

## Original papers

# The enzymes and electrolytes profiles in sera of Iranian stray dogs naturally infected with *Neospora caninum*

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**ABSTRACT.** The protozoan *Neospora caninum* (Apicomplexa: Sarcocystidae), respectively infects canids and ruminants as the most definitive and intermediate hosts. A diagnosis of neosporosis is generally made on the basis of clinical signs together with the detection of high levels of antibodies in serum. The present study compares the serum electrolyte profile (Ca, K, Mg, Na, P) and serum enzyme level (ALP, AST, CPK) of non-infected dogs with those of stray dogs naturally infected with *N. caninum*. The indirect fluorescent antibody test (IFAT) revealed that 17 of the 137 analysed serum samples (12.41%) of the stray dogs were seropositive (Sp) to *N. caninum*. Serum levels of the electrolytes and the enzymes were evaluated in the 17 Sp and 28 seronegative (Sn, 20.44%) dogs using common enzyme kits, spectrophotometry and flame photometry techniques. The average serum level of ALP, AST, and CPK were found to be significantly higher in the Sp stray dogs. Measurements of the average serum levels of Ca, K, Mg, and P were higher in Sp than Sn stray dogs, with the average K level being significantly higher in seropositive stray dogs. It was concluded that evaluation of serum enzyme and electrolyte levels may be used to screen *N. caninum* infection in stray dogs.

**Key words:** electrolytes, enzyme, stray dog, *Neospora caninum*

## Introduction

Neosporosis is a multiple-feature disease, manifesting as a progressive paralysis in young dogs and various clinical signs such as stiffness of the pelvic limbs, paralysis distinguished by gradual muscle atrophy and the progression to rigid contraction of the limbs at different ages. *Neospora caninum* was identified as major cause of abortion in cattle by Dubey [1]. *Neospora caninum* is a cyst-producing coccidian which was isolated for the first time from paralyzed dogs [2]. A range of animals are reported as intermediate hosts, as it causes abortion and economic loss in horse, cattle, sheep, and goats [3,4].

Neosporosis occurs throughout the world, with *N. caninum* infection being reported from Europe (0.5–17%) [1], America (2%) [5], Mexico (37.8%)

[6], New Zealand (22%) [1], Italy (6.4%) [7], Germany (4%) [8], Brazil (10–25%) [9] and Japan (7–31%) [10]. In Iran, the first *N. caninum* infection was recorded in cattle herds of eastern part of the country [11]. Many researchers have also reported the occurrence of *Neospora* infection in domestic ruminants in Iran [12–15]. The prevalence of infection was found to be 11.3% in Iranian urban and rural dogs [15], while in the central part of the country, its prevalence was found to be 20% in household dogs and 46% in farm dogs [16]. The prevalence of infection in stray dogs in north-western Iran was found to be 27% [17]. From neighbouring countries like Turkey and Iraq, the reported prevalence of *N. caninum* infection in domestic dogs was found to be 10% and 1.6%, respectively [18,19].

Most dogs infected with *N. caninum* show

muscular, coetaneous and neurologic signs. Myelography like biochemical parameters and haematology are useful for differential diagnosis. The post-mortem diagnosis of *N. caninum* infection is based on histological, histopathological, and immunohistochemical analyses. Foetal serology is also possible if antibodies against the parasite are present in the foetal fluids [20]. As direct diagnosis of *Neospora* infection is difficult in host tissues such as muscles when low quantities of parasites are present [21], serological methods are useful diagnostic tools in epidemiological studies and experimental infections. Several serological techniques, i.e., enzyme linked immune-sorbent assay (ELISA), western blot (WB), immunofluorescent antibody test (IFAT), and direct agglutination test (DAT) can be used to detect *N. caninum* infection [22,23]. Of those, IFAT is widely used to identify canine neosporosis, and confirmation of infection is provided using immunohistochemistry and polymerase chain reaction (PCR) methods [24]. However, as all these diagnostic tools, as well as their clinical and field applications, are time consuming and expensive, the present study evaluates serum electrolyte profile and serum enzyme level as possible indicators of canine neosporosis.

## Materials and Methods

**Dogs sampling.** A total of 137 stray dogs were randomly selected and 5 ml of blood was taken from the saphenous vein of each dog using venepuncture. The sera were removed after centrifugation at 1000×g for 10 minutes and stored at -20°C until laboratory evaluation (Table 1). All stray dogs were recorded and subjected for a clinical examination including general body condition, clinical and

neurological signs, sex and age.

**Serological examination.** The sera were examined to detect anti-IgG to *N. caninum* using the indirect fluorescent antibody test (IFAT; Mega Screen Fluoneospora, Horbranz, Austria) according to Yakhchali et al. [18]. Briefly, the sera were screened at dilutions of 1:50 in phosphate-buffered saline (pH 7.2). Serum samples showing fluorescence at a dilution of 1:50 (cut-off value) were further subjected to twofold serial dilutions [25,26].

**Haematological test.** Complete cell blood counts, PCV values, and haemoglobin concentration were measured using an automated haematology analyser (NIHON KOHDEN, celltac αMEK-6400 series, JALPan) [27].

**Measurement of serum level of enzymes.** The activity of creatine phosphokinase (CPK) (Roche Diagnostics, Mannheim, Germany), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) were measured (Enzymatic Assay XpressBio, USA).

**Measurement of serum level of electrolytes.** Analyses of calcium and magnesium serum levels were performed using colorimetric methods (Randox, Antrim, UK; Stanbio, Boerne, USA, respectively). Serum levels of Na and K were evaluated using a Flame photometer and Na-K standards (Zist Chimi, Iran). The reference ranges (RR) were as those described by Schalm et al. [28].

**Statistical analysis.** The non-parametric Chi-square test ( $\chi^2$ ) was used to evaluate the relationship between seroprevalence and sex or age. The serum levels of enzymes and electrolytes were also assessed using the non-parametric *t*-test with a confidence interval of 95% (SPSS 11.5). A probability score of  $p \leq 0.05$  was regarded as significant.

Table 1. The prevalence of antibodies in different sex and age groups of examined stray dogs by IFAT (N=137)

No. of examined animals	IFAT	Sex	Age (year) (n/N, %)			
			<1	1-2	3-4	>4
137	Sp n=17	M	3(17.65)	6 (35.29)	4 (23.53)	2 (11.76)
		F	0	0	1 (5.88)	1 (5.88)
	Sn n=120	M	30 (25)	33 (27.5)	6 (5)	6 (5)
		F	0	24 (20)	21 (17.5)	0

F: female; M: male; n: number of infected animals; N: number of examined animals

Table 2. Mean numbers of blood cells from infected (Sp=17) and non-infected (Sn=28) dogs with *N. caninum* (N=137)

Blood parameters	WBC (ml)		Bas (%)		Eo (%)		Mo (%)		Lym (%)		Ne (%)		Hb (mg/dl)		PCV (%)	
	Sn	Sp	Sn	Sp	Sn	Sp	Sn	Sp	Sn	Sp	Sn	Sp	Sn	Sp	Sn	Sp
Mean	7334	11635	3	2	5	2	10	7	14	24	31	62	13	14	41	42
Min-Max	1400-34000	8000-18000	1-34	1-12	0-34	0-11	0-34	2-18	8-45	11-42	36-90	42-78	29-48	33-50	29-48	33-50

Bas: basophils; Eo: eosinophils; Hb: hemoglobin; Lym: lymphocytes; Mo: monocytes; Ne: neutrophils; PCV: packed cell volume; WBC: white blood cells

## Results

The IFAT examination indicated that 12.41% (17 out of 137) of the stray dogs were seropositive (Sp) to *N. caninum* (Table 1). The stray dogs were clinically healthy and no neurologic, coetaneous, respiratory, and cardiac signs were recorded.

The average serum levels of enzymes and electrolytes in different age groups of Sp and seronegative dogs (Sn) are tabulated in Tables 3–4. A total of 28 healthy, Sn dogs (20.44% of total) in good body condition were selected as a control group against the seropositive stray dogs to *N. caninum*. The average serum levels of ALP (25.75 U/L), AST (74.24 U/L), and CPK (84.21 U/L) were significantly higher in Sp stray dogs than in Sn stray dogs ( $P=0.0001$ ) (Table 4).

The average serum levels of the examined electrolytes, i.e. K, Ca, Mg, and P were higher in the Sp stray dogs than the Sn stray dogs. However, significant differences were only observed for K ( $P=0.0001$ ), the other values being insignificant ( $P>0.05$ ).

The haematological findings indicated no

significant differences in PCV and haemoglobin levels between the Sp and Sn stray dogs ( $P>0.05$ ) (Table 2). In addition, no differences were found with regard to the average lymphocyte or WBC numbers ( $P>0.05$ ). The mean number of neutrophils was higher in the Sp stray dogs than the Sn stray dogs; however, in contrast, mean eosinophils numbers were higher in the Sp stray dogs than the Sn stray dogs ( $P>0.05$ ) (Table 2).

## Discussion

A diagnosis of neosporosis is generally made on the basis of clinical signs together with the detection of high levels of antibodies, such as immunoglobulin G (IgG), in serum by IFAT, Western blot, ELISA or DAT [22–24]. *Neospora caninum* causes meningoencephalitis, myositis, and polyradiculoneuritis in dogs throughout the world. Adult infected dogs may display various neurologic signs depending on the site of infection in the neural system [17]. A diagnosis of neosporosis in a living animal is difficult [29], and clinical signs are restricted to the muscles and nerve roots of the

Table 3. The average serum level of aspartate aminotransferase (AST), creatine-phosphokinase (CPK) and alkaline phosphatase (AP), and mineral profiles (Ca, K, Mg, Na, P) in non-infected stray dogs to *N. caninum* (N=28)

Materials Parameters	AST (U/L)	AP (U/L)	CPK (U/L)	Ca (mg/dl)	K (mEq/l)	Na (mEq/l)	P (mg/dl)	Mg (mg/dl)
RR	28-40	80-84	40-255	11.7	141.1-152.3	141.1-152.3	9.9-11.1	2.1
Mean	59.07	65.4	242.21	7.69	344	136	5.92	1.82
Min-Max	35-96	109-157	215-294	7.21-8.57	384-311	131-152 <sup>NSig</sup>	4.09-8.92	1.70-2.24
SD	1.865	1.307	18.118	0.415	0.448	6.935	1.438	0.170

RR: reference range; SD: standard deviation; NSig: no significant association ( $P>0.05$ )

Table 4. Mean serum level of creatine-phosphokinase (CPK), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and electrolytes profiles (Na, K, Ca, Mg, P) in stray dogs infected with *N. caninum* (N=17).

Mineral	CPK (U/L)	AST (U/L)	AP (U/L)	Na (mEq/l)	K (mEq/l)	Ca (mg/dl)	P (mg/dl)	Mg (mg/dl)
RR	80-84	28-40	40-255	11.7	141.1-152.3	141.1-152.3	9.9-11.1	1.8-2.5
Mean	25.75 <sup>Sig</sup>	74.24 <sup>Sig</sup>	84.21 <sup>Sig</sup>	7.91	351 <sup>Sig</sup>	149	6.08	2.18
Min-Max	121-165	59-98	515-894	7.41-8.57	301-367	139-142	4.07-8.22	1.56-2.58
SD	1.746	2.098	118.12	0.326	0.448	6.936	1.314	0.230

RR: reference range; SD: standard deviation; Sig: significant association (P=0.0001)

posterior limbs in young dogs. Neurological and clinical findings may suggest the presence *N. caninum* infection in a puppy; however, more diagnostic tools like IFAT are needed to confirm this [15,17].

The seroprevalence of 12.41% identified in the present study was less than the proportions reported by Malmasi et al., Haddadzadeh et al., and Yakhchali et al. from different parts of Iran [15–17]. Higher seroprevalence has been also reported in earlier studies on dog neosporosis throughout the world [18,30–32].

In the present study, the average serum levels of CPK (reference range: 40 to 255 U/L [33]), ALP (reference range, 12 to 106 U/L), and AST (reference range, 28–40 U/L) were significantly higher in Sp stray dogs. These findings were in accordance with an infected case by Clooten et al. [33]. According to Aktas et al. [34], CPK was mostly present in the skeletal muscles, myocardium, brain and intestine, and sex has no influence on plasma CPK activity, which was higher in young dogs than in adults. Muscle infection was the main source of plasma CPK elevations. Plasma CPK was also found to be elevated in cases of experimental myocardial infarction. In addition, a relationship was proposed between increases in serum enzyme levels (CPK and AST) observed in canine myositis and *N. caninum* infection [35]. CPK serum levels in infected dogs were found to be 500 U/l by Lorenzo et al. and 130 U/L by Clooten et al. [24,33]. Furthermore, the serum CPK level was found to be much higher in infected stray dogs, with Dubey et al reporting a level of 1370 U/l [29]. Dubey et al. [29] also reported increased serum ALP levels of 140 U/L in infected dogs with *N. caninum*.

No significant differences were observed between the two groups of stray dogs with regard to

their mean Na, Ca, P or Mg levels. This finding contradicts those of Crookshanks et al. and Boyd et al., who noted that serum levels of P in Sp dogs were 2.37 mmol/L (reference range, 0.82 to 1.87 mmol/L) and 2.14 mmol/L (reference range: 1–2.52 mmol/L), respectively [36,37], and that average serum level of Na (142 mmol/L, normal range: 145–155 mmol/L) and Ca (2.30 mmol/L, normal range: 2.2–2.85 mmol/L) also differed between the two samples [36,37].

In the current study, the average serum level of K was significantly higher in both examined groups of stray dogs. Boyd et al. [36] reported an average serum level of K of 4.7 mEq/l (normal ranges: 4.5–5 mEq/l) in Sp dogs. While serum level of K was higher in the Sp dogs. In addition, as a major intracellular ions like P and K play significant role in the maintenance and stability of cell membranes, and the normal activities of tissues were the main reasons of importance this finding which should be investigated in further experimental researches.

No significant differences regarding PCV or haemoglobin were found between the Sp and Sn groups of stray dogs. In addition, no differences were found between the mean number of lymphocytes and WBC count. The average number of neutrophils was higher in Sp stray dogs than Sn stray dogs. In contrast, Sp stray dogs demonstrated higher mean numbers of eosinophils than Sn stray dogs. Lorenzo et al. report that WBC count ( $2.02 \times 10^4/\mu\text{l}$ ; reference range 6 to  $17 \times 10^9/\mu\text{l}$ ) decreased, and neutrophil (87%, reference range 60–80%), lymphocytes (8042 cells/ $\mu\text{l}$ ), and eosinophil (1431 cells/ $\mu\text{l}$ ) number increased in dog neosporosis [24,33]. Clooten et al. [33] report moderate regenerative anaemia (haematocrit: 0.23 L/L, reference range: 0.37–0.55 L/L; reticulocytes 9.4%) in dogs with *N. caninum* myositis.

Our present findings indicate that further experimental studies are recommended to confirm whether or not these findings from stray dogs infected with *N. caninum* can be used as a screening method for dog neosporosis in the case of no serological possibilities.

### Acknowledgements

The authors wish to thank the technical members of the Parasitology Division and Central Lab, especially, A. Badali and F. Farhang-Pajuh at the Urmia University.

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Received 27 June 2016

Accepted 16 February 2017