

## Effects of latent Q fever infection on selected hormonal, antioxidant, biochemical and haematological parameters in sheep

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### ABSTRACT

The aim of this retrospective study was to compare various endocrine, antioxidant, haematological and biochemical parameters between *Coxiella (C.) burnetii* positive and negative ewes in order to evaluate the possible effects of infection on selected blood parameters. For this purpose, serum leptin and haptoglobin (Hp) concentrations, whole blood glutathione peroxidase (GSH-Px) activity and haematological parameters were compared between *C. burnetii* positive and negative ewes that were sampled in the autumn and winter months of 2006, before the Q fever infection outbreak in the spring of 2007. *C. burnetii* antibodies and haptoglobin concentrations were measured by commercial ELISA kits, leptin concentrations by a commercial RIA kit and GSH-Px activity by a commercial reagent kit. Out of 26 clinically healthy ewes, 15 exhibited *C. burnetii* antibodies in May 2007; among them, 8 had already been *C. burnetii* positive in the autumn months. Rises in Hp and leptin concentrations as well as of GSH-Px activity, haematocrit and mean cell volume were observed in samples taken between September and December 2006 in positive and negative ewes. Red blood cell count and haemoglobin values remained unchanged, while a decrease in white blood cell and platelet count was detected in the same period in both groups of ewes. The majority of the selected blood parameters remained in the reference range for sheep. The observed changes of the studied parameters are most probably a result of seasonally induced fluctuations or physiological changes, such as pregnancy progression, and cannot be attributed to the influences of the infection with *C. burnetii*. Therefore, the subclinical form of Q fever is not a factor which should be considered when evaluating values of circulating leptin, GSH-Px or haematological parameters in sheep.

**Key words:** Q fever, sheep, leptin, haptoglobin, glutathione peroxidase, haematology

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## Introduction

Q fever is a highly contagious zoonotic disease, occurring worldwide (ANGELAKIS and RAOULT, 2010; GUATTEO et al., 2011). It is caused by an intracellular Gram-negative bacterium *Coxiella (C.) burnetii* which can infect humans, a wide range of domestic and wild mammals, birds, reptilians and ticks. In most cases the infection is asymptomatic but in sheep 5 - 50% of a flock can be affected. Aborting sheep and goats are often reported as a source of infection in humans (ANGELAKIS and RAOULT, 2010; GUATTEO et al., 2011; RODOLAKIS, 2009; RUIZ-FONS et al., 2010). Animals usually become infected with *C. burnetii* by inhalation. After the primary multiplication of the bacteria in the regional lymph nodes, bacteraemia follows, lasting 5 - 7 days. The infection then progresses into the mammary glands and the placenta (ANGELAKIS and RAOULT, 2010; GUATTEO et al., 2011). *C. burnetii* grows and proliferates within the phagolysosomes, and persists in fixed macrophages, due to subversion of macrophage functions and the impairment of T-cell responses (ANGELAKIS and RAOULT, 2010; GUATTEO et al., 2011; HONSTETTRE et al., 2003). The majority of the mentioned studies of Q fever are focused on the characteristics of *C. burnetii*, epidemiology, the determination of the agent, and clinical signs of Q fever in man and animals, but reports considering the influences of the infection on various physiological parameters, such as haematological or endocrinological factors, were not found.

In the spring of 2007, after an outbreak of Q fever in employees and students which were in contact with sheep, *C. burnetii* antibodies were confirmed in a flock of sheep at the research facility of Ljubljana Veterinary Faculty. Among the infected animals there were also ewes which were included in an extensive research project investigating the physiological characteristics of Slovene autochthonous sheep breeds during the whole of 2006, and the results of this project became questionable due to the unexpected *C. burnetii* infection.

The present retrospective study was conducted in order to avoid any erroneous conclusions considering the project results obtained in 2006. To this purpose, the results of serum leptin and haptoglobin (Hp), whole blood glutathione peroxidase (GSH-Px) and haematological parameters in these sheep, obtained during the autumn of 2006, were evaluated with respect to *C. burnetii* infection status, as indicated by serum antibody levels.

## Materials and methods

The study was performed on 26 adult ewes of the Istrian breed kept at a research facility of the Veterinary Faculty (at the Vremščica Centre for Sustainable Recultivation). The ewes were kept on pasture from March to September and housed over the rest of the year. They were lambing during the early spring of 2006. The next breeding season started in the autumn months, and in December 2006 all the ewes, with the exception

of one, were pregnant and due to give birth in February or March 2007. The animals were clinically healthy and did not exhibit any reproductive disorders. Their blood was sampled monthly from January to December 2006, always starting on the first Tuesday of the month between 8 and 10 a.m. Additional sampling was performed in May 2007. Blood samples were collected by jugular venipuncture using evacuated tubes without anticoagulant (Vacutainer®, 5 mL, Becton Dickinson, Heidelberg) for serum leptin, haptoglobin and *C. burnetii* antibodies determination, with lithium heparin (Vacutainer®, LH 119 I.U., 7 mL, Becton Dickinson, Heidelberg) for GSH-Px determination and with EDTA (Vacutainer®, K3E, 5 mL, Becton Dickinson, Heidelberg) for haematological analyses. The latter were performed immediately after the delivery of blood samples to the laboratory on the day of blood samples collection. All other samples were aliquoted and kept frozen below -20 °C until analysed.

After the confirmation of *C. burnetii* antibodies in May 2007, samples which were collected in September and December 2006, were also tested for the same antibodies. Based on the presence of these antibodies, previously measured results (leptin concentration, GSH-Px activity, haematological values) were distributed into two groups, originating from *C. burnetii* positive or negative ewes. In our original study, leptin concentrations were measured each month, and GSH-Px activity and haematological parameters were measured every 3 months. Therefore, in the present study leptin concentrations were evaluated in the period from September to December, GSH-Px activity and haematological parameters in September and December. Additionally, haptoglobin concentrations were measured for the purpose of this study in stored samples collected in September and December 2006.

Serum *C. burnetii* antibodies were determined by a commercial ELISA kit (CHEKIT\* Q Fever Antibody ELISA, IDEXX, Switzerland). A commercial ELISA kit (PHASE™ RANGE Haptoglobin Assay, Tridelata, Ireland) validated for sheep serum was used for haptoglobin determination. Serum leptin concentrations were measured by a commercial RIA test (Multi species Leptin RIA Kit, Linco, USA), validated for sheep serum. GSH-Px activity in whole blood haemolysates was determined spectrophotometrically with an automated biochemistry analyser, RX-Daytona (Randox, Crumlin, UK), using the commercial reagent kit Ransel (#RS 504, Randox Laboratories, Crumlin, UK), and expressed as units per gram of haemoglobin (U/g Hgb). Haematological analyser ABC Vet (ABX, France) was used for haematological analyses that included red blood cell count (RBC), haematocrit, mean cell volume (MCV), haemoglobin concentration, white blood cell count (WBC) and platelet count.

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS for Windows. Release 8.0.0.), with subprograms Paired *t*-test, independent *t*-test

and one-way ANOVA. Results were considered significant at the level  $P < 0.05$  and are presented in the text as mean  $\pm$  standard error of the mean ( $\bar{x} \pm \text{SEM}$ ).

The study was performed in accordance with the ethical and regulatory guidelines regarding the care and use of animals for experimental procedures.

### Results

Out of 26 clinically healthy ewes included in the research project from 2006, 15 (57.69%) of them exhibited *C. burnetii* antibodies in May 2007. Among these 15 positive ewes, *C. burnetii* antibodies were confirmed in stored samples of 8 ewes (22.22%) sampled in September and December 2006, while another 7 ewes were still negative in this period. Negative results were also obtained for another 11 ewes, sampled in 2006.

Mean leptin levels in positive and negative ewes from September to December are represented in Figure 1. Measured leptin concentrations ranged from 0.17 ng/mL to 4.96 ng/mL. The lowest mean leptin levels were measured in both groups of ewes in September, the highest in December ( $P < 0.001$  for negative;  $P > 0.05$  for positive ewes). Differences between positive and negative ewes were found to be insignificant ( $P > 0.05$ ) at each monthly sampling

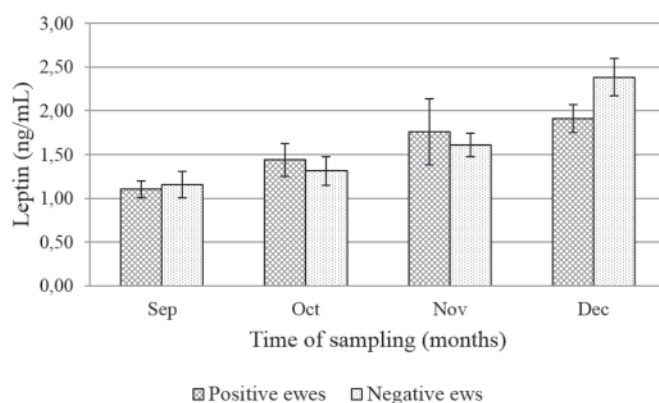


Fig. 1. Monthly leptin concentrations ( $\bar{x} \pm \text{SEM}$ ) in negative and positive ewes from September to December. Sep - September, Oct - October, Nov - November, Dec - December.

Mean GSH-Px activities and Hp concentrations in positive and negative ewes in September and December are represented in Table 1. Measured GSH-Px activity ranged from 184.47 U/gHb to 864.95 U/gHb. At each monthly sampling mean values in negative ewes were lower than in positive, but they did not reach a significant level ( $P > 0.05$ ).

For both positive and negative ewes, the mean values in September were lower than in December ( $P < 0.05$  for negative ewes). Measured Hp values were in the range between 0.25 mg/mL and 2.00 mg/mL. Mean Hp values were insignificantly higher in positive than in negative ewes at both sampling times, and in September they were insignificantly lower than in December for both groups of ewes ( $P > 0.05$ ).

Mean RBC, haematocrit, MCV, Hgb concentrations, WBC and platelet counts of positive and negative ewes in September and December are also presented in Table 1. RBC values ranged from  $7.33 \times 10^{12}/L$  to  $11.99 \times 10^{12}/L$ . The differences between the positive and negative ewes, as well as between all monthly RBC means were insignificant ( $P > 0.05$ ). The measured haematocrit values were in the range of 0.24 to 0.40. Mean haematocrit in December was significantly higher than in September for both negative ( $P < 0.05$ ) and positive ewes ( $P < 0.01$ ), but the differences between the positive and negative ewes in September and December were not significant ( $P > 0.05$ ).

Table 1. Whole blood glutathione peroxidase (GSH-Px) activity, haptoglobin (Hp) concentrations and haematological parameters (RBC - red blood cell count; MCV - mean corpuscular volume;

Hgb - haemoglobin concentration; WBC - white blood cell count) in *C. burnetii* positive and negative ewes in September and December ( $\bar{X} \pm SEM$ ).

| Parameter (unit)*           | Positive (n = 8)              |                              | Negative (n = 18)               |                                 |
|-----------------------------|-------------------------------|------------------------------|---------------------------------|---------------------------------|
|                             | September                     | December                     | September                       | December                        |
| GSH-Px (U/gHgb)             | 452.50 $\pm$ 40.15            | 519.53 $\pm$ 27.71           | 384.45 $\pm$ 30.18 <sup>a</sup> | 473.11 $\pm$ 20.40 <sup>a</sup> |
| Hp (mg/mL)                  | 0.42 $\pm$ 0.03               | 0.72 $\pm$ 0.19              | 0.35 $\pm$ 0.02                 | 0.44 $\pm$ 0.03                 |
| RBC ( $10^{12}/L$ )         | 9.20 $\pm$ 0.38               | 9.70 $\pm$ 0.24              | 9.87 $\pm$ 0.30                 | 9.94 $\pm$ 0.24                 |
| Haematocrit (L/L)           | 0.31 $\pm$ 0.01 <sup>b</sup>  | 0.35 $\pm$ 0.01 <sup>b</sup> | 0.30 $\pm$ 0.01 <sup>a</sup>    | 0.34 $\pm$ 0.01 <sup>a</sup>    |
| MCV (fL)                    | 32.25 $\pm$ 0.67 <sup>b</sup> | 35.5 $\pm$ 0.71 <sup>b</sup> | 31.72 $\pm$ 0.35 <sup>c</sup>   | 34.89 $\pm$ 0.36 <sup>c</sup>   |
| Hgb (g/L)                   | 94.9 $\pm$ 3.40               | 103.10 $\pm$ 2.8             | 99.3 $\pm$ 2.9                  | 104.2 $\pm$ 2.6                 |
| WBC ( $10^9/L$ )            | 10.21 $\pm$ 0.92              | 8.99 $\pm$ 0.72              | 11.681 $\pm$ 0.50 <sup>a</sup>  | 10.29 $\pm$ 0.36 <sup>a</sup>   |
| Platelet count ( $10^9/L$ ) | 275 $\pm$ 31.39               | 254.13 $\pm$ 58.04           | 345.00 $\pm$ 12.69 <sup>b</sup> | 273.00 $\pm$ 18.41 <sup>b</sup> |

Values in a row with the same letter are significantly different: <sup>a</sup> =  $P < 0.05$ ; <sup>b</sup> =  $P < 0.01$ ; <sup>c</sup> =  $P < 0.001$

The measured MCV ranged from 30 fL to 40 fL. Mean MCVs in December were significantly higher than in September for both negative ( $P < 0.001$ ) and positive ewes ( $P < 0.01$ ). The differences between the positive and negative ewes were insignificant in both months of sampling ( $P > 0.05$ ). The measured Hgb concentrations were in the range between 77 g/L and 121 g/L. The Hgb concentrations in December were higher than in September for both groups of ewes ( $P > 0.05$ ) and the differences between the positive and negative ewes were insignificant in both months ( $P > 0.05$ ). The WBC ranged from  $5.70 \times 10^9/L$  to  $16.60 \times 10^9/L$ . The differences between positive and negative ewes were insignificant ( $P > 0.05$ ) in both sampling months, but in positive ewes the mean value in

September was significantly higher than in December ( $P < 0.05$ ). The platelet count was in the range of  $76 \times 10^9/L$  to  $616 \times 10^9/L$ . The differences between the positive and negative ewes at both samplings as well as between the positive ewes in September and December were insignificant ( $P > 0.05$ ). In negative ewes, the mean platelet count in September was significantly higher than in December ( $P < 0.01$ ).

### Discussion

In the studied flock of 26 ewes the incidence of positive cases with *C. burnetii* infection increased from 22.22% in September 2006 to 57.69% in May 2007, confirming the expansion of the disease, which remained clinically unobvious. The ewes were impregnated between September and October 2006 and were due to give birth in the early spring of 2007. The only exception was a barren ewe which had *C. burnetii* antibodies already present in September 2006. No clinical signs, such as abortions, stillbirths, retained placentas, endometritis or small and weak offspring, were observed, confirming the possibility of an asymptomatic form of Q fever in the affected flock (ANGELAKIS and RAOULT, 2010; GUATTEO et al., 2011; RODOLAKIS 2009; RUIZ-FONS et al., 2010).

Leptin is a hormone mainly produced by white adipose tissue. It plays an important role in the regulation of energy balance, metabolism, thermoregulation and reproduction (CHILLIARD et al., 2005; ZIEBA et al., 2005). Increased leptin levels were detected during infections and inflammations, probably representing a protective component of the host response (LAGO et al., 2008, OTERO et al., 2005). Considering this finding we expected elevated leptin levels in *C. burnetii* positive ewes compared to negative ones. In the investigated ewes, leptin concentrations were in the ranges reported for other sheep breeds (ADAM et al., 2003). Though not significantly, leptin concentrations increased from September to December in both our groups of ewes, which can be explained by the progression of pregnancy (ADAM et al., 2003; CHILLIARD et al., 2005; ZIEBA et al., 2005). Pregnancy can promote appetite and food intake, and therefore it facilitates energy storage in the form of adipose tissue, subsequently leading to gradual elevation of leptin levels (ADAM et al., 2003). The leptin concentrations of *C. burnetii* positive ewes were found to be lower in September and December, but higher in October and November than in negative ewes. The differences were insignificant for all samplings and therefore did not confirm our hypothesis of accelerated leptin excretion during infection with *C. burnetii* (LAGO et al., 2008; OTERO et al., 2005).

The influence of Q fever on the whole-blood GSH-Px activity was also studied. GSH-Px is an integral part of the antioxidant defence system against reactive oxygen species (ROS) in the body. Its activity in blood is regarded as sensitive marker of oxidative stress and an indicator of selenium status of animals, including sheep (PAMUKCU et al., 2000; VALKO et al., 2007). Both increased and decreased levels of antioxidant enzymes have

been reported in different diseases as a consequence of enhanced ROS production, either by up-regulation of enzyme activity or utilization of the antioxidant enzymes to counter the ROS (VALKO et al., 2007). The measured GSH-Px activity in the examined ewes was similar to that reported in the literature (PAMUKCU et al., 2000). Although not significantly, GSH-Px activity was higher in positive than in negative ewes for both samplings, which could be ascribed to enhanced synthesis of this antioxidant enzyme as an in-built compensatory mechanism in *C. burnetii* infected ewes. In both groups of ewes slightly increased GSH-Px activity was observed from September to December, which can be attributed to seasonal fluctuations of this enzyme (HEMINGWAY, 2003; PAMUKCU et al., 2000). To make any final conclusions about the role of GSH-Px activity in the response to Q fever, further studies should be performed.

In ruminants, concentrations of Hp and serum amyloid A are the major acute phase proteins suitable as biomarkers of infectious disease in sheep. Haptoglobin belongs to a group of acute phase proteins, which change their concentrations during infections, inflammation, surgical trauma and stress. (GRUYS et al., 2005; PETERSEN et al., 2004). Haptoglobin has been proven to be a useful marker for the presence of bacterial infections and tissue injury in sheep, rising 100 or even 1000 fold within 24 to 48 hours after stimulation (GRUYS et al., 2005; PETERSEN et al., 2004). Therefore we expected elevated Hp values in *C. burnetii* positive ewes in comparison with negative ewes. The Hp levels in investigated ewes exceeded the upper value of the reference interval reported for healthy sheep (LEPHERD et al., 2009). Contrary to our expectations of noticing highly increased Hp concentrations in positive ewes, elevated Hp levels were also observed in negative ewes, indicating the possible presence of another unidentified agent. Another explanation of elevated Hp values could be pregnancy (GRUYS et al., 2005), as all examined ewes, with the exception of one, were in the 3<sup>rd</sup> to 4<sup>th</sup> month of pregnancy at the time of sampling.

To evaluate any possible influences of Q fever on blood cell characteristics, a comparison was performed of haematological parameters between positive and negative ewes. In humans *C. burnetii* infection is first spread haematogenously. It is then followed by a spread of bacteria to the liver, spleen, bone marrow, reproductive tract and other organs, followed by formation of granulomatous lesions in the liver and bone marrow (ANGELAKIS and RAOULT, 2010; GUATTEO et al., 2011). In animals that are naturally infected with *C. burnetii*, no significant lesions are reported (ANGELAKIS and RAOULT, 2010; GUATTEO et al., 2011).

Haematological parameters (RBC, WBC, platelet count, Hgb, haematocrit, MCV) in the studied ewes were in the range of the reference values for sheep (BICKHARDT et al., 1999; JAIN, 1993; KRAMER, 2000; LEPHERD et al., 2009). However, December levels were higher than September levels in both groups of ewes for RBC, PCV, MCV and Hb, but lower for WBC and platelet count. In *C. burnetii* positive ewes, RBC, WBC, platelet

count and Hb were lower, but PCV and MCV were higher than in negative ewes at both samplings. Significant differences between samplings were established in both groups for PCV and MCV as well as for WBC and platelet counts in negative ewes, and are most probably a result of seasonal or pregnancy influences (BICKHARDT et al., 1999; JAIN, 1993; KRAMER, 2000). Differences between positive and negative ewes were insignificant at all samplings and cannot be attributed to the influences of the infection as expected.

### Conclusions

Differences between the positive and negative *C. burnetii* infected ewes, as well as between samplings in individual groups of ewes, were observed in our study for all evaluated parameters, and may be attributed to various external and internal factors, such as seasonal variations, influences of food quality or pregnancy. On the basis of our findings, we may conclude that the observed variations of investigated biochemical, antioxidant, hormonal and haematological parameters are not a consequence of *C. burnetii* infection. In our opinion, subclinical *C. burnetii* infection is not a factor which should be considered when evaluating values of circulating leptin, GSH-Px activity or haematological parameters in sheep.

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**ČEBULJ-KADUNC, N., B. KRT, A. ŠKIBIN, A. NEMEC-SVETE: Učinci latentne Q-groznice na odabrane hormonske, antioksidacijske, biokemijske i hematološke pokazatelje u ovaca. Vet. arhiv 84, 615-624, 2014.**

**SAŽETAK**

Cilj ovog retrospektivnog istraživanja bio je usporediti različite endokrine, antioksidacijske, hematološke i biokemijske pokazatelje u ovaca pozitivnih i negativnih na Q-groznicu s namjerom da se procijene mogući učinci infekcije na odabrane krvne pokazatelje. U tu svrhu bile su uspoređene koncentracije serumskoga leptina i haptoglobina, aktivnost glutation peroksidaze i hematološki pokazatelji između ovaca pozitivnih na bakteriju *C. burnetii* i onih negativnih na tu bakteriju. Uzorci krvi bili su uzeti u jesenskim i zimskim mjesecima 2006. prije pojave Q-groznice u proljeće 2007. Protutijela specifična za bakteriju *C. burnetii* i koncentracije haptoglobina bile su određivane komercijalnim ELISA kompletima, koncentracije leptina komercijalnim RIA kompletima te aktivnost glutation peroksidaze također komercijalnim reagensima. U 15 od 26 klinički zdravih ovaca dokazana su protutijela za *C. burnetii* u svibnju 2007. Od njih je osam bilo pozitivno već u jesenskim mjesecima. Povećane koncentracije haptoglobina i leptina kao i povećana aktivnost glutation peroksidaze, vrijednost hematokrita, srednja vrijednost zapremine eritrocita bile su dokazane u uzorcima uzetim između rujna i prosinca 2006. u pozitivnih i negativnih ovaca. Broj crvenih krvnih stanica i vrijednost hemoglobina ostali su nepromijenjeni, dok je u istom razdoblju broj bijelih krvnih stanica i broj trombocita bio smanjen u obje skupine ovaca. Većina odabranih krvnih pokazatelja ostala je u granicama referentnih vrijednosti za ovce. Ustanovljene promjene istraživanih pokazatelja najvjerojatnije su rezultat sezonski uvjetovanih kolebanja ili fizioloških promjena vezanih uz bređost i ne mogu se pripisati utjecaju infekcije bakterijom *C. burnetii*. Stoga se supklinički oblik Q-groznice ne može uzeti kao čimbenik utjecaja kod procjene cirkulacijskog leptina, glutation peroksidaze ili hematoloških pokazatelja u ovaca.

**Ključne riječi:** Q-groznica, ovce, leptin, haptoglobin, glutation peroksidaza, haematologija

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