Correlation between some hematological parameters, acute phase proteins and serum immunoglobulins in experimental caprine besnoitiosis

A. Hosseini · F. Namazi · A. Oryan ·
S. Nazifi

Received: 31 December 2011 / Accepted: 21 April 2013 / Published online: 15 May 2013
© Indian Society for Parasitology 2013

Abstract The present study was undertaken to investigate correlation between some hematological parameters, acute phase proteins and immunoglobulins in the experimentally infected goats with *Besnoitia caprae* from the time of infection till 360 days post infection (DPI). Six male goats, approximately 12–16 months old, were inoculated subcutaneously with approximately 1.3×10^8 bradyzoites of *B. caprae* and blood samples were collected at weekly intervals from the jugular vein of the goats. Total leukocyte count and differential leukocyte counts were determined. Acute phase proteins (APPs) including serum amyloid A (SAA), haptoglobin (Hp), fibrinogen and ceruloplasmin were undertaken at weekly intervals. We evaluated an enzyme-linked immunosorbent assay (ELISA) (using a somatic antigen of bradyzoite) to detect anti-*B. caprae* antibodies in caprine sera. Cysts were present in the skin biopsies of the distal parts of the leg of the infected goats from 28 DPI. From 30 to 360 DPI, results showed that the APPs concentrations including SAA, Hp, fibrinogen and ceruloplasmin were enhanced in the serum of infected goats. However, there were some variation in hematological parameters; the differences were not significant with those of the normal values. Some variations were seen in the levels of specific antibodies against this parasite and they had correlation with some hematological parameters and acute-phase proteins.

Keywords *Besnoitia caprae* · Acute phase proteins · Immunoglobulin · Hematological parameters

Introduction

Besnoitiosis is an economically important parasitic disease (Heerden et al. 1993) in a wide range of domestic and wild animals that is previously identified in nine species of animals including cattle, goats, sheep, equids, reindeer, caribou, opossums, rabbits, rodents, and lizards (Leighton and Gajadhar 2001; Dubey et al. 2003). *Besnoitia* infections in goats have been reported in wild and domestic goats in Iran (Cheema and Toofanian 1979; Oryan and Sadeghi 1997) and Kenya (Bwangamoi et al. 1989; Bwangamoi and Njenga 1993). Thickening, alopecia and hyperkeratosis in the skin of the infected goat occurs due to localization of the *Besnoitia* cysts in the dermis and subcutaneous tissues. Localization of the *Besnoitia* cysts in these areas results in tissue necrosis and infiltration of mononuclear and eosinophils in the affected area (Oryan and Sadeghi 1997; Njagi et al. 1998; Oryan et al. 2008b). Substitution of the cysts in the parenchyma of the testis and epididymis and its adverse effect on spermatogenic activity and goat production in male animals is previously reported (Kafi et al. 2007; Oryan et al. 2008a). Its extensive skin damage criteria together with condemnation of infected carcass organs and the parasite's adverse effects on growth and weight gain in association with reduction in male fertility makes this disease of great economic concern to the mutton and leather industries as well as animal breeders (Bwangamoi and Njenga 1993; Oryan and Sadeghi 1997; Ellis et al. 2000; Cortes et al. 2005; Oryan et al. 2008a). The life cycle of *Besnoitia caprae* is not yet completely known and the intermediate host range and the definitive

A. Hosseini · F. Namazi · A. Oryan
Department of Pathobiology, School of Veterinary Medicine,
Shiraz University, PO Box 1731, 71345 Shiraz, Iran

S. Nazifi (✉)
Department of Clinical Studies, School of Veterinary Medicine,
Shiraz University, PO Box 1731, 71345 Shiraz, Iran
e-mail: nazifi@shirazu.ac.ir

host(s) are not known. There is also a controversy between the investigators in the main natural route(s) of transmission of this protozoon from definitive host(s) to the intermediates (Oryan and Sadeghi 1997; Njenga et al. 1999).

Although the changes in the biochemical and hematological parameters in the naturally infected domestic goats by *B. caprae* were studied previously (Nazifi et al. 2002; Oryan et al. 2008b) but no report is yet available on investigation of correlation between hematological parameters, acute phase proteins and immunoglobulins in the infected goats with *B. caprae*. Therefore, this experiment was conducted to investigate correlation between some hematological parameters, acute phase proteins and immunoglobulins in the experimentally infected goats with *B. caprae* from the time of infection till 360 DPI.

Materials and methods

Isolation of *Besnoitia caprae*

Goats with chronic besnoitiosis were identified by observing *Besnoitia* cysts in their conjunctiva and subsequent confirmation of caprine besnoitiosis on the histopathological sections of the skin covering tarsal or carpal area (Bigalke and Naude 1962; Oryan and Sadeghi 1997). The naturally infected goat, exhibiting clinical features of caprine besnoitiosis was culled and then painlessly euthanized according to the Iranian Veterinary Organization rules and the skin and subcutaneous tissues from this animal was collected and stored at 4 °C. To remove surface contaminations, tissue pieces were separately washed in a petri dish containing Dulbecco's modified eagle medium (DMEM) supplemented with 2 % fetal calf serum (FCS), 1 % antibiotic solution (10,000 IU penicillin and 10,000 mg streptomycin/ml solution) and 1 % Amphotericin B (250 mg/ml). Subsequently, the tissue sections were cut into 1–2 mm³ pieces with scalpel. The DMEM with liberated bradyzoites was collected and centrifuged at 770×g for 15 min at 4 °C. The pellets were resuspended in DMEM and the *B. caprae* bradyzoites were counted using a Neubauer chamber. Cryopreservation of bradyzoites was conducted by resuspending the bradyzoites in FCS containing 10 % DMSO, and freezing and storage in liquid nitrogen.

Infection of goats

Six male goats approximately 12–16 months old, from a non-infected area were carefully examined to be free of *Besnoitia* cysts in the scleral conjunctiva and in the biopsy from the carpal skin under local anesthesia. No *Besnoitia* cysts were reported from at least 120 km surrounding the

area that the male goats were collected. Each goat was inoculated subcutaneously (SC) with approximately 1.3×10^8 bradyzoites of *B. caprae*. The goats were located in separate boxes, each one in a different room and the animals were observed daily for development of clinical besnoitiosis and skin biopsy from the distal parts of the limbs were collected at weekly intervals and histologically examined for *Besnoitia* cysts. Five healthy goats were bought from the herd and were used as control.

Hematological and biochemical analyses

Blood samples were collected at weekly intervals from the jugular vein of the goats. For hematological parameters, blood samples were collected in vacutainers containing EDTA as an anticoagulant. For the serum biochemical analysis, blood samples were collected into vacutainers, and serum was separated by centrifugation at 750×g for 15 min and stored in a freezer of –20 °C until use. Total leukocyte count was determined using standard routine techniques. For differential leukocyte counts, the blood smears were prepared and stained with giemsa stain (Jain, 1986). Hp was measured with a commercial colorimetric kit and SAA by a solid phase sandwich ELISA (Tridelta Development Plc, Wicklow, Ireland). The analytical sensitivities of these tests in serum have been determined as 0.3 µg/ml for SAA and 0.0156 g/l for Hp by the manufacturer. Ceruloplasmin was measured using Sunderman and Nomoto (1970) method and fibrinogen by precipitation-refractory method as described by Thrall et al. (2004).

Immunoglobulin assay with ELISA

The *Besnoitia*-ELISA was carried out by the following steps: frozen *B. caprae* bradyzoite pellets were resuspended in PBS 1×. Subsequent treatment by five freezing-thawing cycles (–70 ± 37 °C) and final processing by ultrasonication (3 × 20 s at 65 W) at 1 °C yielded a soluble *B. caprae*-extract (Somatic antigen). The extract was sedimented at 10000×g at 4 °C for 10 min. The soluble supernatant was used as somatic ELISA antigen. Coating High-binding plates (Greiner Bio One, Germany) was done at a concentration of 1 and 1.5 µg protein/ml PBS 1× (for IgG and IgM respectively) at room temperature overnight. All protein concentrations were assessed by the procedure of Lowry et al. (1951) using bovine albumin as the standard. The washing and blocking solution was PBS containing 0.05 % Tween 20 (PBS-Tween) and PBS-BSA 3 %. Serum incubations were for 60 min at 37 °C. The second antibodies were horseradish peroxidase-conjugated rabbit anti-goat antibodies (Sigma) diluted at 1:10,000 in PBS-BSA 3 %. The conjugate was incubated for 45 min at 37 °C. The substrate used was H₂O₂ and absorbance values

were determined at $A_{405\text{ nm}}$ using ELISA reader (ImmunoScan plast, BDSL, USA).

Statistical analysis

The data are presented as mean $\pm$ standard deviation (SD) and the level of significance was set at $p < 0.05$. The data were analyzed statistically using statistical analysis system (SAS) software. The correlation between hematological parameters and APPs was analyzed by correlation test.

Results

Cysts were present in the skin biopsies of the distal parts of the leg of the infected goats from 28 DPI. The mean $\pm$ SD of hematological and APPs values from different samples of each goat at weakly intervals are presented in Table 1. However, there were some variation in hematological parameters; the differences were not significant with those of the normal values. From 30 to 360 DPI, results showed that the APPs concentrations including SAA, Hp, fibrinogen and ceruloplasmin were enhanced in the serum of infected goats.

Some variations were seen in the levels of specific antibodies against this parasite. There were similar changes in process of these antibodies and some hematological parameters and acute-phase proteins values of infected goats (Figs. 1, 2, 3, 4) and they had correlation with each other. IgG values had correlation with white blood cells ($r = 1$; $p < 0.01$), lymphocytes ($r = -1$; $p < 0.01$) and segmented ($r = 1$; $p < 0.01$) on 15 DPI, with white blood cells ($r = 0.966$; $p < 0.05$) on 30 DPI, with white blood cells ($r = 0.669$; $p < 0.05$), lymphocytes ($r = 0.787$; $p < 0.05$) and segmented ($r = -0.639$; $p < 0.05$) on 60 DPI and with lymphocytes ($r = -0.438$; $p < 0.05$) and

segmented ($r = 0.435$; $p < 0.05$) on 180 DPI. IgM values had correlation with band cells on 15 ($r = -1$; $p < 0.01$) and 120 ($r = -0.522$; $p < 0.05$) DPI.

IgG values had correlation with serum amyloid A ($r = 0.498$; $p < 0.01$), haptoglobin ($r = 0.403$; $p < 0.05$), fibrinogen ($r = 0.515$; $p < 0.01$) and ceruloplasmin ($r = 0.445$; $p < 0.01$) on 360 DPI and with serum amyloid A and fibrinogen ($r = 1$; $p < 0.01$) on 15 DPI. IgM values had correlation with serum amyloid A and haptoglobin ($r = 0.367$; $p < 0.05$) and fibrinogen ($r = 0.353$; $p < 0.05$) on 360 DPI, serum amyloid A ($r = 0.515$; $p < 0.05$), haptoglobin ($r = 0.505$; $p < 0.05$), fibrinogen ($r = 0.520$; $p < 0.05$) and ceruloplasmin ($r = 0.507$; $p < 0.05$) on 120 DPI, serum amyloid A ($r = 0.752$; $p < 0.05$), haptoglobin ($r = 0.733$; $p < 0.05$) and ceruloplasmin ($r = 0.679$; $p < 0.05$) on 60 DPI and with serum amyloid A and fibrinogen ($r = 1$; $p < 0.01$) on 15 DPI.

Discussion

Despite presence of variation in hematological values, there was no significant difference between hematological parameters in experimentally infected goats. Nazifi et al. (2002) studied the changes of hematological parameters of the naturally infected domestic goats with *B. caprae*. They observed macrocytic-normochromic anemia, leukocytosis, neutrophilia, lymphopenia, basophilia, eosinophilia and monocytosis in naturally infected goats. Naturally infected animals probably had mixed infections with other protozoa that might affected the hematological parameters.

The serum Hp, SAA, fibrinogen and ceruloplasmin concentrations of the infected animals were significantly higher than those of the normal animals and they enhanced by increasing of post-infection days. The acute phase proteins are a group of blood proteins that their

Table 1 Mean $\pm$ SD of hematological and acute phase proteins values on different days post infection in experimentally infected goats

Parameters	Days post-infection					
	Control	30	60	120	180	360
WBC ($\times 10^9/l$)	9.72 $\pm$ 2.94	7.71 $\pm$ 1.72	12.74 $\pm$ 1.88	8.69 $\pm$ 0.85	9.04 $\pm$ 3.76	9.37 $\pm$ 4.98
Segmented ($\times 10^9/l$)	3.24 $\pm$ 1.29	4.77 $\pm$ 1.64	7.47 $\pm$ 0.84	4.79 $\pm$ 0.61	4.89 $\pm$ 0.43	5.57 $\pm$ 2.62
Lymphocyte ($\times 10^9/l$)	5.31 $\pm$ 1.16	2.61 $\pm$ 0.99	4.69 $\pm$ 0.73	3.32 $\pm$ 0.25	3.88 $\pm$ 0.92	3.38 $\pm$ 1.53
Monocyte ($\times 10^9/l$)	0.63 $\pm$ 0.48	0.19 $\pm$ 0.08	0.24 $\pm$ 0.01	0.11 $\pm$ 0.03	0.11 $\pm$ 0.04	0.11 $\pm$ 0.05
Eosinophil ($\times 10^9/l$)	0.43 $\pm$ 0.28	0.00 $\pm$ 0.00	0.29 $\pm$ 0.08	0.39 $\pm$ 0.15	0.05 $\pm$ 0.10	0.22 $\pm$ 0.13
Band ($\times 10^9/l$)	0.11 $\pm$ 0.06	0.14 $\pm$ 0.13	0.05 $\pm$ 0.02	0.08 $\pm$ 0.02	0.11 $\pm$ 0.14	0.09 $\pm$ 0.05
Serum amyloid A (mg/ml)	5.64 $\pm$ 1.67	5.93 $\pm$ 1.48	7.51 $\pm$ 2.93	8.54 $\pm$ 3.81	9.51 $\pm$ 4.48	11.26 $\pm$ 7.51
Haptoglobin (g/l)	0.10 $\pm$ 0.05	0.09 $\pm$ 0.01	0.09 $\pm$ 0.02	0.10 $\pm$ 0.02	0.11 $\pm$ 0.04	0.14 $\pm$ 0.09
Fibrinogen (g/l)	1.99 $\pm$ 0.07	2.01 $\pm$ 0.05	2.21 $\pm$ 0.24	2.22 $\pm$ 0.26	2.35 $\pm$ 0.29	2.47 $\pm$ 0.68
Ceruloplasmin (g/l)	0.06 $\pm$ 0.01	0.05 $\pm$ 0.01	0.06 $\pm$ 0.01	0.06 $\pm$ 0.01	0.07 $\pm$ 0.01	0.08 $\pm$ 0.02

Fig. 1 Mean $\pm$ SD of IgG and WBC values of the goats experimentally infected with *B. caprae*. There are similar changes in processes of serum IgG concentration and WBC numbers of the infected goats at different post infection intervals

Fig. 2 Mean $\pm$ SD of IgM and WBC values of the goats experimentally infected with *B. caprae*. There were similar changes in process of IgM concentration and WBC numbers of the infected goats at different post infection intervals

concentration in the blood of animals change subjected to external or internal challenges such as infection, inflammation, surgical trauma or stress (Eckersall 2004; Murata et al. 2004; Gruys et al. 2005). They are mainly synthesized in the liver, mediated by pro-inflammatory cytokines, and their concentration can increase (positive APPs) or decrease (negative APPs) as a consequence of inflammatory stimuli. It has been suggested that APPs may be useful in the assessment of animal welfare (Eckersall 2000; Murata et al. 2004; Murata 2007). On the other hand, it is suggested that various infections and inflammatory processes may be associated with different APP pattern

Fig. 3 Mean $\pm$ SD of IgG and haptoglobin values of the goats experimentally infected with *B. caprae*. There are similar changes in processes of serum IgG and haptoglobin concentrations of the infected goats at different post infection intervals

Fig. 4 Mean $\pm$ SD of IgM and haptoglobin values of the goats experimentally infected with *B. caprae*. There are similar changes in processes of serum IgM and haptoglobin concentrations of the infected goats at different post infection intervals

details, which may lead to APP parameters being used for diagnostic purposes (Gruys et al. 2005). APPs and their changes due to various inflammatory and non-inflammatory conditions have been studied intensively in many animal species (Kaneko 1997; Eckersall 2000; Murata et al. 2004; Gruys et al. 2005; Murata 2007). The specific type of APPs and the time course for alterations in these proteins vary in different species on the basis of the initiating disorder or underlying inflammatory process (Feldman et al. 2000). Serum amyloid A and Hp have been proposed to be markers of stress in cattle and other species (Alsemgeest et al. 1995; Deak et al. 1997; Hicks et al. 1998; Arthington et al. 2003; Hickey et al. 2003; Pieiro et al. 2007).

Hp is a major acute phase protein in numerous species of livestock and domesticated animals. Elevated Hp concentrations occur not only with inflammation but also with some conditions not generally associated with inflammation or tissue damage (Murata et al. 2004). Statistically significant increase in Hp has been observed between clinically healthy and diseased cows in similar researches with same inflammatory diseases (Alsemgeest et al. 1994; Katoh and Nakagawa 1999; Ganheim et al. 2003).

SAA proteins comprise a superfamily of apolipoproteins and are considered one of the major acute phase reactants in vertebrates. SAA proteins are highly conserved across evolutionary distinct vertebrate species with respect to both their sequence and inductive capacity, and it is therefore generally assumed that they have a crucial, yet ill defined, protective role during inflammation (Uhlir and Whitehead 1999). Elevated SAA levels are found following inflammation and also under conditions unrelated to inflammation such as physical stress or at parturition (Murata et al. 2004). Glass et al. (2003) reported that following experimental infection with *Theileria annulata* in cattle, SAA appeared first, followed by a rise in alpha-1-acid glycoprotein in all animals, whereas Hp only appeared in low level in some of the animals.

Plasma fibrinogen in all species is synthesized in the liver and hyperfibrinogenemia, which is characteristic of a broad range of bacterial infections and other inflammatory conditions, has been reported in a wide range of vertebrates (Gentry 2004).

The levels of specific antibodies against this parasite had correlation with some hematological parameters and acute-phase proteins. The degenerating *Besnoitia* cysts caused necrosis and granulomatous inflammation in tissues and the main inflammatory cells consisted of macrophages and lymphocytes. Any tissue damage leads to release of pro-inflammatory cytokines (Van Miert 1995). The cytokines that are produced during and participate in inflammatory processes are the chief stimulators of the production of APPs (Wigmore et al. 1997) and the most important sources of cytokines are macrophages and monocytes at inflammatory sites (Cem Gabay and Irving Kushner 1999). Stimulation of cytokines such as IL-6, IL-1 and TNF- α activates hepatocytic receptors and APPs especially C-reactive protein, serum amyloid A and haptoglobin are produced by hepatocytes (Heinrich et al. 1990, 1998; Ingenbleek and Young 1994). In this study increased APPs levels results from the presence of inflammation and the effects of cytokines on hepatocytic receptors.

The positive APPs are regarded as having general functions in opsonization and trapping of micro-organisms and their products, in activating complement, scavenging free radicals and in modulating the host's immune response (Gruys et al. 2005). Haptoglobin binds to CD11b/CD18

integrines representing major receptors on the cell membranes of leukocytes and has anti-inflammatory capabilities (El Ghmati et al. 1996). Serum amyloid A has also been reported to cause adhesion and chemotaxis of phagocytic cells and lymphocytes (Berliner et al. 1995). Although APPs increase in acute inflammations they become chronic despite their name during permanent stimulation and release of cytokines. During permanent stimulation (chronic infection) positive acute phase protein levels remain elevated in comparison to normal values (Gruys et al. 2005).

Local inflammation induces a specific immune response in the tissue and production of cytokines. IL-1 and TNF produced by monocyte-macrophages at the site of local inflammation induce chemotaxis of lymphocytes (Burger and Dayer 2002). The pro-inflammatory cytokines activate white blood cell precursors in the bone marrow and growth of inflammatory tissue fibroblasts and macrophages (Dinarello 1989; Heinrich et al. 1990; Van Miert 1995). Increase of lymphocytes is caused by increased lymphopoiesis in response to chronic antigenic or cytokine stimulation (Stockham and Scott 2008). Inflammatory intermediates including interleukins and cytokines are interrelated in causing neutrophil proliferation, maturation and release from the bone marrow into blood (Latimer et al. 2003).

Macrophages also present antigens to lymphocytes and cause induction immune response and production of IFN γ . IFN γ increases phagocytic activity of macrophages (McGavin and Zachary 2007). During chronic diseases, enhancement of chronic phase proteins concentrations, γ -globins, is seen. Concentration of IgG and IgM increases in response to chronic infectious agents as γ -globins. Furthermore, increase of APPs causes to elevate globulins concentration (Latimer et al. 2003). Besnoitiosis is a chronic disease related to presence of granulomatous inflammation and during the disease increase of APPs such as SAA and Hp together with some variation in white blood cells and immunoglobulins was seen. In addition, immunoglobulins (IgG and IgM) values had correlation with APPs and white blood cells and the process of their changes was similar. This seems that summon and enhancement inflammatory cells, increase of APPs level and immunoglobulins value as chronic phase proteins in infected goats are related to presence of chronic inflammation and products of tissue injury and these changes result from release of inflammatory intermediates.

Acknowledgments The authors would like to thank the members of the research committee for providing financial support. They would like to thank Dr. M. Ansari-Lari for her assistance in statistical evaluations. We also thank Mr. M. Rahsepar for his technical assistance in hematological evaluations.

References

- Alsemgeest SPM, Kalsbeek HC, Wensing T, Koeman JP, Van Ederen AM, Gruys E (1994) Concentrations of serum amyloid A (SAA) and haptoglobin (Hp) as parameters of inflammatory diseases in cattle. *Vet Q* 16:21–23
- Alsemgeest SPM, Lambooy IE, Wierenga HK, Dieleman SJ, Meerkerk B, Van Ederen AM, Niewold TA (1995) Influence of physical stress on the plasma concentrations of serum amyloid A (SAA) and haptoglobin (Hp) in calves. *Vet Q* 17:9–12
- Arthington JD, Eicher SD, Kunkle WE, Martin FG (2003) Effect of transportation and commingling on the acute-phase protein response, growth, and feed intake of newly weaned beef calves. *J Anim Sci* 81:1120–1125
- Berliner JA, Navab M, Fogelman AM (1995) Atherosclerosis: basic mechanism-oxidation, inflammation, and genetics. *Circulation* 91:2488–2496
- Bigalke RD, Naude TW (1962) The diagnostic value of cysts in the scleral conjunctivain bovine besnoitiosis. *J S Afr Vet Med Assoc* 33(1):21–27
- Burger D, Dayer J (2002) Cytokines, acute-phase proteins, and hormones. *Ann NY Acad Sci* 966:464–473
- Bwangamoi O, Njenga JM (1993) A review of besnoitiosis with special reference to goats. *Zimbabwe Vet J* 24:23–27
- Bwangamoi O, Carles AB, Wandera C (1989) An epidemic of besnoitiosis in goats in Kenya. *Vet Rec* 125:461
- Cem Gabay MD, Irving Kushner MD (1999) Acute-phase proteins and other systemic responses to inflammation. *New Engl J Med* 340(6):448–454
- Cheema AH, Toofanian F (1979) Besnoitiosis in wild and domestic goats in Iran. *Cornell Vet* 69:159–168
- Cortes H, Leita A, Vidal R, Vila-Vicosa MJ, Ferreira ML, Caeiro V, Hjerpe CA (2005) Besnoitiosis in bulls in Portugal. *Vet Rec* 27:262–264
- Deak T, Meriwether JL, Fleshner M, Spencer RL, Abouhamze A, Moldawer LL, Grahn RE, Watkins LR, Maier SF (1997) Evidence that brief stress may induce the acute phase response in rats. *Am J Physiol* 273:1998–2004
- Dinareello CA (1989) Interleukin-1 and its biologically related cytokines. *Adv Immunol* 44:153–205
- Dubey JP, Sreekumar C, Lindsay DS, Hill D, Rosenthal BM, Venturini L, Venturini MC, Greiner EC (2003) *Besnoitia oryctofelisi* n. sp. (protozoa: apicomplexa) from domestic rabbits. *Parasitology* 126:521–539
- Eckersall PD (2000) Recent advances and future prospects for the use of APPs as markers of disease in animals. *Rev Méd Vét* 151:577–584
- Eckersall PD (2004) The time is right for acute phase protein assays. *Vet J* 168:3–5
- El Ghmati SM, van Hoeyveld EM, van Strijp JG, Ceuppens JL, Stevens EA (1996) Identification of haptoglobin as an alternative ligand for CD11B/CD18. *J Immunol* 156:2542–2552
- Ellis JT, Holmdahl OJM, Ryce C, Njenga JM, Harper PAW, Morrison DA (2000) Molecular phylogeny of *Besnoitia* and the genetic relationships among *Besnoitia* of cattle, wildebeest and goats. *Protistologica* 151:329–336
- Feldman BF, Zinkl JG, Jain NC (2000) Schalm's veterinary hematology. Lippincott Williams & Wilkins, Philadelphia
- Ganheim C, Hulten C, Carlsson U, Kindahl H, Niskanen R, Waller KP (2003) The acute phase response in calves experimentally infected with bovine viral diarrhoea virus and/or *Mannheimia haemolytica*. *J Vet Med B* 50:183–190
- Gentry PA (2004) Comparative aspects of blood coagulation. *Vet J* 168:238–251
- Glass EJ, Graigmile SC, Springbett A, Preston PM, Kirvar E, Wilkie GM, Eckersall PK, Hall FR, Brown CG (2003) The protozoan parasite *Theileria annulata* induces a distinct acute phase protein response in cattle that is associated with pathology. *Int J Parasitol* 33:1409–1418
- Gruys E, Toussaint MJM, Niewold TA, Koopmans SJ (2005) Acute phase reaction and acute phase proteins. *J Zhejiang Univ Sci B* 6:1045–1056
- Heerden JV, Els HJ, Raubenheimer EJ, Williams JH (1993) Besnoitiosis in a horse. *J S Afr Vet Assoc* 64:92–95
- Heinrich PC, Castell TA, Andus T (1990) Interleukin-6 and the acute phase response. *Biochem J* 265:621–636
- Heinrich PC, Behrmann I, Müller-Newen G, Schaper F, Graeve L (1998) Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. *Biochem J* 334:297–314
- Hickey MC, Drennan M, Early B (2003) The effect of abrupt weaning of suckler calves on the plasma concentrations of cortisol, catecholamines, leukocytes, APPs and in vitro interferon gamma production. *J Anim Sci* 81:2847–2855
- Hicks TA, Mcglone JJ, Whisnant CS, Kattesh HG, Norman RL (1998) Behavioral, endocrine, immune and performance measures for pigs exposed to acute stress. *J Anim Sci* 76:474–483
- Ingenbleek M, Young V (1994) Transthyretin (prealbumin) in health and disease: nutritional implications. *Annu Rev Nutr* 14:495–533
- Jain NC (1986) Schalm's Veterinary Hematology, 4th edn. Lea & Febiger, Philadelphia
- Kafi M, Oryan A, Morgan-Azghadi N (2007) Pathology of testis and epididymis in native goats in southern Iran. *Com Clin Pathol* 16:201–205
- Kaneko JJ (1997) Serum proteins and dsproteinemias. In: Kaneko JJ, Harvey JW, Bruss ML (eds) Clinical biochemistry of domestic animals, 5th edn. Academic, San Diego, pp 117–138
- Katoh N, Nakagawa H (1999) Detection of haptoglobin in the high-density lipoprotein fractions from sera of calves with experimental pneumonia and cows with naturally occurring fatty liver. *J Vet Med Sci* 61:119–124
- Latimer KS, Mahaffey EA, Prasse KW (2003) Veterinary laboratory medicine. clinical pathology, 4th edn. Iowa State Press, Iowa
- Leighton FA, Gajadhar AA (2001) *Besnoitia* spp. and besnoitiosis. In: Samuel B, Pybus M, Kocan AM (eds) Parasitic diseases of wild animals. Iowa State University Press, Iowa, pp 468–478
- Lowry OH, Rosebrough NJ, Farr AL, Randall RL (1951) Protein measurement with the folin phenol reagent. *J Biol Chem* 193:265–275
- McGavin MD, Zachary JF (2007) Pathologic basis veterinary disease, 4th edn. Elsevier Mosby, London
- Murata H (2007) Stress and acute phase protein response: an inconspicuous but essential linkage. *Vet J* 173:473–474
- Murata H, Shimada N, Yoshioka M (2004) Current research on APPs in veterinary diagnosis: an overview. *Vet J* 168:28–40
- Nazifi S, Oryan A, Mohebbi H (2002) Evaluation of hematological parameters in caprine besnoitiosis. *J Appl Anim Res* 21:123–128
- Njagi ON, Ndarathi CM, Nyaga PN, Munga LK (1998) An epidemic of besnoitiosis in cattle in Kenya. *Onderstepoort J Vet Res* 65:133–136
- Njenga MJ, Munyua SJ, Bwangamoi O, Kang'ethe EK, Mugeru GM, Mutiga ER, Wamukoya JP (1999) Prevalence of besnoitiosis in domestic ruminants in Kenya: a preliminary survey. *J S Afr Vet Assoc* 70:151–153
- Oryan A, Sadeghi MJ (1997) An epizootic of besnoitiosis in goats in fars province of Iran. *Vet Res Commun* 21:559–570
- Oryan A, Kafi M, Morgan-Azghadi N (2008a) Besnoitiosis of the reproductive tract of male goats. *Com Clin Pathol* 17:185–191
- Oryan A, Nazifi S, Mohebbi H (2008b) Pathology and serum biochemical changes in natural caprine besnoitiosis. *Rev Méd Vét* 159:27–32

- Pieiro M, Pineiro C, Carpintero R, Morales J, Campbell FM, Eckersall PD, Toussaint MJ, Lampreave F (2007) Characterization of the pig acute phase protein response to road transport. *Vet J* 173:669–674
- Stockham SL, Scott MA (2008) *Fundamentals of veterinary clinical pathology*, 2nd edn. Iowa, USA
- Sunderman FW Jr, Nomoto S (1970) Measurement of human serum ceruloplasmin by its *p*-phenylenediamine oxidase activity. *Clin Chem* 160:903–907
- Thrall M, Baker AD, Lassen E, Campbell DT, Denicola D, Fettman M, Alan R, Weiser G (2004) In: Thrall MA (ed) *Veterinary hematology and clinical chemistry*. Lippincott Williams and Wilkins, Philadelphia
- Uhlar CM, Whitehead AS (1999) Serum amyloid A, the major vertebrate acute-phase reactant. *Eur J Biochem* 265:501–523
- Van Miert ASJPAM (1995) Pro-inflammatory cytokines in a ruminant model: pathophysiological, pharmacological, and therapeutic aspects. *Vet Q* 175:41–50
- Wigmore SJ, Fearon KCH, Maingay JP, Lai PBS, Ross JA (1997) Interleukin-8 can mediate acute-phase protein production by isolated human hepatocytes. *Am J Physiol* 273:720–726