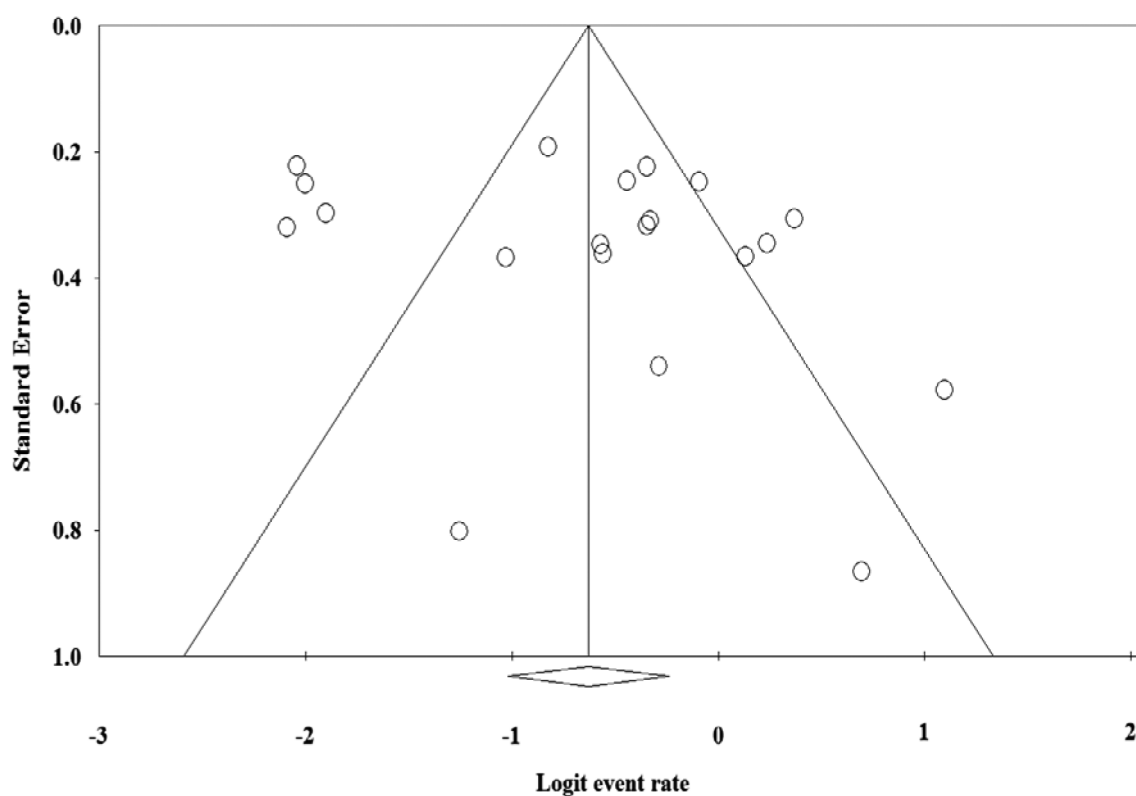
Figure 4. Funnel plot of *Neospora caninum* infection with 95% confidence limits in aborted bovine fetuses in Iran. The figure displays each study's estimated effect plotted against its standard error for examination of publication bias.

Discussion

There was no apparent asymmetry in the funnel plot, and Egger's statistic was not significant (intercept=2.52; 95% CI: -1.52, 6.57; P=0.21), indicating no publication bias. According to the mean apparent detection rate of *N. caninum* in each province, distribution in the country is shown in Fig. 5.

Discussion

Based on the results of the present meta-analysis, 34.8% of aborted bovine fetuses in Iran are infected with *N. caninum*. It should be acknowledged that these types of studies (i.e., studies on aborted fetuses) are commonly based on referral samples and inherently suffer in terms of sample representativeness, because a considerable number of aborted fetuses are not referred anywhere and therefore are not screened for responsible infectious agents. Therefore, the overall estimate may overestimate the true one, and should be interpreted with caution due to this inevitable limitation.

Another point which should be noted is that 12 out of 20 total included studies had low sample size. Small sample size may be a source of random error and leads to imprecision of the pooled estimate. To compensate for this shortcoming, in subgroup meta-analysis, studies with sample size <50 were compared with studies with sample size ≥ 50. The result was significant and showed that overall estimate for infection in the latter group (23.1%) is significantly lower than the first category (45.7%). This indicates that difference in sample size in different studies is responsible for at least part of the observed heterogeneity. Furthermore, it could be concluded that the pooled estimate for the subgroup with ≥ 50 sample size would be a more precise, conservative and acceptable approximation for the overall picture of the infection in aborted bovine fetuses at the country level. This figure is also supported by similarity to the result of the recently published meta-analysis for bovine neosporosis, which revealed that a pooled serologic prevalence of *N. caninum* in the dairy cattle population in Iran is 23.6% with 95% CI: 19.8–27.9 [22].

Three studies reported that they examined fetuses exclusively from *N. caninum* seropositive mothers which all had lower than 50 sample size [14–16]. Selection of fetuses from seropositive dams may be an obvious source of bias toward overestimation of the infection in aborted fetuses. This is evident from decrease in the overall estimate
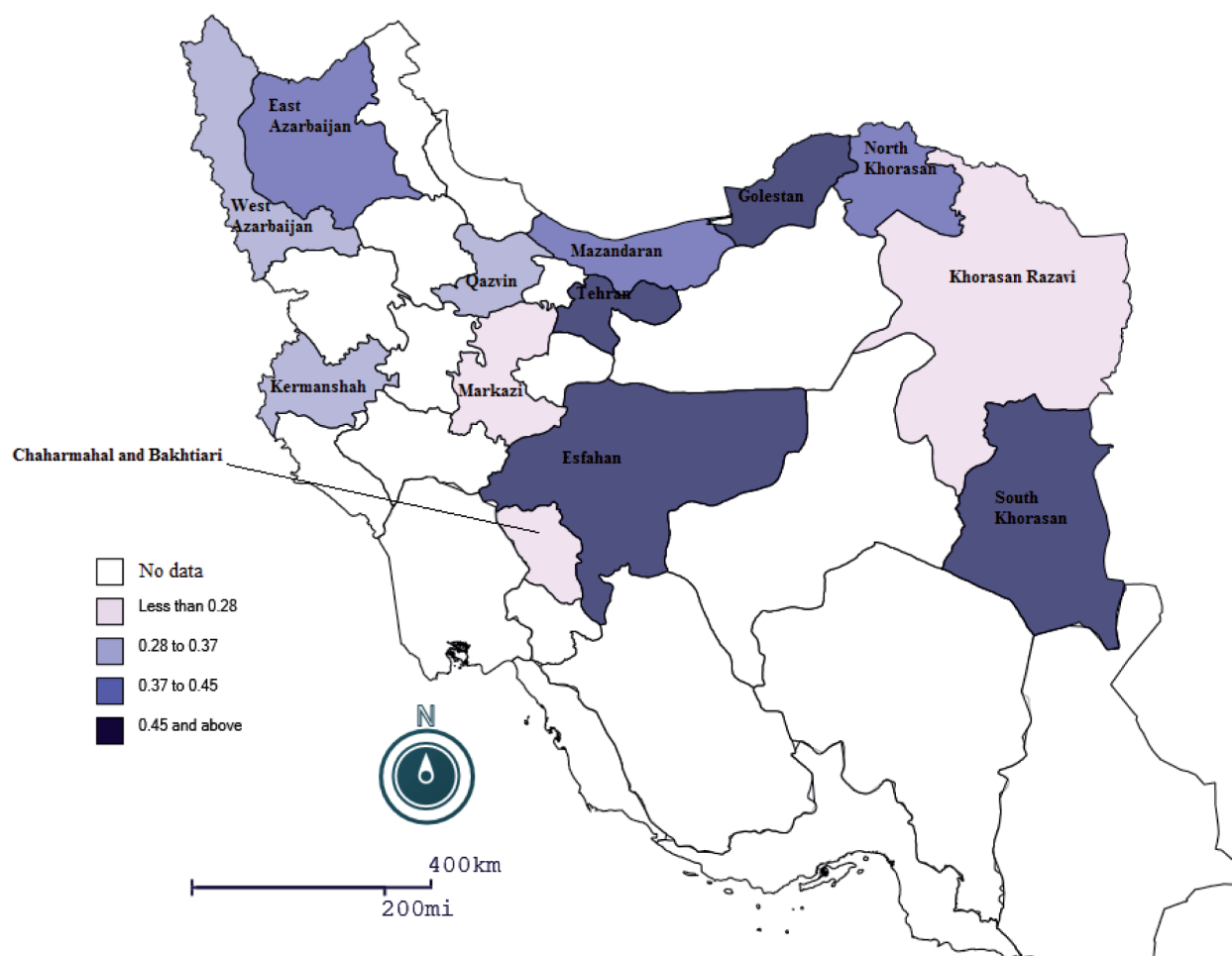
Figure 5. Spatial distribution of *Neospora caninum* infection in aborted bovine fetuses in Iran

(from 34.8 to 31.7%) after removing these three studies in the sensitivity analysis. The conservative estimate mentioned above (23.1%) does not include these studies because of the low sample size. and therefore, controlling this source of bias in the subgroup meta-analysis. According to this conservative estimate, approximately one bovine aborted fetus from every four in the country, is infected with *N. caninum*. The estimate of the infection prevalence in fetuses is high enough to warrant prompt action toward prevention and control of this parasite at a national level. Spatial distribution of *N. caninum* infection in aborted bovine fetuses using choropleth map shows that infection is widespread in the northern half of the country with relatively high variation. There is lack of data for southern half of Iran and it is recommended to pay more attention to this part in future researches.

Since the late 1980s, when the *N. caninum* was recognized for the first time, it has become the most commonly diagnosed cause of abortion in many

parts of the world. As an example, Wilson et al. [23] reported that of the total 236 fetal submissions from dairy herds by active and passive surveillance in British Colombia during 2007–2014, *N. caninum* was diagnosed in 18.2% of cases and was the most commonly identified infectious agent associated with fetal loss. They reported that active surveillance was associated with higher rate of *N. caninum* (41%) compared to passive surveillance (13.3%). A similar study from southern Brazil showed that 23% of the 161 bovine aborted fetuses were infected with *N. caninum* based on histopathological criteria [24]. In a review of *Neospora caninum* in dairy and beef cattle from a Canadian perspective, authors indicated that diagnosis of *N. caninum*-associated abortions in dairy cattle in Ontario increased from 1.6% of abortion submissions in 1993–1994 to 14–15% in 1997 to 2000, and since 1994, *N. caninum* has been the most commonly diagnosed cause of abortion in dairy herds in Ontario [25]. In the Netherlands, it has been recognized as a leading cause of abortion

in cattle [26] and similarly *Neospora* infection was the major cause of abortion (42.5%) identified in dairy herds in California, USA [27]. More generally, studies from around the world indicated that 12–42% of aborted fetuses from dairy cattle are infected with *N. caninum* [4]. Previously, I have suggested that in comparison with the reports from other countries, Iran could be considered among the intermediately infected countries for *N. caninum* seroprevalence in dairy cattle population [22]. Based on the results of the present meta-analysis, seems that this is true for the infection with *N. caninum* in bovine aborted fetuses and emphasizes the large impact of *N. caninum* on dairy producing sector in Iran.

All selected studies in the present work, have used brain tissue PCR as the diagnostic method for infection with *N. caninum*. This eliminates the source of bias due to detection of infection between studies. On the other hand, the risk of bias within studies is also negligible because PCR techniques have been useful as diagnostic tools for detection of the parasite in aborted bovine fetuses [28] and generally have a higher sensitivity than IHC methods and also a high specificity [29–30]. Furthermore, while lesions of neosporosis are found in several organs of aborted fetus, brain is the most consistently affected organ [4].

In conclusion, the present meta-analysis confirms the previous findings that *Neospora caninum* is a major cause of abortion in cattle population in Iran. However, it is worth noting that in the present study the conclusions of the previous validated publications were supported by a multi-variable statistical analyzes. Regarding high burden of economic impact of abortion in dairy industry, appropriate action for prevention and control of this parasite by implementation of educational programs and developing national policies is recommended.

Acknowledgements

References

[1] McAllister M.M. 2016. Diagnosis and control of bovine neosporosis. *Veterinary Clinic of North America Food Animal Practice* 32: 443-463. doi:10.1016/j.cvfa.2016.01.012

[2] Dubey J.P., Schares G., Ortega-Mora L.M. 2007. Epidemiology and control of neosporosis and *Neospora caninum*. *Clinical Microbiology Review* 20: 323-367. doi:10.1128/CMR.00031-06

[3] Reichel M.P., Ayanegui-Alcérreca M.A., Gondim L.F., Ellis J.T. 2013. What is the global economic impact of *Neospora caninum* in cattle – the billion dollar question. *International Journal of Parasitology* 43: 133-142. doi:10.1016/j.ijpara.2012.10.022

[4] Lindsay D.S., Dubey J.P. 2020. Neosporosis, toxoplasmosis, and sarcocystosis in ruminants: an update. *Veterinary Clinic of North America Food Animal Practice* 36: 205-222. doi:10.1016/j.cvfa.2019.11.004

[5] Trees A.J., Williams D.J. 2005. Endogenous and exogenous transplacental infection in *Neospora caninum* and *Toxoplasma gondii*. *Trends in Parasitology* 21: 558-561. doi:10.1016/j.pt.2005.09.005

[6] Keshavarzi H., Sadeghi-Sefidmazgi A., Kristensen A.R., Stygar A.H. 2017. Abortion studies in Iranian dairy herds: I. Risk factors for abortion. *Livestock Science* 195: 45-52. doi:10.1016/j.livsci.2016.11.004

[7] Razmi G.R., Maleki M., Farzaneh N., Talebkhan Garoussi M., Fallah A.H. 2007. First report of *Neospora caninum*-associated bovine abortion in Mashhad area, Iran. *Parasitology Research* 100: 755-757. doi:10.1007/s00436-006-0325-6

[8] Ansari-Lari M., Rowshan-Ghasrodashti A., Jesmani H., Masoudian M., Badkoobeh M. 2017. Association of *Neospora caninum* with reproductive performance in dairy cows: A prospective study from Iran. *Veterinary Research Forum* 8: 109-114.

[9] Salehi N., Haddadzadeh H.R., Shayan P., Vodjgani M., Bolourchi M. 2010. Serological study of in pregnant dairy cattle in Tehran, Iran. *International Journal of Veterinary Research* 4: 113-116. doi:10.22059/IJVM.2010.21364

[10] DerSimonian R., Laird N. 1986. Meta-analysis in clinical trials. *Controlled Clinical Trials* 7: 177-188. doi:10.1016/0197-2456(86)90046-2

[11] Kamali A., Seifi H.A., Movassaghi A.R., Razmi G.R., Naseri Z. 2014. Histopathological and molecular study of *Neospora caninum* infection in bovine aborted fetuses. *Asian Pacific Journal of Tropical Biomedicine* 4: 990-994. doi:10.12980/APJTB.4.201414B378

[12] Hoseini A., Merat E., Samani S., Soltan Nezhad S., Danandeh R. 2018. Comparison of *Neospora caninum* infected tissues in aborted fetal bovine by PCR. *Journal of Veterinary Research* 73: 377-382. doi:10.22059/JVR.2018.203195.2450

[13] Rafati N., Jaafarian M. 2014. The determination of prevalence of *Neospora caninum* in aborted fetuses in dairy cattle of Shahrekord area, Chahar Mahal Bakhtiari province, by Nested-PCR. *Journal of Veterinary Laboratory Research* 6: 45-50. doi:10.22075/JVLR.2017.1257

[14] Salehi N., Gottstein B., Haddadzadeh H.R. 2015. Genetic diversity of *bovine Neospora caninum* determined by microsatellite markers. *Parasitology International* 64: 357-361. doi:10.1016/j.parint.2015.05.005

[15] Nematollahi A., Moghaddam G.H., Jaafari R., Helan J.A., Norouzi M. 2013. Study on outbreak of *Neospora caninum*-associated abortion in dairy cows in Tabriz (Northwest Iran) by serological, molecular and histopathologic methods. *Asian Pacific Journal of Tropical Medicine* 6: 942-946. doi:10.1016/S1995-7645(13)60168-6

[16] Sadrebazzaz A., Habibi G., Haddadzadeh H., Ashrafi J. 2007. Evaluation of bovine abortion associated with *Neospora caninum* by different diagnostic techniques in Mashhad, Iran. *Parasitology Research* 100: 1257-1260. doi:10.1007/s00436-006-0417-3

[17] Amouei A., Sharif M., Sarvi S., Bagheri Nejad R., Aghayan S.A., Hashemi-Soteh M.B., Mizani A., Hosseini S.A., Gholami S., Sadeghi A., Sarafrazi M., Daryani A. 2019. Aetiology of livestock fetal mortality in Mazandaran province, Iran. *PeerJ* 6: e5920. doi:10.7717/peerj.5920

[18] Razmi G.R., Zarea H., Naseri Z. 2010. A survey of *Neospora caninum*-associated bovine abortion in large dairy farms of Mashhad, Iran. *Parasitology Research* 106: 1419-1423. doi:10.1007/s00436-010-1820-3

[19] Khani M., Arabkhazaeli F., Hosseini S.D., Shayan P. 2018. Molecular detection of *Neospora caninum* in aborted fetuses of cattle farms in Arak. *Journal of Veterinary Research* 73: 457-463. doi:10.22059/JVR.2018.218656.2535

[20] Kaveh A., Merat E., Saman S., Danandeh S., Soltan Nezhad S. 2017. Infectious causes of bovine abortion in Qazvin Province, Iran. *Archive of Razi Institute* 72: 225-230. doi:10.22092/ARI.2017.113299

[21] Razmi G.R., Zarae H., Nourbakhash M.F., Naseri Z. 2013. Estimating the rate of transplacental transmission of *Neospora caninum* to aborted fetuses in seropositive dams in Mashhad area, Iran. *Iranian Journal of Veterinary Medicine* 7: 253-256. doi:10.22059/IJVM.2013.36284

[22] Ansari-Lari M. 2020. Bovine neosporosis in Iran: A systematic review and meta-analysis. *Preventive Veterinary Medicine* 176: 104913. doi:10.1016/j.prevetmed. 2020.104913

[23] Wilson D.J., Orsel K., Waddington J., Rajeev M., Sweeny A.R., Joseph T., Grigg M.E., Raverty S.A. 2016. *Neospora caninum* is the leading cause of bovine fetal loss in British Columbia, Canada. *Veterinary Parasitology* 218: 46-51. doi:10.1016/j.vetpar.2016.01.006

[24] Corbellini L.G., Pescador C.A., Frantz F., Wunder E., Steffen D., Smith D.R., Driemeier D. 2006. Diagnostic survey of bovine abortion with special reference to *Neospora caninum* infection: importance, repeated abortion and concurrent infection in aborted fetuses in Southern Brazil. *Veterinary Journal* 172: 114-120. doi:10.1016/j.tvjl.2005.03.006

[25] Haddad J.P., Dohoo I.R., VanLeewen J.A. 2005. A review of *Neospora caninum* in dairy and beef cattle – a Canadian perspective. *Canadian Veterinary Journal* 46: 230-243.

[26] Wouda W., Moen A.R., Visser I.J., van Knapen F. 1997. Bovine fetal neosporosis: a comparison of epizootic and sporadic abortion cases and different age classes with regard to lesion severity and immunohistochemical identification of organisms in brain, heart, and liver. *Journal of Veterinary Diagnostic Investigation* 9: 180-185. doi:10.1177/104063879700900212

[27] Anderson M.L., Palmer C.W., Thurmond M.C., Picanso J.P., Blanchard P.C., Breitmeyer R.E., Layton A.W., McAllister M., Daft B., Kinde H. et al. 1995. Evaluation of abortions in cattle attributable to neosporosis in selected dairy herds in California. *Journal of American Veterinary Medicine Association* 207: 1206-1210.

[28] Baszler T.V., Gay L.J., Long M.T., Mathison B.A. 1999. Detection by PCR of *Neospora caninum* in fetal tissues from spontaneous bovine abortions. *Journal of Clinical Microbiology* 37: 4059-4064. doi:10.1128/JCM.37.12.4059-4064.1999

[29] Ortega-Mora L.M., Fernández-García A., Gómez-Bautista M. 2006. Diagnosis of bovine neosporosis: Recent advances and perspectives. *Acta Parasitologica* 51: 1-14. doi:10.2478/s11686-006-0001-0

[30] Jenkins M., Baszler T., Björkman C., Schares G., Williams D. 2002. Diagnosis and seroepidemiology of *Neospora caninum*-associated bovine abortion. *International Journal of Parasitology* 32: 631-636. doi:10.1016/s0020-7519(01)00363-0