Serological status, epidemiology and prevalence of bovine *Neospora caninum* infection in Turkey: a review of published studies

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**Introduction**

*Neospora caninum*, a protozoan parasite of animals firstly recognized in 1984 in dogs in Norway [17], has an important role causing abortions and neonatal mortality in cattle [41, 42, 44-46]. It is deemed a major pathogen for dog and cattle [43].

Bovine neosporosis has been reviewed in many studies throughout the world [6, 20, 33, 40-47, 53, 66, 67]. However up to date, only limited aetiological and epidemiological information was available regarding the status of cattle neosporosis in Turkey, and most of the studies were independently reported with different methodologies in different provinces. Consequently, the real prevalence of the disease remains unclear in Turkey. The aim of this review was to update and summarize current knowledge of *N. caninum* in dairy and beef cattle in Turkey. After presenting the neosporosis repartition in worldwide, it was focused on epidemiology and *N. caninum* seroprevalence in Turkish dairy and beef cattle. Between 2003 and 2007, the neosporosis serological status of Turkish cattle mainly investigated with ELISA tests varied from 2% to 13.96%, and would be affected by climatic conditions, breeding types, animal susceptibility and dosage performance.

**Keywords**: Cattle, Neosporosis, Seroprevalence, Turkey.

**Serological prevalence of *N. caninum* in cattle in worldwide**

Seroprevalences of *N. caninum* in dairy and beef cattle have been investigated worldwide throughout in many countries [45, 46]. Although the obtained results are not comparable because of the different serological methods used and the different threshold values chosen, they prove the presence of the parasite and suggest that many species of mammals have been probably exposed to it. In a well designed research study, seroprevalences were compared, randomly in dairy and beef cattle from Germany, The Netherlands, Spain, and Sweden via enzyme-linked immunosorbent assays (ELISA) [15]. The seroprevalence of neosporosis among cattle of Sweden was much lower than the other neighbouring countries and among beef and dairy cattle prevalences in beef cattle were lower [15].

*Neospora caninum* infections have been reported from several different parts of the world comprising Europe, Asia, Australia, New Zealand, Thailand, and the Americas [45, 46]. The seroprevalence rates observed on European dairy cattle ranged from 1.3% to 65%: the less infected countries were the Czech Republic [139], Hungary [62, 63], and the Ireland [87], the most infected were the Sweden [15, 19, 52, 130], the United Kingdom [31, 32], the Portugal [23, 134], the Netherlands [15, 38], the Spain [15, 22, 80-83, 85, 111] and the Italy [50, 84, 99, 116] whereas intermediate rates were observed in Belgium [34], in Poland [21, 153], in Denmark...
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However, within the same country the neosporosis rates greatly varied according to investigated regions. For example, in Sweden the seroprevalence fluctuated from 1.3% to 65% [15, 19, 52, 130]. On the American continent, the disease prevalence varied from 11.2% to 64.5% in South America (Argentina, Brazil, Chile, Paraguay and Uruguay) [46], from 16% to 59% in Mexico and in Costa Rica [46] and from 5.6% to 25.5% in Canada and in USA according to explored regions [46]. In the same way, neosporosis was also spread with some heterogeneity in Asian (Vietnam, Japan and Korea) (prevalences were comprised between 5.5% to 48.7%), in Iran (from 15.1% to 46%), in New Zealand (from 10.9% to 53%) and in Australia (from 10.2% to 24%) [46]. In contrast, rather few studies regarding serological prevalence of *N. caninum* in beef cattle in worldwide have been reported: in Europe (Andorra, Hungary, Germany, France, Italy, the Netherlands, Belgium and Spain) [46], the infection rates were comprised between 7.4% in Andorra [8] to 17.9% in Spain [110]. Like in this continent, neosporosis seemed to infect beef cattle in a lesser extend than dairy cows in Americas (prevalences were ranged from 4.7% in Argentina to 29.9% in Brazil), in Asia (1.5% in Japan and 4.1% in Korea) and in Oceania (2.8% in New Zealand and 14.9% in Australia) [46]. However, some herds remained highly infected in USA (79% in Nebraska [86]).

The serological prevalences of *N. caninum* summarized previously [46] evidence marked differences among countries, regions, and beef and dairy cattle [15, 39, 74, 76, 96]. However, comparisons between the different studies would be cautions because variability in the serological methods used, in the study design and in the sample size would interfere with results. The serological prevalences of *N. caninum* as

<table>
<thead>
<tr>
<th>Country</th>
<th>Dairy cattle</th>
<th><em>N. caninum</em> seroprevalences (%)</th>
<th>Beef cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Andorra</td>
<td>-</td>
<td>0.7-9.2 [8]</td>
<td></td>
</tr>
<tr>
<td>Belgium</td>
<td>12.2 [34]</td>
<td>14 [35]</td>
<td></td>
</tr>
<tr>
<td>Czech Republic</td>
<td>3.1 - 3.9 [139]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Denmark</td>
<td>22 [68]</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>5.6 - 26.0 [72, 73, 100, 108, 109]</td>
<td>4.1 [72]</td>
<td></td>
</tr>
<tr>
<td>Hungary</td>
<td>3.3 - 10.0 [62, 63]</td>
<td>1.8 [63]</td>
<td></td>
</tr>
<tr>
<td>Ireland</td>
<td>3.0 - 12.6 [87]</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>11.4 - 30.8 [50, 84, 99, 116]</td>
<td>6 [99]</td>
<td></td>
</tr>
<tr>
<td>Netherlands</td>
<td>1.8 - 10.8 [15, 38]</td>
<td>13.3 [15]</td>
<td></td>
</tr>
<tr>
<td>Poland</td>
<td>9.3 - 15.6 [21, 153]</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Portugal</td>
<td>28 - 49 [23, 134]</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Spain</td>
<td>11.2 - 36.8 [15, 22, 80-83, 85, 111]</td>
<td>15.8 - 17.9 [15, 110]</td>
<td></td>
</tr>
<tr>
<td>Sweden</td>
<td>1.3 - 63 [15, 19, 52, 130]</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>United Kingdom</td>
<td>17.1 - 60 [31, 32]</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Americas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Argentina</td>
<td>16.0 - 64.5 [92, 93, 142, 143]</td>
<td>4.7 - 20.3 [92, 93, 94]</td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>11.2 - 46.0 [26, 27, 36, 37, 56, 57, 78, 79, 89, 91, 95, 112, 144]</td>
<td>6.7 - 29.9 [1, 7, 28, 60, 89, 112, 122]</td>
<td></td>
</tr>
<tr>
<td>Canada</td>
<td>5.6 - 25.5 [30, 48, 58, 61, 70, 101, 127, 136, 140, 141]</td>
<td>5.2 - 9.1 [140, 141, 148-150]</td>
<td></td>
</tr>
<tr>
<td>Chile</td>
<td>15.7 - 30.2 [106]</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Costa Rica</td>
<td>39.7 - 43.3 [118, 119]</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Mexico</td>
<td>16 - 59 [ 54, 88, 90]</td>
<td>10 [88]</td>
<td></td>
</tr>
<tr>
<td>Paraguay</td>
<td>35.7 [98]</td>
<td>26.6 [98]</td>
<td></td>
</tr>
<tr>
<td>USA</td>
<td>10.3 - 60.6 [49, 77, 97, 102-104, 117]</td>
<td>5.2 - 79 [12, 70, 86, 121]</td>
<td></td>
</tr>
<tr>
<td>Iran</td>
<td>15.1 - 46 [113, 120]</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Asia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Japan</td>
<td>5.7 - 20 [74, 75]</td>
<td>1.5 [74]</td>
<td></td>
</tr>
<tr>
<td>Korea</td>
<td>12.1 - 48.7 [2, 10, 65]</td>
<td>4.1 [71]</td>
<td></td>
</tr>
<tr>
<td>Vietnam</td>
<td>5.5 [64]</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Oceania</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Australia</td>
<td>10.2 - 24.0 [9, 59]</td>
<td>14.9 [132]</td>
<td></td>
</tr>
<tr>
<td>New Zealand</td>
<td>10.9 - 53.0 [29, 107, 114, 115, 123, 135, 152]</td>
<td>2.8 [133]</td>
<td></td>
</tr>
</tbody>
</table>

TABLE I: Compared seroprevalences of *N. caninum* in dairy cattle and in beef cattle in the world.
referred to locations within a country and between beef and dairy cattle [46].

Some epidemiological criteria (i.e. age, sex, breed, breeding conditions, herd size) would affect neosporosis seroprevalence. Some of them may constitute risks for direct contamination of cattle such as the presence of definitive hosts (dogs and coyotes) [27, 85, 105, 126, 146], other carnivores [100] and intermediate hosts [14, 99, 100], human population density [125], the consumption of potential contaminated food through grazing, fodder and water [13, 99, 121] or colostrum / milk ingestion [138] and the environmental conditions favourable to Neospora survival (climate [116, 126] and vegetation index [99, 116]). Interestingly in Germany, human population density has been reported as a risk factor correlating positively with dog density and therefore similarly to dog density, which could be used to evaluate the prevalence of bulk milk- positive herds in districts and cities [125]. It has been speculated that as the dog density has been identified as a putative risk factor for neosporosis, it may be suggested that human population density could have the same effect [125]. Other risk factors (like cattle stocking density and size of farmland [12, 13, 121] or herd size [99, 126], source of replacement heifers [51, 131], calving management [13] and housing type [100]) are more related to the increased transmission of these pathogens. Finally, other epidemiological criteria (age [49, 68, 116, 121], breed [15] and occurrence of antibodies against various infectious agents [19]) are linked to an increased sensitivity of cattle. Therefore, these mentioned criterias/risk factors should also be encountered when evaluating seroprevalence rates.

As aforementioned above, a detailed review on the epidemiology and prevalence of neosporosis in Turkish dairy and beef cattle is lacking. However, the objective of the present article is to synthesize the neosporosis published prevalences observed among different regions and provinces in Turkey. Most importantly for Turkish bovine practitioners, the distribution of the infection was also discussed. In the most of these studies, an available competitive inhibition ELISA (c-ELISA) was used for diagnosis [3, 66, 96, 128] whereas an immunocomb diagnostic test kit was assessed in only one previous study [76].

Based on the literature review (Table II) (except of preliminary results of national congress proceedings), a seroprevalence rate of 10.87% (10 out of 92 cow sera) determined with a c-ELISA test was found in Sakarya in 2003 [96], then using similar kits, serum anti- N. caninum antibodies were found in 7.5% (23/305) cows from Sanliurfa city [128] and in 7.0 % (36/513) cows from the Eastern Anatolia [4] in 2005. Moreover, in the latter study, the neosporosis seroprevalence rates were heterogeneous according to the considered cities: 15.00%, 4.86%, 4.69% and 4.00% in Elazığ, Muş, Bingöl and Malatya respectively [4]. By contrast, the overall Neospora caninum seroprevalence determined in Central Anatolia from 3287 sera of cows belonging to 32 herds in 8

<table>
<thead>
<tr>
<th>Region / references</th>
<th>Number of cattle</th>
<th>Seroprevalence (%)</th>
<th>Test type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ankara [76]</td>
<td>60</td>
<td>10</td>
<td>ImmunoComb</td>
</tr>
<tr>
<td>Kayseri [66]</td>
<td>186</td>
<td>7</td>
<td>c-ELISA</td>
</tr>
<tr>
<td>Sakarya [96]</td>
<td>92</td>
<td>10.87</td>
<td>c-ELISA</td>
</tr>
<tr>
<td>Sanliurfa [128]</td>
<td>305</td>
<td>7.5</td>
<td>c-ELISA</td>
</tr>
<tr>
<td>Kars [3]</td>
<td>301</td>
<td>2</td>
<td>c-ELISA</td>
</tr>
</tbody>
</table>

Eastern Anatolia [4] 513 7.0 c-ELISA

Central Anatolia [147] 3287 13.96 ELISA-IDEXX

Ankara 453 10.15
Cankiri 418 6.93
Neveshir 392 5.10
Kirsehir 409 19.55
Kayseri 425 10.82
Kirikkale 434 32.72
Eskisehir 387 5.43
Yosgat 369 20.32

Tekirdag and Kirklareli [16] 124 5.6 ELISA
Gebze [5] 97 5.1 ELISA
Elazig [129] 183 8.19 c-ELISA

**Table II: Seroprevalence of N. caninum in dairy cattle in Turkey.**
different provinces was higher: 13.96% [147]. The results in Central Anatolia were also heterogeneous according to the different cities and herds. In the Kars region, 6 out of 301 (2%) cows were positive for antibodies to *N. caninum* [3]. On 2005 a prevalence rate of 5.6%, among 214 cattle tested, was reported in Tekirdag and Kirkareli [16]. A seroprevalence rate of 7% (13/186) was found in Kayseri in 2006 [66] and KURTDEDE et al. [76] reported a prevalence rate of 10% (6 out of 60 cattle) using immuno-chromatography with immunochromatographic test kits, in Ankara. In the Gebze region, *N. caninum* antibodies were detected using ELISA with low overall neosporosis prevalence (5.15%) [5], despite the fact that the 97 dairy cows investigated in this study were stemming from 3 dairy herds with signs of abortion. Finally, in Elazig province of the Eastern Turkey, 15 out of 183 cows tested (8.19%) were found seropositive using a competitive enzyme linked immunosorbent assay (cELISA) [129] (Table II).

These results indicate that a substantial proportion of cattle in different parts of Turkey were infected with *N. caninum* and that infection may be associated with pregnancy. It should be mentioned that there was higher neosporosis seroprevalence in aborting cows. Indeed, a statistically significant (p<0.05) difference was found among the seropositivity rates of aborting and non-aborting cows, specific antibodies being more often detected in aborting cows [19]. The apparent prevalence of *N. caninum* infection in cattle in Turkey ranged from 2% to 13.96% probably because of great variations in the study population, sampling methods, and diagnostic procedures. As the modified agglutination tests and ELISA [124] only detect IgG antibodies [18], and as *N. caninum* antibodies in bovine serum samples [24] can also belong to the IgM [137], the specificity of the serological test towards the different Ig classes and the evaluation of the Ig type (Ig G, Ig M or others) concentrations should also be taken into consideration for analysis of the neosporosis seroprevalence. The positive findings of serological tests may only indicate that the cow has been infected with *N. caninum*, but they do not completely provide a definitive diagnosis in abortions [55]. Furthermore, the resistance of the parasite in the environment according to the variable climatic conditions and the role of other species (reservoir) as contamination sources should also be encountered even in serological evaluations. However, those mentioned criteria influencing seropositivity rates were generally lacking in most of the studies performed in Turkey.

When analysing neosporosis prevalence it should also be suitable to discuss about the biological interest of determining neosporosis seroprevalence instead of direct parasite detection for evaluating infection rates in cattle. Serum analysis from an aborting cow is solely indicative of exposure to *N. caninum*, and for a definitive diagnosis histological examination of the foetus may be required [45, 46]. It is suggested that antibody detection in serum and in individual or bulk milk samples by the indirect fluorescent antibody test (IFAT) and various ELISAs [124] are optimal for the identification and recognition of infected herds [46, 145]. Several serological tests such as various ELISAs, indirect fluorescent antibody test (IFAT) [24], and Neospora agglutination test (NAT) and immunoblots may be capable of detecting *N. caninum* antibodies [18]. These tests may be useful for the control of neosporosis in the international animal trade [46, 92], because infected cattle may introduce and transmit parasite to naïve herds or in locations where the disease does not previously exist or in where prevalence is very low [46]. In this way, the *N. caninum* antibodies were not detected in local cattle breeds [3] from the Kars region, whereas imported cattle were found to be seropositive.

Based on the information showed here it should be suggested at least that the disease is prevalent in many provinces in Turkey. However risk factors and associated statistical analyses regarding neosporosis are lacking in most of the studies performed in Turkey, thus suggesting the necessity for more detailed surveys.

### References


