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Original papers

Relationships between certain metabolic diseases and selected serum biochemical parameters in seropositive dairy cows against *Neospora caninum* infection in different stages of lactation

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ABSTRACT. *Neospora caninum* is an important cause of abortion in dairy cattle. The general health of affected cows has not been investigated before. Therefore, the main objective of this study was to identify possible relationships between certain metabolic diseases and selected serum biochemical parameters in seropositive dairy cows against *N. caninum* antibodies in different stages of lactation. The study was carried out using 72 *N. caninum* seropositive cows and 61 seronegative dairy cows (control). Serum from all cows was tested to determine their *N. caninum* status (seropositive vs seronegative) using commercially available indirect enzyme-linked immunosorbent assay test kit (iELISA). In addition, serum biochemical parameters including beta-hydroxybutyrate (BHB), glucose, creatinine, blood urea nitrogen, total protein, albumin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH) and gamma-glutamyltranspeptidase (GGT) were determined using routine laboratory methods. The stage of lactation was obtained at the time of sampling from farm records. Student independent t-test showed that there was a significant difference in the serum concentrations of BHB, AST, ALT, and LDH between seropositive and seronegative cows. There was no significant association between seropositivity and the stage of lactation. However, multivariable logistic regression analysis showed that there was a strong association between seropositivity and BHB concentrations. Results of this study indicate a possible relationship between *N. caninum* seropositivity and certain metabolic diseases such as ketosis and fatty liver syndrome in dairy cows.

Key words: *Neospora caninum*, BHB, liver enzymes, ketosis, dairy cows

Introduction

Neosporosis is considered one of the most economically important diseases of both dairy and beef cattle. The disease is caused by the protozoan *Neospora caninum* which is well-recognized as an important cause of abortion in cattle [1,2]. Until recently, the dog was the only known definitive host of *N. caninum.* However, recent scientific research suggested that coyotes and wild wolfs might also be definitive hosts of *N. caninum* [3,4].

The life cycle of *N. caninum* in cattle has not been well characterized. The definitive host sheds environmentally-resistant unsporulated oocysts. Intermediate hosts such as cattle become infected upon ingestion of oocysts-contaminated food or water [5]. In cattle, infection can exist in two different stages: the tachyzoite and the bradyzoite. The tachyzoite stage occurs predominantly during the acute stage of infection. In this stage, tachyzoites actively multiply within the host cells causing significant damage in different body tissues. Histopathologically, tachyzoites can be detected in various tissues including neuronal cells, hepatocytes, placenta and skeletal muscular tissues and myocardium [6]. The bradyzoite stage is

reportedly associated with chronic infection where bradyzoites remain inactive within the infected cells in the form of tissue cysts. Inactive cysts are commonly found in the heart, brain and skeletal muscles [6].

In recent literature, an abundance of research could be cites regarding the prevalence, risk factors and new developments in the diagnosis, treatment and prevention of *N. caninum* in different animal species [4,8–11]. To the authors' knowledge however, there has been no reported data evaluating the association between common metabolic diseases, serum biochemical parameters and seropositivity against *N. caninum* in dairy cows in different stages of lactation. Therefore, the main objective of this study was to identify possible relationships between certain metabolic diseases and selected serum biochemical parameters in seropositive dairy cows against *N. caninum* antibodies in different stages of lactation.

Materials and Methods

Study area and study population. A total of 133 dairy cows were randomly selected from 30 different herds located in different regions in Jordan. Only apparently healthy cows over two years of age were enrolled in the study. Data regarding the age of the cow and stage of lactation as well as current and past medical history of each cow was obtained from the farm records using a pre-structured questionnaire. Additional data collected included the presence other concurrent diseases particularly; retained placenta, abortion, mastitis, milk fever, and left or right abomasal displacement (LDA or RDA). All cattle were kept in open stalls with fence line separation between heifers and adult cows. The cows were typically milked twice a day. Cows were subjected to a complete physical examination and were considered healthy if they appeared healthy and manifested no symptoms of clinical illness.

Sample collection. Whole blood samples were collected from the coccygeal vein of each animal in non-EDTA plain vacutainer tubes (Occidem Biotech, Middleses, UK) and transported on ice to the laboratory. Serum was collected by centrifugation of clotted blood samples at 5000g for 10 min. Sera were stored in Eppendorf tubes at −20°C until analysis.

Table 1. Serum biochemical analysis in seropositive and seronegative dairy cows against *N. caninum* antibodies

Parameter	*N. caninum* status	Mean±SD	Reference values [18]
BHB (mmol/l)*	–	0.39±0.23	<1.4
	+	1.56±1.26	
Glucose (mg/dl)	–	39.85±17.68	31-77
	+	54.53±22.50	
Creatinine (mg/dl)	–	1.04±2.02	0.4-1.0
	+	1.03±3.11	
Blood urea nitrogen (mg/dl)	–	6.38±2.03	10-25
	+	5.97±1.98	
Total protein (g/dl)	–	7.57±6.38	7.2-9.0
	+	7.07±1.50	
Albumin (g/dl)	–	3.40±0.40	3.2-4.2
	+	3.10±0.34	
ALP (IU/l)	–	41.40±18.56	23-78
	+	44.57±27.10	
AST (IU/l)*	–	62.82±22.26	53-162
	+	544.29±16.22	
ALT (IU)*	–	21.03±16.96	25-74
	+	313.01±8.26	
LDH (IU/l)*	–	710.98±322.38	659-1231
	+	328.08±64.48	
CGT (IU/I)	–	31.02±14.46	11-39
	+	35.29±25.94	

*P value ≤ 0.05; BHB- beta-hydroxybutyrate; ALP- alkaline phosphatase; AST- aspartate transaminase; ALT- alanine aminotransferase; LDH- lactate dehydrogenase; GGT- gamma-glutamyltranspeptidase

Laboratory analysis. Seropositivity against *N. caninum* was determined using a commercially available indirect enzyme-linked immunosorbent assay kit (The Herd Chek Anti-*Neospora caninum* Antibody TestKit, IDEXX Laboratories Inc., Westbrook, ME, USA) according to the manufacturers' instructions. Test sensitivity and specificity are 98.6% and 98.9%, respectively.

Serum biochemical analyses. The following serum biochemical parameters were determined using routine laboratory methods [12]: glucose, creatinine, blood urea nitrogen, total protein, albumin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH) and gamma-glutamyltranspeptidase (GGT). In addition, serum beta-hydroxybutyrate (BHBA) was measured using commercially available colorimetric enzymatic assay kit (Bio Scientific Corporation, USA) according to the manufacturer's instructions.

Statistical analysis. All statistical analyses were performed using the statistical software package SPSS (version 19.0, SPSS Inc., Chicago, IL, USA). Associations between *N. caninum* seropositivity and various biochemical parameters were initially screened in a univariable analysis using Pearson's chi-square test. Only significant variables and variables with no collinearity ($r<0.50$) were considered for the final multivariable logistic regression. Collinearity was evaluated using Pearson's correlation test. A multivariable binary regression model was constructed using „Enter" method. The Hosmer-Leme show test was used to evaluate the goodness-of-fit for the developed logistic regression model. Student independent t-test was used to evaluate significant differences between the selected biochemical parameters between seropositive and seronegative cows. Analysis was considered significant if $P \leq 0.05$.

Results and Discussion

Neospora caninum infection is a significant cause of reproductive loss in dairy cow industry. This is the first clinical study that investigated the possible association between seropositivity against *N. caninum* and common metabolic diseases of dairy cows. In the serum biochemical analyses, there was a significant difference in BHB, AST, ALT and LDH concentrations between seropositive and seronegative cows (Table 1). Serum concentrations of BHB, AST, and ALT were significantly elevated in seropositive cows compared to their values in seronegative cows. On the other hand, serum concentrations of LDH were significantly lower in seropositive cows. In this study, only 7 out of 72 cows were at their early stage of lactation, which is the period expected for the highest AST and ALT enzymes activity in dairy cows [13,14]. Since these enzymes are highly sensitive indicators of liver damage or injury, their increase in all stages of lactation might indicate a pathological cause due to hepatocyte injury or dysfunction caused by the parasite. Furthermore, low LDH levels points out either a laboratory error that could have been avoided by repeating the test, or due to a lack of productive cells due todamage or injury caused by hypothesized tachyzoites in different parts of the infected animals' body including the liver.

In this study, the significant increase in BHB levels in seropositive cows may suggest a shift in the main energy producing pathways (trichloroacetic acid cycle) towards increased mobilization of ketone bodies which has been suggested to be detrimental to the health of the animal [15,16]. Energy balance is one of the most critical factors affecting animal health, lactation, and reproductive performance. At present, the current "gold standard" test for ketoacidosis in cattle industry is blood β-hydroxybutyric acid (BHB). This ketone body is reportedly considered more stable in blood than other ketone bodies including acetone or acetoacetate [16]. Ketone bodies are produced by the liver from fatty acids during periods of negative energy balance, which freely diffuse across cell membrane to provide energy when shortage occurs due to the assembly of the growing parasites [17].

In this study, we proposed that negative energy balance in seropositive cows due to increased energy demands and high metabolic rate in infected

Table 2. Chi-square analysis of the association between *N. caninum* seropositivity and stage of lactation in dairy cows

Stage of lactation	Seronegative	Seropositive	P value
Dry	5	20	0.32
Early	9	7	
Mid	22	25	
Late	24	20	

body tissues by the proliferating parasites. This theory was fortified by the finding of no significant differences between *N. caninum* seropositivity and stage of lactation (Table 2). Indeed, these findings suggests that this energy deficit in infected cows is not associated with early lactation ketosis. More interestingly, clinical association between the infection and negative energy balance was further confirmed by the strong association between seropositivity and BHB concentrations in seropositive cows. In negative energy states, more lipolysis is expected to occur in order to provide more energy to the host cells [17]. These results may also explain the slightly lower albumin levels in seropositive cows.

In conclusion, the study presented here reports for the first time a possible association between negative energy balance and ketosis and *N. caninum* infection in dairy cows. In addition, significant changes in selected serum biochemical parameters were reported in infected cows which fortified our conclusions. However, further clinical and pathological studies are warranted to determine the exact role of negative energy balance in the pathogenesis of *N. caninum* infection in dairy cattle.

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