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Seroepidemiology and biochemical alterations associated with *Neospora caninum* infection in dairy cows from the northeastern region of Algeria

Seroepidemiología y alteraciones bioquímicas asociadas con la infección por *Neospora caninum* en vacas lecheras de la región noreste de Argelia

Aya BENSALEM*, **Sana HIRECHE**, **Amina AMRAOUI**, **Samia AMEZIANE**, **Amir AGABOU**

University of Constantine 1-Frères Mentouri, Institute of Veterinary Sciences El-Khroub, PADESCA Research Laboratory. Constantine, Algeria.
**Corresponding author:* drayabensalem@gmail.com

ABSTRACT

Bovine neosporosis is a major cause of abortion and stillbirth in cattle, causing significant economic losses worldwide. This study aimed to determine *Neospora caninum* seroprevalence, identify its potential risk factors and investigate alterations in selected biochemical traits in cattle from five Northeastern Algerian provinces. From March 2022 to April 2025, 368 cows belonging to 36 herds were randomly sampled and analyzed using an indirect enzyme-linked immunosorbent assay kit. Risk factors were determined using multivariable logistic regression model. For biochemical disturbances, sera from 30 seropositive and 30 seronegative cows were tested for glucose, total protein, albumin, calcium, phosphorus, urea, creatinine, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, lactate dehydrogenase and β- hydroxybutyrate, using standard laboratory methods. Biochemical values were compared statistically and the effects of N. caninum infection were evaluated using generalized linear models adjusting for confounding factors. According to results, herd and individual animal prevalence were 63.88 % (23/36; 95 % CI: 46.20 – 79.20) and 16.30 % (60/368; 95 % CI: 12.70 – 20.50) respectively. At individual level, the risk of being seropositive increased with age, history of reproductive problems, mastitis and types of vaccines. The use of artificial insemination, vaccination and birth of healthy calves were protective factors. At herd-level, quarantine and vaccination were protective factors. Infected cows showed significantly elevated serum levels of alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, urea, total protein and globulin along with a significant decrease in calcium and albumin/globulin ratio. This survey highlights the widespread establishment of *N. caninum* within local cattle population and elucidates its associated risk factors as well as the metabolic and reproductive disorders it may generate.

Key words: Neosporosis; cattle; Algeria; seroprevalence; risk factors; biochemical changes; metabolic disorders.

RESUMEN

La neosporosis bovina es una de las principales causas de abortos y mortinatalidad en el ganado vacuno, lo que provoca importantes pérdidas económicas en todo el mundo. El objetivo de este estudio fue determinar la seroprevalencia de *Neospora caninum*, identificar sus posibles factores de riesgo e investigar las alteraciones en determinados parámetros bioquímicos en el ganado vacuno de cinco provincias del noreste de Argelia. Entre marzo de 2022 y abril de 2025, se tomaron muestras aleatorias de 368 vacas pertenecientes a 36 rebaños y se analizaron utilizando un kit ensayo por inmunoabsorción ligado a enzimas indirecto. Los factores de riesgo se determinaron mediante un modelo de regresión logística multivariable. En cuanto a las alteraciones bioquímicas, se analizaron las muestras de suero de 30 vacas seropositivas y 30 seronegativas para determinar los niveles de glucosa, proteína total, albúmina, calcio, fósforo, urea, creatinina, alanina aminotransferasa, aspartato aminotransferasa, gamma-glutamil transferasa, fosfatasa alcalina, lactato deshidrogenasa y β-hidroxibutirato, utilizando métodos de laboratorio estándar. Los valores bioquímicos se compararon estadísticamente y los efectos de la infección por N. caninum se evaluaron mediante modelos lineales generalizados ajustados por factores de confusión. Según los resultados, la prevalencia en el rebaño y en los animales individuales fue del 63,88 % (23/36; IC del 95 %: 46,20 – 79,20) y del 16,30 % (60/368; IC del 95 %: 12,70 – 20,50), respectivamente. A nivel individual, el riesgo de ser seropositivo aumentaba con la edad, los antecedentes de problemas reproductivos, la mastitis y los tipos de vacunas. El uso de la inseminación artificial, la vacunación y el nacimiento de terneros sanos fueron factores protectores. A nivel de rebaño, la cuarentena y la vacunación fueron factores protectores. Las vacas infectadas mostraron niveles séricos significativamente elevados de alanina aminotransferasa, aspartato aminotransferasa, gamma-glutamil transferasa, urea y proteína total y globulina, junto con una disminución significativa del calcio y de la relación albúmina/globulina. Este estudio pone de relieve la amplia diseminación de *N. caninum* en nuestra población bovina local y aclara los factores de riesgo asociados, así como los trastornos metabólicos y reproductivos que puede provocar.

Palabras clave: Neosporosis; ganado; Argelia; seroprevalencia; factores de riesgo; cambios bioquímicos; trastornos metabólicos.

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INTRODUCTION

Neospora caninum is a cyst-forming coccidian protozoan parasite of the Toxoplasmatinae subfamily, the Sarcocystidae family and the Apicomplexa phylum. It is worldwide distributed and infects a large variety of endothermic species, especially ruminants and canids [1].

The global economic impact of *N. caninum* infection is estimated at over 1.3 billion USD per year in the dairy and beef industries, resulting from direct losses (related to abortions, stillbirths, fetal deaths and milk production drops) and indirect ones (due to Veterinary, diagnosis and control measures costs) [2].

The parasite is characterized by a complex biological cycle that includes sexual and asexual stages. Domestic dogs (*Canis lupus familiaris*), coyotes (*Canis latrans*) and dingoes (*Canis lupus dingo*) serve as definitive hosts that excrete sporulated oocysts in their feces and contaminate the environment [3, 4]. Cattle (*Bos taurus*), sheep (*Ovis aries*), goats (*Capra hircus*), horses (*Equus caballus*), chickens (*Gallus gallus domesticus*), dogs, cats (*Felis catus*) and rabbits (*Oryctolagus cuniculus*) act as intermediate hosts that get infected through a horizontal route by ingesting oocysts-contaminated food or water [1, 5].

Vertical transmission can also occur in cattle, when pregnant infected cows transfer the parasite transplacentally to their offspring [6, 7]. *N. caninum's* life cycle includes three main infectious stages: sporozoites (contained within sporulated oocysts), bradyzoites (also termed cystozoites) and tachyzoites [8].

Neosporosis is characterized by the proliferation of tachyzoites in multiple cell types, inducing their lysis [9, 10]. Histopathological examination of *N. caninum* infected aborted calves reveals inflammatory lesions, such as non-suppurative hepatitis, myocarditis, pericarditis and encephalitis [11]. Polymerase chain reaction (PCR)-positive fetal tissues from aborted lambs show mononuclear cell infiltration, cerebral lesions, cardiac and hepatic necrosis (characterized by parenchymal destruction and hepatocytes atrophy) and fibrosis [12].

Reproductive disorders are the principal outcomes of bovine neosporosis and have been related with late first calving, reduced conception rates and prolonged calving intervals [13, 14]. The infection may manifest as sporadic, endemic or epidemic abortion, mostly at 5 - 6 months of gestation. Furthermore, the infection can lead to the birth of neurologically impaired calves [15, 16], or persistently-infected apparently-healthy calves [1,5, 17].

The occurrence of *N. caninum* in cattle has been recognized worldwide, showing regional fluctuations. Reported prevalence ranges from 4.1 to 43 % in Asia, 0.5 to 27.7 % in Europe, around 10.2 % in Oceania, 7.6 to 41 % in America and 10.7 to 19.6 % in Africa [18]. In Algeria, available studies indicate a prevalence of 12.4 to 36.2 % [19], with a very scarce data for the Northeastern region. Thus, we have undertaken the current study to define *N. caninum* seroprevalence and determine its potential risk factors.

Recently, the epidemiology and diagnostic strategies of N. caninum have been widely documented, however, studies focusing on metabolic and biochemical alterations in dairy cows affected by neosporosis are rare. In this context, the present study also aimed to examine the effect of *N. caninum* on selected serum biochemical parameters in naturally infected cattle.

MATERIALS AND METHODS

Study design, population and sampling

From march 2022 till april 2025, 368 dairy cows of more than two years old were randomly sampled from 36 herds located in Oum El Bouaghi (n = 33), Constantine (n = 78), Mila (n = 82), Guelma (n = 85) and Skikda (n = 90) provinces (Northeastern Algeria) (FIG.1) (TABLE I) [20, 21, 22].

For significance representativeness, a two-stages sampling was performed. First, the minimum required sample size (355 serum samples) was calculated using formulas for simple random sampling as recommended by Thrusfield [23], using a confidence level of 95 %, a required precision of 5 % and an estimated prevalence of 36.2 % [19]. Then, at herd level, simple random sampling was performed using the records of the ministry of Agriculture. Random number tables have been employed to take random samples of cows from each herd. The minimal number of cows to be selected, so as to detect the presence of the disease, was calculated using the following formula (Eq.1):

TABLE I
Characteristics of the five study provinces in northeastern Algeria

Characteristics	Constantine	Mila	Skikda	Guelma	Oum El Bouaghi
Latitude (N)	36°17’	36°44’	36°52’	36°27’	35°53’
Longitude (E)	6°37’	6°15’	6°54’	7°25’	7°07’
Area (Km²)	2 297	3 407	4 137.68	3 686.84	7 638
Mean altitude(m)	656	789	85	613	952
Mean annual precipitation(mm/year)	569.28	501.7	774.36	505.07	348.55
Climate	Csa[a]	Csa[a]	Csa[a]	Csa[a]	Csa[a]
Mean temperature (C°)	16.07	15.3	19	18.4	15.73
Cattle population (heads)	41 874	86 130	138 424	101 707	34 137

[a]Hot-summer mediterranean climate (Köppen-Geiger climate classification).

$$\text{Eq. 1:}\ n = \left[1-(1-P)^{\frac{1}{d}}\right] \times \left(N - \frac{d}{2}\right) + 1$$

Where n is the sample size, P is the probability of detection of at least one seropositive cow, N is the herd size, and d is the number of seropositive cows in the herd. The probability to detect at least one seropositive cow in a herd was determined at 95 % (P = 0.95), while the number of seropositive cows in each herd (d) was estimated assuming that within herd prevalence equals 10 %.

Blood samples (10 mL) were obtained from the jugular vein, using disposable needles and plain tubes. They were transported on ice to the laboratory and centrifuged at 0.805 G for 10min (Sigma, model 1–6P, maximum speed: 2.837 G, Germany). Serum was divided into two separated aliquots (for serological and biochemical analyses) that were kept at – 20 °C until testing (ENIEM, model CF 1301, 350 L, Algeria).

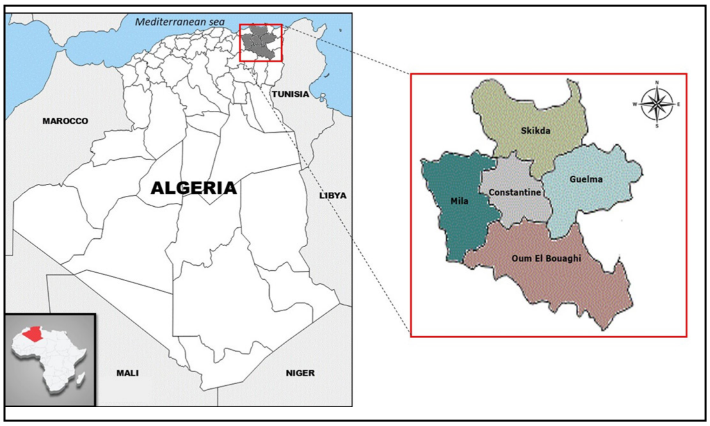

FIGURE 1. Geographic map of Algeria showing the locations of the sampled dairy herds for *Neospora caninum* infection

Epidemiological data collection

A pre-structured questionnaire based on previously reported associated factors to *N. caninum* infection was administered to farmers. Farm-level variables included farming system (extensive/intensive/semi-intensive), farm type (dairy/mixed), presence of other animal species (dogs, rodents, cats, sheep, poultry, and wild animals), forest access, water source, feeding practices, and disinfection methods. Individual animal data covered age, breed, vaccination and deworming status, lactation phase, parity, pregnancy status, and reproductive disorders (abortion, stillbirths, mastitis, and infertility) during the last 24 months.

Serological analysis

Serum antibodies (IgG) against *N. caninum* were detected by an indirect enzyme-linked immunosorbent assay (ELISA) kit (ID Screen®, Innovative Diagnostics, France) as recommended by the manufacturer. Both specificity and sensitivity of the test attain 100 % (95 % CI: 99.41 – 100 % and 95 % CI: 98.8 – 100 %, respectively). Optical density (OD) values were measured at 450 nm using an ELx800 microplate reader (BioTek Instruments Inc., USA). Results were calculated as sample to positive (S/P) ratios, with values ≥ 0.5 considered as positive.

Biochemical analyses

To investigate the relationship between certain biochemical parameters and *N. caninum* serostatus, sera from 30 seropositive and 30 seronegative animals were selected. In order to minimize confounding variables, each seropositive cow was matched with a seronegative one of similar age, breed, lactation phase and pregnancy status.

Serum biochemical parameters including calcium, phosphorus, glucose, total protein, albumin, creatinine, urea, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), were measured colorimetrically using biochemistry reagent kits (Biosystems S.A., Spain) on a BA-88A analyzer (Mindray, China).

Globulin concentrations were obtained by calculating the difference between serum total protein and albumin concentration. Albumin/globulin ratio (A/G) was also calculated. Serum β- hydroxybutyrate (BHB) was determined using a rapid test kit (On Call GK Dual, Acon Laboratories, USA).

Statistical analysis

Data from questionnaires, serological and biochemical analyses were analyzed using SPSS (Version 26, IBM Corp, USA). Chi-square tests were performed to identify factors associated with *N. caninum* seropositivity at individual and herd levels. Variables with P ≤ 0.25 were entered to multivariate logistic regression analysis.

After normality testing (Shapiro-Wilk test), biochemical parameters (mean ± SD) were compared between seronegative and seropositive cattle using Student's t test or Mann-Whitney U test. Generalized linear models (GLMs) evaluated the independent effect of *N. caninum* seropositivity on each biochemical parameter. *N. caninum* serostatus (seropositive/seronegative) was entered as the main predictor factor. The following variables were also included in each model as independent variables: age (< 3, [3 – 6], and > 6 years), breed (Holstein, Atlas Brown, Montbeliarde), lactation phase (early, mid, late, dry, gravid heifer) and parity (0, 1, 2, 3, 4, 5, and > 5). For all analysis, P < 0.05 indicated statistical significance.

RESULTS AND DISCUSSION

Seroprevalence

Out of the 368 dairy cattle tested for *N. caninum*, 60 samples (16.30 %) were found seropositive. The prevalence varied across provinces: 15.15 % (5/33) in Oum El Bouaghi, 15.29 % (13/85) in Guelma, 15.38 % (12/78) in Constantine, 17.07 % (14/82) in Mila and 17.78 % (16/90) in Skikda. The over-all herd-level prevalence was 63.88 % (23/36).

Published data on bovine *N. caninum* epidemiology in Algeria are limited, with only five studies documented between 2012 and 2025, yielding prevalence rates ranging from 12.22 to 36.2 % across different regions [19, 24, 25, 26, 27]. The present study recorded a prevalence of 16.30 %, consistent with this range and comparable to findings of Derdour *et al*. [27] of 15 % and Ghalmi *et al*. [25] of 19.64 %, but it is higher than those (12.37 and

12.22 %) described by Achour *et al*. and Miroud *et al*. [24, 26], respectively, and lower as compared to prevalence of 36.2 % documented by Abdeltif *et al*. [19].

At the international level, the observed prevalence is comparable to those observed in Portugal (17.2 %) [28] and Egypt (18.6 %) [29]. However, this rate is lower than those reported in Brazil (47.36 %) [30] and Greece (21.03 %) [17] and higher than those found in Ethiopia (13.3 %) [31] and China (13.6 %) [32].

In neighboring countries, there are also fewer surveys of *N. caninum* in domestic animals in general. In Morocco (on the western borders of Algeria), Zouagui et al. found 25 % of dairy cattle to be seropositive for this protozoan [33]; while in Tunisia (on the eastern borders), this parasite had been molecularly identified in genital apparatus of 21.1 % naturally infected cows [34] which confirms the establishment of this pathogen. For the other surrounding countries, no data could be found in the published scientific literature.

The observed inter-regional and international variations in prevalence may be ascribed to differences in environmental conditions, herd management, diagnostic methods, study design, and sampling strategies [35].

Associated risk factors

At individual level, multivariable logistic regression analysis (TABLE II) identified age, type of vaccines used, mastitis and reproductive disorders as significant risk factors. However, vaccination, artificial insemination and birth of healthy calves were protective factors.

At herd level, there were no significant risk factors, vaccination and quarantine were identified as protective (TABLE III).

TABLE III

Results of multivariable logistic regression analysis of risk factors associated with Neospora caninum seropositivity at the herd- level in cattle from Northeastern, Algeria

Variables	B[a]	SE[b]	OR[c]	95 % CI[d] (OR)	P value
Constant	1.61	2.77	5.01	–	0.561
Vaccination (yes)	– 2.70	1.20	0.07	[0.01 – 0.70]	0.024*
Quarantine (yes)	– 1.47	0.64	0.23	[0.07 – 0.80]	0.021*

[a]Logistic regression coefficient, [b]Standard error, [c]Odds ratio, [d]Confidence interval, *Significant at P < 0.05. Model chi-square 15.225 with df of 7, Model-2 log likelihood 31.809, Chi square goodness to fit (Hosmer-Lemeshow) = 3.797, P = 0.803

In this study, older animals were more likely to be seropositive to *N. caninum*, in agreement with the findings of Selim *et al*. in Egypt [36] and Llano *et al*. in Colombia [37], who observed a positive association between age and *N. caninum* seroprevalence. This could be attributed to prolonged exposure time, that increases the probability of acquiring the infection via horizontal transmission and contact with definitive hosts [1]. Conversely, in Iran, Noori *et al.* [38] observed a seropositivity decrease with age, in relation to gradual antibody titers decline (in absence of re-infection), reduced longevity or selective culling of seropositive cattle [1, 39].

Cattle with reproductive disorders history had significantly increased odds of seropositivity, specifically, those with metritis, fertility disorders and multiple reproductive issues. These findings support earlier studies that identified various reproductive problems, like abortion, stillbirth and uterine infections, to be significantly associated to *N. caninum* seropositivity [8, 14, 17, 36].

In addition, mastitis was significantly associated with increased risk of *N. caninum* seropositivity, in accordance with Thurmond and Hietala [40], who reported seropositive cows to

TABLE II

Multivariable logistic regression analysis of risk factors associated with Neospora caninum seropositivity at the individual-level in cattle from northeastern Algeria

Variables	Category	Total (n)	Negative (n)	Positive (n)	Seroprevalence (%)	B[a]	SE[b]	OR[c]	95 % CI[d]	P value
Constant	**–**	**–**	**–**	**– –**		**1.20**	**1.13**	**3.31**	**–**	***0.288***
Age (years)	< 3	89	82	7	7.9	0.73	0.32	2.07	[1.09 – 3.91]	0.026*
	3 ≥ 6	203	168	35	17.2					
	> 6	76	58	18	23.7					
Vaccination	No	70	45	25	35.7	– 4.43	0.97	0.01	[0.00 – 0.08]	0.000*
	Yes	298	263	35	11.5					
Type of vaccines used	Against (*Aphtovirus* + *Rhabdovirus*)	200	185	15	7.5	1.09	0.40	2.99	[1.39 – 6.43]	0.005*
	Against (*Aphtovirus* + *Rhabdovirus* + *E. coli* + *Coronavirus* + *Rotavirus* + *Clostridium*)	65	56	9	13.8					
	Against (*Aphtovirus* + *Rhabdovirus* +*Clostridium* + *Pasteurella*)	33	22	11	33.3					
Mastitis	No	346	299	47	13.6	2.42	0.62	11.26	[3.36 – 37.69]	0.000*
	Yes	22	9	13	59.1					
Reproductives disorders	Without disorders	277	266	11	4.0	1.10	0.18	3.01	[2.11 – 4.28]	0.000*
	Fertility disorders	43	16	27	62.8					
	Metritis	17	5	12	70.8					
	Retained placenta	16	15	1	6.3					
	Multiple disorders	15	6	9	60.0					
Breeding methods	Natural breeding	255	206	46	18.0	– 1.83	0.63	0.16	[0.05 – 0.54]	0.003*
	Artificial insemination	113	99	14	12.4					
Birth of healthy calf	No	23	14	9	39.1	– 2.15	0.75	0.12	[0.03 – 0.51]	0.004*
	Yes	345	294	51	14.8					

[a]Logistic regression coefficient, [b]Standard error, [c]Odds ratio, [d]Confidence interval, *Significant at P < 0.05. Model chi-square 129.801 with df of 12, Model-2 log likelihood 197.486, Chi square goodness to fit (HosmerLemeshow) = 3.149, P = 0.925

be more susceptible to mastitis, with lower milk production and a duple risk of being culled due to udder disorders. This suggests that chronic *N. caninum* infection may alter mammary immunity and increase vulnerability to intramammary pathogens.

On the other hand, Peregrine *et al*. [41] found better udder health markers in seropositive cows. Although artificial insemination may control bovine neosporosis [42], Algerian family farms (especially small holders) still share a same bull to breed all their cows, even between herds, facilitating horizontal transmission of the parasite.

In the present study, artificially inseminated cows had significantly lower risk compared to naturally bred ones.

Farms practicing quarantine showed significantly lower odds to be seropositive to *N. caninum*. However, this protective effect is not necessarily attributed to quarantine itself. In the visited farms, quarantine consisted of isolating newly introduced animals up to 15 days to evaluate their overall health.

Since *N. caninum* infection is asymptomatic in cattle, and serological diagnosis of the parasite is not systematically performed in Algeria, this measure cannot identify infected animals or directly reduce seropositivity. The lower odds observed may reflect better farm management, including good hygiene practices and rigorous biosecurity measures that control horizontal transmission [35].

To date, no specific commercial vaccines for *N. caninum* are available [43]. Although, cattle vaccinated against other pathogens had significantly lower *N. caninum* seropositivity, at both individual and herd level. Regular vaccination reflects good herd management. Farms that invest in routine vaccination demonstrate greater hygiene vigilance and apply good biosecurity measures, both of which are known to reduce the risk of horizontal transmission of *N. caninum* [1].

On the contrary, cattle receiving multiple vaccines (*Aphtovirus, Rhabdovirus, Pasteurella, Clostridium*) had higher seropositivity than those vaccinated only against *Aphtovirus* and *Rhabdovirus*. Similar associations between specific vaccination protocols and increased *N. caninum* seropositivity have been recorded by Cavirani *et al.* [44]. This increase may result from vaccine-induced immune stress, that can reactivate latent encysted bradyzoites. Furthermore, as cattle are vulnerable to endotoxins, the administration of vaccines against Gram-negative bacteria such as Pasteurella, which contain lipopolysaccharide endotoxins, may alters host immunity and increase the likelihood of acquiring secondary infections [45].

Cows that gave birth to healthy calves had less chance of being seropositive for *N. caninum*, as confirmed by the pathophysiology of vertical transmission. In fact, infected cows can transmit the parasite transplacentally to their fetuses, leading to fetal losses, stillbirths or birth of weak or non-viable calves [1, 6].

Serum biochemical alterations

Serum biochemical concentrations in *N. caninum*-seropositive and seronegative cattle are presented in TABLE IV. Results of generalized linear models for significant biochemical parameters associated with *N. caninum* serostatus and predictor factors (age, breed, parity and lactation phase) are detailed in TABLE V.

TABLE IV

Univariate analysis of serum biochemical parameter levels in Neospora caninum seropositive and seronegative cattle from northeastern, Algeria

Parameter	Unit	Seronegative (n = 30)	Seropositive (n = 30)	P value	Test
		Mean ± SD[a]	Mean ± SD[a]		
BHB	mmol/L	0.51 ± 0.50	0.52 ± 0.40	0.639	MW[c]
Glucose	g/L	0.65 ± 0.11	0.64 ± 0.15	0.697	ST[b]
Total protein	g/L	68.10 ± 10.03	82.22 ± 9.77	0.000*	ST[b]
Albumin	g/L	21.53 ± 9.28	19.65 ± 4.00	0.525	MW[c]
Globulin	g/L	46.58 ± 10.82	62.56 ± 9.79	0.000*	STa
A/G ratio		0.53 ± 0.40	0.32 ± 0.09	0.003*	MW[c]
AST	U/L	42.66 ± 13.46	57.73 ± 24.70	0.005*	ST[b]
ALT	U/L	13.08 ± 7.17	17.18 ± 7.45	0.034*	ST[b]
GGT	U/L	9.20 ± 4.29	11.66 ± 5.17	0.054	MW[c]
LDH	U/L	872.06 ±	951.16 ± 181.96	0.078	MW[c]
ALP	U/L	217.05	118.03 ± 57.46	0.033*	MW[c]
Urea	g/L	97.80 ± 69.97	0.34 ± 0.09	0.000*	MW[c]
Creatinine	mg/L	0.23 ± 0.06	12.29 ± 4.15	0.007*	MW[c]
Calcium	mg/L	10.09 ± 3.00	63.69 ± 9.86	0.000*	ST[b]
Phosphorus	mg/L	79.38 ± 12.55	51.52 ± 8.06	0.361	ST[b]

[a]Standard deviation, [b]Student's test, [c]Mann-Whitney U test, *Significant at P < 0.05. BHB: β hydroxybutyrate, A/G: Albumin/Globulin, AST: Aspartate aminotransferase, ALT: Alanine amino-transferase, GGT: Gamma-glutamyl transferase, LDH: Lactate dehydrogenase, ALP: Alkaline phosphatase

TABLE V

Results of significant factors in generalized linear models of biochemical parameters according to Neospora caninum serostatus and confounding factors (lactation phase, age, breed and Parity)

Biochemical parameters	Predictor	Category	Mean±SD	B[a]	SE[b]	Wald Chi-square	95 % CI[c]	P value	AIC[d]
Total protein	*N. caninum*	+	82.22 ± 9.77	14.15	2.30	37.82	[9.64 ; 18.66]	0.000*	459.514
	serostatus	–	68.11 ± 10.03	0					
Globulin	*N. caninum*	+	62.57 ± 9.79	15.17	2.53	35.86	[10.20 ; 20.13]	0.000*	471.015
	serostatus	–	46.58 ± 10.82	0					
	Parity	0	53.06 ± 1.33	27.46	12.50	4.83	[2.96 ; 51.96]	0.028*	
A/G ratio	*N. caninum*	+	0.32 ± 0.09	– 0.18	0.07	6.85	[– 0.32 ; – 0.05]	0.009*	39.760
	serostatus	–	0.53 ± 0.40	0					
	Parity	0	0.40 ± 0.19	– 1.03	0.34	8.99	[– 1.70 ; 0.36]	0.003*	
		1	0.39 ± 0.12	– 0.94	0.31	8.90	[– 1.55 ; 0.32]	0.003*	
		2	0.41 ± 0.34	– 0.94	0.29	10.06	[– 1.52 ; 0.36]	0.002*	
		3	0.44	– 0.90	0.40	5.05	[– 1.69 ; 0.11]	0.025*	
		4	0.43 ± 0.31	– 0.96	0.29	10.32	[– 1.54 ; 0.37]	0.001*	
		5	0.31 ± 0.07	– 0.91	0.36	6.42	[–1.63 ; 0.21]	0.011*	
Urea	*N. caninum*	+	0.34 ± 0.09	0.10	0.02	30.59	[0.07 ; 0.15]	0.000*	–112.956
	serostatus	–	0.23 ± 0.06	0					
ALT	*N. caninum*	+	17.18 ± 7.45	3.12	1.56	3.99	[0.06 ; 6.17]	0.046*	412.825
	serostatus	–	13.08 ± 7.17	0					
	Breed	Holstein	14.16 ± 7.15	– 8.72	2.21	15.64	[– 13.05 ; – 4.40]	0.000*	
AST	*N. caninum*	+	57.73 ± 24.70	17.73	4.23	17.57	[9.44 ; 26.02]	0.000*	532.577
	serostatus	–	42.67 ± 13.46	0					
	Breed	Holstein	45.64 ± 19.05	– 26.26	5.98	19.25	[– 37.77 ; – 14.53]	0.000*	
	Parity	2	48.23 ± 20.68	– 39.71	17.95	4.89	[– 74.90 ; – 4.52]	0.027*	
		5	37.00 ± 24.04	– 69.46	22.00	9.96	[– 112.59 ; – 26.33]	0.002*	
GGT	*N. caninum*	+	11.67 ± 5.17	2.54	1.10	5.37	[0.39 ; 4.69]	0.020*	370.595
	serostatus	–	9.20 ± 4.286	0					
	Breed	Holstein	11.23 ± 5.21	4.17	1.55	7.22	[1.13 ; 7.21]	0.007*	
Calcium	*N. caninum*	+	63.69 ± 9.86	– 17.70	2.37	55.74	[– 22.35 ; – 13.06]	0.000*	463.102
	serostatus	–	79.38 ± 12.55	0					
	Lactation	Mild	64.11±12.50	– 20.15	6.58	9.38	[– 33.04 ; – 7.26]	0.002*	
	phase	Dry	72.21 ± 3.29	– 16.01	6.76	5.61	[– 29.25 ; – 2.75]	0.018*	

[a]beta coefficient, [b]Standard error, [c]Confidence interval, [d]Akaike information criterion, *Significant at P < 0.05, A/G: Albumin/Globulin, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma-glutamyl transferase. +: seropositive, –: seronegative, breed (Holstein, Atlas Brown, Montbeliarde), lactation phase (early, mid, late, dry, gravid heifer) and parity (0, 1, 2, 3, 4, 5, > 5)

Univariate comparative analysis revealed significant differences in serum levels of total protein, globulin, A/G ratio, AST, ALT, ALP, urea, creatinine and calcium between *N. caninum* seropositive and their seronegative counterparts (TABLE IV). After adjusting by generalized linear models (TABLE V), seropositive cattle showed significantly higher levels of total protein (P < 0.0001), globulin (P < 0.0001), AST (P < 0.0001), ALT (P = 0.046), GGT (P = 0.020) and urea (P = 0.000), and significantly lower levels of calcium (P < 0.0001) and A/G ratio (P = 0.009). No significant associations were noticed for BHB, glucose, albumin, LDH, ALP, creatinine or phosphorus (P > 0.05). After adjusting for confounding factors, GGT became statistically significant (P = 0.054 vs P = 0.020) while, the ALP (P = 0.033 vs P = 0.231) and creatinine (P = 0.007 vs P = 0.520) turned into non-significant (TABLES IV and VI).

TABLE VI

Generalized linear model results evaluating the effect of Neospora caninum seropositivity on serum biochemical parameters in dairy cattle from Northeastern Algeria

Parameter	β[a]	SE[b]	95 % CI[d]	P value
BHB	– 0.07	0.10	[– 0.27 ; 0.13]	0.504
Glucose	– 0.01	0.03	[– 0.07 ; 0.06]	0.810
Total protein	14.15	2.30	[9.64 ; 18.66]	0.000*
Albumin	– 10.02	1.56	[– 4.09 ; 2.05]	0.541
Globulin	15.17	2.53	[10.20 ; 20.13]	0.000*
A/G ratio	– 0.19	0.07	[– 0.32 ; 0.05]	0.009*
AST	17.73	4.23	[9.44 ; 26.02]	0.000*
ALT	3.12	1.56	[0.06 ; 6.17]	0.046*
GGT	2.54	1.10	[0.39 ; 4.69]	0.020*
LDH	60.96	51.83	[– 40.63 ; 162.55]	0.240
ALP	15.43	12.88	[– 9.80 ; 40.66]	0.231
Urea	0.11	0.02	[0.07 ; 0.15]	0.000*
Creatinine	1.76	0.91	[– 0.01 ; 3.54]	0.520
Calcium	– 17.70	2.37	[– 22.35 ; – 13.06]	0.000*
Phosphorus	– 1.05	1.65	[– 4.28 ; 2.18]	0.523

[a]Logistic regression coefficient, [b]Standard error, [c]Odds ratio, [d]Confidence interval, *Significant at P < 0.05. Model chi-square 15.225 with df of 7, Model-2 log likelihood 31.809, Chi square goodness to fit (Hosmer-Lemeshow) = 3.797, P = 0.803

The biochemical profile of dairy cattle can be influenced by various factors including physiological status, nutrition, breed predispositions and pathologies [46, 47]. Based on the literature on *N. caninum* pathogenesis, we targeted 15 biochemical indicators of hepatic, renal and metabolic functions. As shown in TABLE V, certain predictor factors, including parity, breed, and lactation phase, significantly influenced some biochemical parameters. It should be noted, however, that this study was designed to investigate the impact of *N. caninum* serostatus on biochemical parameters, and not to assess the effects of physiological variables that the study will not discuss.

Infected cattle showed significantly increased hepatic enzymes: AST, ALT and GGT compared with healthy ones. Alike previous studies [48, 49], this could result from the hepatocellular damage caused by *N. caninum*. As well, elevated AST may also reflect necrosis in other organs [1, 12].

Regarding mineral metabolism, no significant differences in phosphorus concentrations were observed between seropositive and seronegative cows. But, seropositive cows had significantly lower calcium levels. Under physiological conditions, the coordinated action of 1.25-dihydroxyvitamin D3, parathyroid hormone (PTH) and calcitonin, controls calcium levels [50]. The pathological effects of neosporosis on hepatic and renal functions may disturb the hydroxylation steps of vitamin D to 25-hydroxyvitamin D3 in the liver and to 1.25-dihydroxyvitamin D3 in the kidneys [50], that lower the calcium levels.

Moreover, proinflammatory interleukins produced during the host immune response activated during neosporosis can reduce PTH secretion, causing hypocalcemia [51].

A previous study reported significantly elevated BHB in *N. caninum* infected cows [49]. Differently to these findings, no significant differences were detected in serum levels of BHB and glucose between seropositive and seronegative cows in the present work.

Raised urea and creatinine levels have been reported in sheep with renal damage and dysfunction following infection with *Toxoplasma gondii* [48], another protozoan coccidian parasite that shares many similarities with *N. caninum* [1]. In the current survey, comparable results were observed, but, elevated creatinine in seropositive cows was not significant after GLM adjustment, suggesting confounding factors. In contrast, elevated urea remained significantly associated with seropositivity.

Although, this dissociation between urea and creatinine indicates that the observed hyperuremia may reflect increased proteins catabolism due to inflammation process or may be related to other factors rather than renal dysfunction, such as nutrition, that were not explored in this study.

As opposed to Alekich *et al*. [49], who observed a non-significant decrease in total protein in *N. caninum* seropositive cows, the findings of the current study showed a significant increase, even after adjustment. This may be due to the non-suppurative inflammation induced by *N. caninum* in multiple organs [1, 11, 47].

The elevated total protein amount was associated with a significant increase in globulin levels and a decrease in A/G ratio in seropositive cattle, which reflects the immunoglobulin production during the immune response [1,47,52]. These findings are reinforced by those of Appelt *et al.* [53].

CONCLUSION

The present study provides evidence of subclinical hypocalcemia in dairy cows in relation to neosporosis. Altered hepatic enzymes reflect an overall metabolic disorder beyond the well-known reproductive issues. Further researches must be conducted to better understand pathological mechanisms of this infection on dairy cows' metabolism.

The detection of *N. caninum* in five provinces of Northeastern Algeria indicate its widespread distribution in that region and may be the whole country (taking into consideration cattle and feed trade and definitive/intermediate hosts' displacements). Mastitis and reproductive disorders are significantly associated to *N. caninum* infection, confirming its major role in reproductive failure and reduced productivity.

Conflicts of interest

The authors declare no conflicts of interest.

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