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RESEARCH ARTICLE

Hematological and Serum Biochemical Changes in Sheep Naturally Infected with *Toxoplasma gondii* in Southern Punjab (Pakistan)

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ABSTRACT

Toxoplasma (T.) gondii is a blood parasite which causes variations in hematological and serological values which can lead to economic losses in animals like reduced fur, wool, meat and milk productions. This study was conducted to determine the hematological and serological changes in sheep naturally infected with *T. gondii* present in districts Multan and Khanewal in southern Punjab, Pakistan. Blood samples were collected from 500 sheep from April 2012 to June 2013 and the parasitological diagnosis was confirmed by using ELISA. Different blood parameters [haemoglobin (Hb), glucose, aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH) and cholesterol] were measured and compared between *T. gondii* positive and negative sheep. Results showed that Hb, glucose and ALT concentrations were lower in *T. gondii* positive sheep as compared to *T. gondii* negative sheep. On the other hand, cholesterol, AST and LDH concentrations were higher in *T. gondii* positive sheep than *T. gondii* negative sheep. Hb, glucose, AST, ALT and LDH values varied non-significantly ($P > 0.05$) between *T. gondii* positive and negative sheep; whereas, difference in cholesterol level was statistically significant ($P < 0.05$). In conclusion, cholesterol level was found higher in sheep infected with toxoplasmosis which may lead to certain complications including coronary artery diseases, myocardial infarction, atherosclerosis, hypertension, hemorrhage and vascular thrombosis in infected sheep.

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INTRODUCTION

Sheep and goats play a very important role in agricultural systems of the developing countries especially by providing valuable animal products such as meat, milk, wool and skins (Devendra, 2005; Ofukwu and Akwuobu, 2010). Small ruminants have natural ability to change the grasses, shrubs, etc. into high quality food such as meat and milk (Afzal and Naqvi, 2004). Livestock farming is a more safe and secure source of earning and employment generation in rural areas for the small and landless farmers (Ugwu, 2007). Sheep are famous for their mutton quality and especially male animals are reared for sales on special occasions as a sacrificial animal (Qureshi et al., 2010). Parasitism is not only a burden on sheep and goats farmers countrywide but also play a vital role in the reduction of per capita mutton and/or milk yield in Pakistan (Jamil et al., 2015) It is also one of the main threats and primary hurdles to the productivity of small

ruminant (Asif et al., 2008). The infection may be responsible for morbidity and mortality in small ruminants which have negative impact on farm output and livestock productivity (Skykes, 1994).

Toxoplasmosis is a common zoonotic disease caused by *Toxoplasma (T.) gondii* which is an obligate intracellular protozoan parasite and source of infection to humans, birds and mammals (Bartova et al., 2009; Abu-Dalbou et al., 2010). Cats are the final hosts of *T. gondii* and excrete infective oocysts with feces to the environment (Dubey, 2010). *T. gondii* is the single species of genus *Toxoplasma* (Dubey, 2008) but it contains three strains, which are classified into I, II and III genetic types. Genetic type I is more virulent in mice, II in sheep and goats and may also causes clinical toxoplasmosis in humans; whereas, type III is avirulent strain (Sibley, 2003).

Small ruminants may get infection after ingestion of forage or feedstuffs polluted with sporulated oocysts or congenitally through placenta (Innes et al., 2009).

Toxoplasmosis causes heavy economic losses to sheep industry throughout the world (Buxton et al., 2007) by inducing abortions, still birth, early embryonic death, fetal resorption or birth of weak kids or lambs and by reducing fertility (Garcia et al., 2012; Edward and Dubey, 2013). Sheep, goats, pigs and humans may be more vulnerable to the *T. gondii* infection than cattle and horses. Cattle show natural resistance to the parasite (Khalil and Elrayah, 2011). Humans may get infection by consumption of contaminated meat. However, in humans it may cause a febrile illness related with swollen lymph nodes, while during pregnancy it may cause fetal death, abortions or fetal abnormalities. Infection during pregnancy with oocysts or tissue cysts may result in transmission of tachyzoites to fetus through placenta and cause blindness and mental abnormalities in congenitally infected fetus. In AIDS patients' toxoplasmic encephalitis, pulmonitis are major clinical signs (Jithendran, 2004; Elmore et al., 2010).

Blood is a most important and reliable medium to determine the health condition of animals (Ramprabhu et al., 2010). Variations in blood parameters may be due to differences in age, sex, breed of animals, altitude, feeding status, temperature, seasonal variations and physiological changes in animals (Mbassa and Poulsen, 2003). The hematological and biochemical values are very important for evaluation of normal physiological status, management practice, nutritional and pathological condition of animals (Opara et al., 2010; Maina and Gathumbi, 2015). Hematological and serum biochemical tests are extensively used to diagnose serious diseases which may cause economic losses in animals due to low production of wool, fur, meat and milk. There are relatively few reports on the prevalence of toxoplasmosis in small ruminants in Southern Punjab, Pakistan (Lashari and Tasawar, 2010; Tasawar et al., 2011). Keeping in view the importance of this parasite, present study was conducted to determine its effects on hematological and serological parameters in infected sheep.

MATERIALS AND METHODS

Five hundred blood samples were collected from sheep population (n=500; Lohi breed) of districts Multan and Khanewal through random sampling. Briefly, the 10 ml blood sample was taken from each sheep with the help of disposable syringes by puncturing the jugular vein. Out of this, 3 ml blood was poured in the plastic tubes containing 100 µL of 0.5 M EDTA as anticoagulant for hematological studies and the remaining 7 ml of blood was poured slowly in anticoagulant free plastic tubes to collect the sera samples. Information related to hosts and herds were recorded on a specially designed questionnaire. The blood samples were taken to

Parasitology Research Laboratory at Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan for further analysis. Antibodies of *T. gondii* in the sera samples were detected with the help of ELISA (ID. Vet, France) and biochemical parameters were analyzed by using commercially available diagnostic kits (RANDOX Diagnostics®, UK). Glucose concentration in blood was determined by using ON-CALL PLUS Active blood glucometer (Germany); whereas, concentrations of haemoglobin (Hb), AST, ALT, LDH and cholesterol were measured by using CHEM-100 Biochemistry Analyzer (Italy) and RANDOX diagnostic kits UK (Catalogue numbers:HG-1539, AS-1267, Al-146, LD-487 and CH-201, respectively) following the manufacturer's instructions.

Statistical analysis

The collected data/information was analyzed by using SPSS version 23.0. Mean±Standard deviation was calculated for quantitative variables such as Hb Level, Glucose, Cholesterol, AST, ALT and LDH Level. Student's t-test was applied for comparison of quantitative variables in both *T. gondii* positive and negative groups. The variables were considered statistically significant at P<0.05.

RESULTS AND DISCUSSION

In the present study, hematological and serum biochemical parameters were studied between *T. gondii* positive and negative sheep (Table 1). The Hb, glucose and ALT concentrations was lower in *T. gondii* positive sheep as compared to *T. gondii* negative sheep. On the other hand, cholesterol, AST and LDH concentrations were higher in *T. gondii* positive sheep than negative ones. The differences between Hb, glucose, AST, ALT and LDH values were statistically non-significant (P>0.05) between *T. gondii* positive and negative sheep; whereas, the difference in cholesterol level was statistically significant (P<0.05). The Hb is an iron containing protein present in erythrocytes and plays an important role in transporting respiratory gases (O₂ and CO₂) from lungs or gills to the various tissues present in different body parts of the vertebrate. At tissues level this O₂ carry on the process of aerobic respiration and release energy in the form of ATP to power the functions of various organs. Blood Hb level was lower in *T. gondii* positive sheep (9.5±2.5 g/dL) as compared to *T. gondii* negative sheep (9.9±3.1g/dL). Devendran et al. (2008) and Jawasreh et al. (2010) also reported lower Hb values in Coimbatore and Awassi sheep breeds, respectively in their genetic studies. Health status, stress, sex, age, geographical changes and co-parasitic infection may also be responsible for low Hb level in *T. gondii* positive sheep (Ramprabhu et al., 2010). Glucose is the most important source of energy for cellular respiration. It may store in liver and muscle

Table 1: Comparison of hematological and serum biochemical parameters in *T. gondii* positive and negative sheep

Parameters	<i>T. gondii</i> positive sheep (n= 137)	<i>T. gondii</i> negative sheep (n= 363)	t-cal	P-value	95 % (CI)
	Mean ± SD	Mean ± SD			
Haemoglobin (g/dL)	9.5±2.5	9.9±3.1	-1.430	0.153	-1.011, 0.159
Glucose (mg/dL)	50.6±11.3	52.1±11.2	-1.290	0.198	-3.691, 0.766
Cholesterol (mg/dL)	214.5±79.9	198.4±73.8	2.136	0.033*	1.294, 30.938
AST (U/L)	217.3±140.7	209.6±605.1	0.148	0.883	-94.286, 109.61
ALT (U/L)	77.8±66.4	97.62±219.8	-1.042	0.298	-57.069, 17.511
LDH (U/L)	606.8±291.5	583.8±295.7	0.784	0.433	-34.716, 80.845

*P-Value <0.05 (Statistically Significant).

cells as glycogen. Blood glucose concentration was lower in *T. gondii* positive sheep (50.6±11.3 mg/dL) as compared to negative sheep (52.1±11.2 mg/dL). Variations in nutrition and metabolic activities may be related with glucose changes in animal (Jawasreh et al., 2010). Reduction in blood glucose level may be due to liver damage or more consumption of glucose by parasites in infected animals (Zulfiqar et al., 2012). However, high metabolic activity due to pyrexia or liver damage may also be a reason for reduction in glucose concentration (Sivajothi et al., 2015). The differences in hematological parameters may be due to the different phase of disease, presence of secondary infection, nutrition and dehydration degree (Heaney et al., 2002; Kataria et al., 2007). Lipids play a vital structural and functional role in our body. Cholesterol act as a raw materials in the biosynthesis of steroids hormones, bile pigments and vitamin D and help in digestion of food, provide energy, act as storage and metabolic fuels of the body, used as components of biomembranes and form insulating layer around neuron cells to increase the speed of nerve impulse conduction and below skin prevent from heat loss. Lipid and lipoproteins disorders in the body can be diagnosed by measuring cholesterol level. Significant variation in their plasma levels may indicate to a variety of clinical disorders (Sitmo, 2014). Cholesterol concentration was higher in *T. gondii* positive sheep (214.5±79.9 mg/dL) than *T. gondii* negative sheep (198.4±73.8 mg/dL). Similarly higher cholesterol values were determined by Devendran et al. (2008) in Coimbatore sheep and Jawasreh et al. (2010) in Awassi sheep. An elevated level of cholesterol concentration has been implicated as one of the several risk factors in coronary artery disease (Njidda et al., 2013). AST found abundantly in heart and liver tissues and plays a significant role in the metabolism of amino acid (Vojta et al., 2011). AST level was higher in *T. gondii* positive sheep (217.3±140.7 U/L) as compared to *T. gondii* negative sheep (209.6±605.1 U/L). Variable AST values indicated that this enzyme level may vary with strains and species of small ruminants. AST found in each tissue of the body, including erythrocytes and highly concentrated in liver and heart muscle, kidney and skeletal muscle have intermediate

value of AST; whereas, other tissues have lower concentration. Measurement of AST values is helpful to diagnose the diseases related to heart attack, liver damage and skeletal muscle disorders due to trauma, kidney failure or various hemolytic conditions (Alex and Laverne, 1983). The elevated values of AST may be due to inflammation or necrosis in the host tissues, particularly in muscle, kidney, liver and heart (Sivajothi et al., 2015). Enzymes are protein catalysts which speed up the rate of reaction in all living organisms while they are regularly and quickly degraded but their supply can be renewed by new synthesis (Coles, 1986). ALT enzyme found in the high concentration in liver and normally used to identify liver injury (Pratt, 2010). ALT level was slightly higher in *T. gondii* negative sheep (97.62±219.8 U/L) than positive sheep (77.8±66.4 U/L). Lower ALT values were observed by Soch et al. (2011) in sheep (10.0±1.1 U/L) present in West African and Mostaghni et al. (2005) in wild sheep (29.15±3.20 U/L). Lack of ALT changes may suggest that this infection was well managed by the liver (Miranda et al., 2015). LDH enzyme causes the conversion of lactate into pyruvic acid which is a vital step in energy production process of a cell and basically present in all tissues involved in the process of glycolysis. Any damage to these tissues causes more production of LDH which leak out into body fluids and interstitial fluids. Hemorrhage, necrosis and vascular thrombosis, especially in the tissues of liver and kidney of infected animals may increase the activity of LDH (Nazifi et al., 2008). LDH level was higher in *T. gondii* positive sheep (606.8±291.5 U/L) than negative sheep (583.8±295.7 U/L). Similar values were reported by Mostaghni et al. (2005) in wild sheep. High concentration of LDH detected in the blood stream of damaged tissues may be a definitive diagnostic and prognostic measure for the confirmation of various disorders and diseases. Similarly elevated level of LDH may be associated with liver abnormalities or dysfunctions (Talkhan et al., 2010; Zufiqar et al., 2012). It is concluded that cholesterol level was found to be higher in sheep infected with toxoplasmosis which may be the cause of coronary artery diseases, myocardial infarction, atherosclerosis, hypertension, hemorrhage and vascular thrombosis.

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Authors' contributions

Sampling of blood, biochemical testing and statistical analysis were performed by MH as a part of his PhD thesis research and all the stages of research and manuscripts preparation were supervised by ZT.

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