Pakistan J. Zool., vol. 54(6), pp 2623-2627, 2022 DOI: https://dx.doi.org/10.17582/journal.pjz/20210906110923

Effect of Subclinical Coxiellosis (Q fever) on Selected Hematological and Serum Biochemical Variables of Naturally Infected Camels

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Article Information
Received 06 September 2021
Revised 05 October 2021
Accepted 27 October 2021
Available online 18 January 2022 (early access)
Published 16 August 2022

Authors' Contribution
SH and MS conceived the study. SH, MS and KA collected and analyzed the samples. SH and ZS compiled data set for manuscript. SH wrote the manuscript.

Key words
Coxiellosis, Camel, Hemato-biochemical variables

ABSTRACT

Coxiellosis, a widely dispersed zoonotic disease due to *Coxiella burnetii*, serve as a key trade barrier and adversely influence the productive and reproductive potential of animals. The current investigation was designed to determine the selective hemato-biochemical variations in dromedary camels due to subclinical coxiellosis. The study encompassed two groups *viz*, one group of 20 *C. burnetii* positive camel identified by Real time PCR and second group of 20 healthy camels (Real time PCR negative) used as control. The student t-test demonstrated significant (p<0.05) rise in packed cell volume (36.30±0.32%), hemoglobin (15.57±0.32g/dL), neutrophils (71.03±0.58%), erythrocyte sedimentation rate (86.19±0.78mm/hr), creatinine (2.13±0.08mg/dl), urea (69.30±1.14mg/dl), blood urea nitrogen (32.38± 0.62mg/dl), gamma-glutamyl transferase (11.66±0.63µ/L), aspartate aminotransferase (70.63±0.65 µ/L), alkaline phosphatase (94.10±0.89 µ/L) and calcium (9.48±0.39mg/dl). On the other hand, a significant decline (p<0.05) in WBC count (11.94±0.04), eosinophils% (3.10±0.20), basophils% (0.76±0.06), platelets count (103.2±0.5610^{3}µ/l), glucose (55.13±0.84mg/dl), albumin (4.25±0.01g/dl) and sodium (110.26±1.21mEq/L) were observed in infected camels. This investigation demonstrated significant differences in hematological and serum biochemical variables of infected and healthy camels. Moreover, to set a reference line of blood variables for the detection of coxiellosis in camels additional investigations are obligatory.

INTRODUCTION

Coxiella burnetii is a strict intra-cellular highly communicable Gram-negative bacteria that inhibit the immune response of macrophages and monocytes in several wild and domestic animals in addition to humans resulting in a disease known as Q fever (Norlander, 2000). Initially, *C. burnetii* categorized as rickettsial microorganism and dubbed as *Rickettsia burnetii,* but then, many studies clearly described that this species is dissimilar from other Rickettsia fellows and recategorized below the genus of Coxiella, Coxiellaceae family of Legionellales order (Eldin *et al*., 2017). *C. burnetii* have progressive difference in its lipopolysaccharide (LPS) structure: A smooth LPS for the phase I (infectious) and a rough LPS for the phase II (noninfectious) (Shah *et al*., 2015). Domestic animals are believed to be the chief reservoirs for Coxiella among animals as well as humans, and the infection can be transmitted either by inhalation of infected aerosols or by ticks (Angelakis and Raoult, 2010; Guatteo *et al*., 2011). The clinical presentation of Q fever varies per host species. The coxiellosis generally appears as sub-clinical disease in animals, with the exception of ruminants where coxiellosis is responsible for reduced fertility, abortions, and stillbirths during late gestation, as well as low birth weight (Saglam and Sahin, 2016). In human, disease manifestations are characterized by pneumonia, neurological symptoms, cirrhosis along with heart involvement (Angelakis and Raoult, 2010). According to writers perception, the information about hemato-biochemical variables of coxiellosis in camel is so far sparse. The contemporary investigation presented here was performed to assess the paradigm of variations and an association of positivity with hemato-biochemical variables in camels. Aforementioned study will be offer a basic description for additional comprehension about the pathophysiology of the sub-clinical coxiellosis in camels.

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0030-9923/2022/0006-2623 $ 9.00/0

MATERIALS AND METHODS

Ethics statement

Blood specimens were taken from camels as per recommendation of the International Animal Care and Use Committee (IACUC). The blood specimens were processed on the basis of approval from the Director Graduate Studies at the University of Agriculture Faisalabad, Pakistan (vide letter No. DGS/ 2169-72, dated January 23, 2020).

Study area and target animals

A cross-sectional sero-epidemiological survey was carried out in Punjab province (31.1704°N and 72.7097°E) from January to June 2020. Punjab is the second biggest province of Pakistan with area of 205,344 square kilometers (Fig. 1). Most areas in Punjab experience extreme weather with the mean temperature varies among -2 to 45°C (Anonymous, 2018). In this survey, blood specimens were accrued from dromedary camels reared in camel populated districts of Punjab. These districts are located in three zones *viz*, Southern, Northern and central Punjab. These zones have different climatic conditions, ecological and animal management systems. For the random selection of animals Survey Toolbox software (EpiSurvery®) was used. The study encompassed two groups *viz*, one group of 20 healthy camels (Real time PCR negative) used as control while second group of 20 °C. *burnetii* positive camel identified by Real time PCR. Of 20 infected camels, 13 were females and 7 males. The age of animals varies from 2 to 14 years and camels were of 3 different local breeds viz. Marecha (n=7), Berella (n=8) and Nondescript (n=5). All animals fell in different categories of body conditions like moderate, poor and good. History of dromedary camels display decreased milk production, progressive loss of body condition, and work inefficiency. Neither of camels revealed apparent signs of disease (fever, conjunctivitis, arthritis, mastitis, and reproductive disorders), the infection was named as subclinical coxiellosis.

Sampling protocol

From each one camel, 8 ml blood was drawn aseptically from jugular vein in gel and clot activator vacutainers (Xinle®, China) and labelled with animal ID, age, sex and location. Instantly after sample collection, positioned vertically in an ice box filled by gel freezer packs, and shifted to lab for further manipulation.

Molecular diagnosis

The infection was confirmed by Coxiella burnetii real time PCR kit (Liferiver ™ Shanghai ZJ Bio-Tech Co., Ltd.). Detection procedure based on fluorogenic 5, nuclease assay. DNA isolation from blood specimen was carried out by DNA extracting buffer supplied with kit. Then amplifications carried out in 36.4 μL volume at following conditions: 37°C for 2 min; 01 cycle, 94°C for 2 min; 01 cycle, 93°C for 15sec, 60°C for 1minute; 40 cycles.

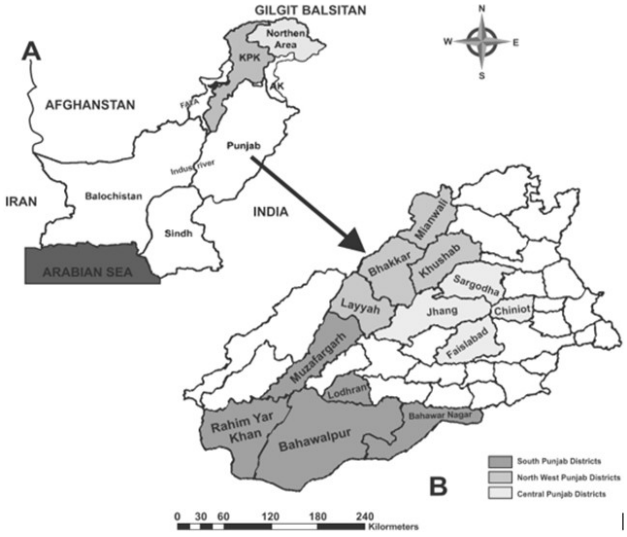

Fig. 1. (A) Map of Pakistan and its contiguous countries, and (B) Map of Punjab, Pakistan showing the districts under study.

Determination of hematological parameters

Determination of hemoglobin (HGB), total leucocytes (TLC), packed cell volume (PCV), Platelets count (PLT) and Red blood cells (RBCs) were carried out by electronic counter (Medonic, Sweden). Red cell indices viz, MCHC (mean corpuscular hemoglobin concentration), MCH (mean corpuscular hemoglobin) and MCV (mean corpuscular volume) were computed by formula described by Coles (Coles, 1986).

Biochemical analysis

Serum biochemistry comprising albumin was determined by bromocresol green method, total protein by Biuret method (Tietz, 1995), blood urea nitrogen and urea level were measured by urease method (Burtis and Ashwood, 1994) and serum creatinine level via Jaffe technique (Swanson *et al*., 1993). The sera were evaluated for "gamma glutamyl transferase" level by kinetic procedure (Tietz, 1995). The aspartate aminotransferase (AST) levels were measured by Kaplan colorimetric method (Kaplan, 1984). Thomas colorimetric method was utilized to determine the alkaline phosphatase (ALP) level in serum (Thomas, 1998). The enzymatic colorimetric method (GPO-PAP) was used to measure triglyceride level and enzymatic colorimetric technique (CHOD-PAP) used to access serum cholesterol level. The level of inorganic

constituents (Ca, Na, K) were measured as previously mentioned by (Hussain *et al*., 2014).

Statistical analysis

A sum of 20 RT PCR positive specimens and 20 control (RT PCR negative) specimens for coxiellosis were taken for hematological and biochemical investigation. Hemato-biochemical variables were denoted as mean ± standard error of mean (SEM). The data collected were statistically assayed by utilizing Student t- test with probability of 5% (p<0.05) by means of SPSS (statistical software version 22.0).

RESULTS

Hematological changes

Coxiella burnetii positive and negative camel blood specimens were subjected to hematological examination to figure out variations in different variables. Coxiella infected camels exhibited a significant decline (p<0.05) in WBC count, basophils, eosinophils, and platelets count. Whereas substantial (p<0.05) rise in PCV, ESR and hemoglobin level were observed. Other hematological variables were within normal range and reveled insignificant (p>0.05) relationship with coxiellosis (Table I).

Table I. Hematological variables (Mean± SEM) of noninfected (Real time PCR negative) and infected (Real time PCR positive) camels in Punjab province, Pakistan.

Parameters (Units)	Non-infected	Infected
PCV(%)	33.18±0.56	36.30±0.32*
Hb Conc. (g/dL)	13.24±0.31	15.57±0.32*
RBCs count (10^6/µL)	9.66±0.36	10.24±0.50
MCV (fL)	34.50±0.71	35.21±0.76
MCH (pg)	14.67±0.20	14.91±0.05
MCHC (gm/dL)	41.60±0.29	41.01±0.35
WBCs count (10^3/µL)	14.16±0.28	11.94±0.04*
Neutrophils (%)	67.06±0.18	71.03±0.58*
Lymphocytes (%)	21.43±0.32	21.56±0.47
Monocytes (%)	3.23±0.09	3.46±0.41
Eosinophils (%)	7.01±0.04	3.10±0.20*
Basophils (%)	1.25±0.08	0.76±0.06*
ESR (mm/hr)	30.01±0.03	86.19±0.78*
Platelets (10^3/µl)	244.6±1.24	103.2±0.56*

*Significant (p<0.05); ESR, erythrocyte sedimentation rate; Hb conc., hemoglobin concentration; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PCV, packed cells volume; RBCs count, red blood cells count; WBCs count, white blood cells count.

Biochemical changes

A significant decrease in albumin (α-globulin, β-globulin, γ-globulin), serum glucose and sodium (Na) level was observed in *C. burnetii* infected camels. Whereas substantial increase (p<0.05) in the average values of urea, BUN, creatinine, ALP, GGT, AST and Ca was noticed in infected animals (Table II).

Table II. Biochemical variables (Mean± SEM) of Non-infected (Real time PCR negative) and infected camels (Real time PCR positive) in Punjab province, Pakistan.

Parameters	Groups	
	Non-infected	Infected
Glucose (mg/dl)	62.30±18.33	55.13±0.84*
Total protein (g/dl)	7.59±1.88	7.71±0.02
Albumin (g/dl)	4.34±0.03	4.25±0.01*
α-globulin (g/dl)	0.72±0.02	0.61±0.02*
β-globulin (g/dl)	0.92±0.01	0.55±0.04*
γ-globulin (g/dl)	1.6±0.17	2.3±0.05*
Total globulin (g/dl)	3.24±0.17	3.45±0.02
A/G ratio	1.34±0.07	1.22±0.00
Creatinine (mg/dl)	1.86±0.01	2.13±0.08*
Urea (mg/dl)	49.45±1.37	69.30±1.14*
BUN (mg/dl)	21.11±0.55	32.38±0.62*
Cholesterol (mmol/L)	12.35±0.22	12.55±0.02
Triglycerides (mmol/L)	12.41±0.53	11.35±0.47
GGT (µ/L)	5.23±0.33	11.66±0.63*
AST (µ/L)	64.48±1.39	70.63±0.65*
ALP (µ/L)	62.82±0.82	94.10±0.89*
Na (mEq/L)	157.93±0.64	110.26±1.21*
K (mEq/L)	4.20±0.41	5.04±0.37
Ca (mg/dl)	8.08±0.28	9.48±0.39*

*Significant (p<0.05). A/G ratio, Albumin to globulin ratio; BUN, Blood urea nitrogen; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ALP, Alkaline Phosphatase; Na, Sodium; K, Potassium, Ca, Calcium.

DISCUSSION

Though Coxiellosis has been recognized by the researcher since 1935, but special consideration was provided to this malady after its massive outbreak during 2007-2010 in Dutch's populace (Hadush *et al*., 2016). Pakistan is based on an agriculture economy in which livestock plays a vital part in livelihood of inhabitants of village areas. About 8 million Pakistani families are connected with animal farming and getting over 35% of their total income from livestock raising business

(Anonymous, 2017). Domestication of camel was critical for pastoral populations in which camels are raised for various purposes comprising milk production, as a meat source, transportation, and conventional medicine (Faye, 2011). Since, due to zoonotic potential of Q fever (coxiellosis), this intimate camel human interaction and absence of consciousness between camel breeders perhaps risk zoonosis and endanger "One Health" approach. Surprisingly, limited case reports are available for alliance of *C. burnetii* with hematological variables that tested in routine in human patients infected with Q fever, however the pathogen detected in animals since 1930s. The disparities in different variables may act as markers to diagnose and to monitor the intensity of disease. During this survey, significant ($p<0.05$) rise in Hb, ESR and PCV was noticed. The reason behind the increase in ESR is that, different inflammatory conditions like, cancer, infection and auto immune diseases leads to an increase in plasma protein level in blood which result in clumping of RBCs and subsequently they settle rapidly. The increase in Hb and PCV occur due to relative change to blood water and absolute increase in RBCs mass (Angelakis and Raoult 2010). These results are in close agreement with those of Cebulj-Kadunc *et al*. (2014). Moreover, a significant decline in platelets count was recorded after mechanism of this decline is that Q fever which may be the result in transient bacteremia which evoke thrombocytopenia by various factors which includes, endothelial damage, disseminated intravascular coagulation and decrease platelet production from bone marrow (Maurin and Raoult 1999). These results closely match with previously reported (Fournier *et al*., 1998). Investigation of hematological variables revealed that RBCs indices does not show significant alterations in values of positive camels compared to negatives; while, reference intervals of WBCs disclosed a significant decline in total WBCs (leucopenia) due to lymphocyte decrease (lymphocytopenia) and substantial rise in granulocyte due to neutrophil increase. This observation is congruent with previous results of Hasanain *et al*. (2020). This result is not corelated with findings of Cebulj-Kadunc *et al*. (2014) and Fournier *et al*. (1998). Sub populations of WBC plays a vital role in body against various invading pathogens and assessment of their levels can be helpful in diagnosis, monitoring of disease and therapeutic actions (Kraft and Durr, 2013). The underlying mechanism of leucopenia could be due to autoimmune disorders, viral infections and bacterial septicemia (Grunder, 2006; Kocaturk *et al*., 2010). In current study, significant ($p<0.05$) rise in BUN, creatinine and urea was observed. In Q fever frequent renal involvement correspond to immune complex glomerulonephritis, which may result in renal insufficiency. Due to impaired glomerular filtration rate urea, BUN and creatinine level is increased (Maurin and Raoult, 1999). These findings are congruent with prior investigation by Abdulrahman, (2014), and Hussein *et al*. (2012) and are in contrast with those of Cebulj-Kadunc *et al*. (2014). In *C. burnetii* infected camels level of GGT, AST and ALP was increased significantly and this closely match with results of previous reports by (Maurin and Raoult, 1999; Fournier *et al*., 1998). This rise in level of hepatic enzymes may be due to hyperplasia of Kupffer cell. Kupffer cells are believed to be the target tissues in liver by *C. burnetii*. This may induce local inflammation and consequently result in fatty changes in liver and hepatic degeneration (Maurin and Raoult, 1999). Level of sodium and serum glucose was substantially ($p<0.05$) reduce in *C. burnetii* positive camels in comparison to healthy camels. During fever aldosterone level is increased which enhance sodium resorption and promotes potassium excretion by its effect on aldosterone receptors in the distal tubules of the kidney (Angelakis and Raoult, 2010). As regard serum glucose is concerned, due to non-availability of any investigation about C. *burnetii* impact on serum glucose level, it is premature to describe pathophysiology of this change. These results are in line with those previously reported by Abdulrahman (2014) and Cebulj-Kadunc *et al*. (2014).

CONCLUSIONS

Results of presented study indicates that sub clinical coxiellosis in camels result in variations in different hemato-biochemical parameters of infected camels. This need additional investigation to describe the worth of these selected variables in coxiellosis. Subclinical coxiellosis badly affects health status of camels that impose an economic load on pastoral clans in form of production losses plus work inefficiency. The findings of hemato-biochemical variables of camel coxiellosis might act as baseline for upcoming investigations in dromedaries in Pakistan and globally.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the camel owners and colleagues for their assistance in the accomplishment of this study.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abdulrahman, A.J., 2014. *Studies on Q fever in farm animals in Kingdom of Saudi Arabia*. M.Sc. thesis,

Sudan University of Science and Technology.

Angelakis, E., and Raoult, D., 2010. Q fever. *Vet. Microbiol*., **140**: 297–309. https://doi.org/10.1016/j.vetmic.2009.07.016

Anonymous, 2017. *Economic survey of Pakistan*. Economic Advisors Wing, Finance Division, Government of Pakistan, Islamabad, Pakistan.

Anonymous, 2018. *Livestock census Punjab*. Livestock and Dairy Development Department. pp. 16-19.

Burtis, C.A. and Ashwood, E.R., 1994. *Tietz textbook of clinical chemistry*, 2nd ed. Saunders, Philadelphia.

Cebulj-Kadunc, N., Krt, B., Skibin, A. and Nemec-Svete, A., 2014. Effects of latent Q fever infection on selected hormonal, antioxidant, biochemical and hematological parameters in sheep. *Vet. Arhiv*., **84**: 615-624.

Coles, E.H., 1986. *Veterinary clinical pathology,* 4th ed. WB. Saunders Co., Philadelphia, Toronto, London.

Eldin, C., Melenotte, C., Mediannikov, O., Ghigo, E., Million, M., Edouard, S. and Raoult, D., 2017. From Q fever to *Coxiella burnetii* infection: A paradigm change. *Clin. Microbiol. Rev.*, **30**: 115-190. https://doi.org/10.1128/CMR.00045-16

Faye, B., 2011. Combating desertification: The added value of the camel farming. *Annls Arid Zone*, **50**: 1–11.

Fournier, P.E., Marrie, T.J. and Raoult, D., 1998. Diagnosis of Q fever. *J. clin. Microbiol*., **36**: 1823-1834. https://doi.org/10.1128/JCM.36.7.1823-1834.1998

Grunder, H.D., 2006. Diseases of the circulatory organs and of the blood. *Internal Med. Surg. Cattle* 5th ed. Parey, Berlin, Germany, **1**: 159–270.

Guatteo, R., Seegers, H., Taurel, A.F., Joly, A. and Beaudeau, F., 2011. Prevalence of *Coxiella burnetii* infection in domestic ruminants: A critical review. *Vet. Microbiol*., **149**: 1–16. https://doi.org/10.1016/j.vetmic.2010.10.007

Hadush, A., Kandi, V. and Pal, M., 2016. Epidemiology and public health implications of Q fever. *Perspect. Med. Res*., **4**: 42-46.

Hasanain, A., Gharban, J. and Yousif, A., 2020. Serological, clinical and hematological prevalence of *coxiella burnetii* in adult cows, *Iraq Biochem. Cell. Arch*., **20**: 67-74.

Hussain, R., A. Khan, A., Mahmood, F., Rehan, S. and Ali. F., 2014. Clinico hematological and tissue changes induced by butachlor in male Japanese quail (*Coturnix japonica*). *Pestic. Biochem. Physiol*., **109**: 58-63. https://doi.org/10.1016/j.pestbp.2014.01.005

Hussein, M. F., Al-Khalifa, I. M., Aljumaah, R.S., Gar Elnabi, A., Mohammed, O., Omer, S. A. and Macasero, W.V., 2012. Serological prevalence of *C. burnetii* in captive wild ruminants in Saudi Arabia. *Comp. clin. Pathol*., **21**: 33-38. https://doi.org/10.1007/s00580-010-1061-y

Kaplan, G.S.A., 1984. *Clinical chemistry*. The C.V Mosby Co. St. Louis, Toronto, Princeton.

Kocaturk, M., Yeþilbao, K. and Yilmaz, Z., 2010. Evaluation of red blood cell and platelet indices in cattle naturally infected with bovine viral diarrhea virus (BVDV). Uludao Universitesi *Vet. Fakul. Derg*., **29**: 17-22.

Kraft, W. and Durr, U.M., 2013. *Clinical laboratory diagnostics in veterinary medicine.* 7th ed. Schattauer, Stuttgart, Germany, **1**: 79-156.

Maurin, M. and Raoult. D., 1999. Q fever. *Clin. Microbiol. Rev*., **12**: 518–553. https://doi.org/10.1128/CMR.12.4.518

Norlander, L., 2000. Q fever epidemiology and pathogenesis. *Microb. Infect.,* **2**: 417-424. https://doi.org/10.1016/S1286-4579(00)00325-7

Saglam, A.G. and Sahin, M., 2016. *Coxiella burnetii* in samples from cattle herds and sheep flocks in the Kars region of Turkey. *Vet. Med*., **61**: 17-22. https://doi.org/10.17221/8678-VETMED

Shah, S.Y., Kovacs, C., Tan, C.D., Pettersson, G., Shrestha, N.K., Lutwick, L. and Gordon, S.M., 2015. Delayed diagnosis of Q fever endocarditis in a rheumatoid arthritis patient. *ID Cases*, **2**: 94-96. https://doi.org/10.1016/j.idcr.2015.09.002

Swanson, A.F., Swartzentruber, M. and Nolen, P.A., 1993. *Multicenter evaluation of the boehringer Mannheim compensated, rate-blanked creatinine/ jaffe application on BM/Hitachi systems*. Advances in Clinic. Diagnostic. Boehringer Mannheim Corp.

Thomas, L., 1998. *Clinical laboratory diagnostics*. 1st ed. T.H. Books verlagsgesellschaft, Frankfurt. pp. 36-46.

Tietz, N.W., 1995. *Clinical guide to laboratory tests*. 3rd ed. W.B. Saunders Co., Philadelphia.