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DYNAMICS OF SERUM PROTEIN DURING THE ESTROUS CYCLE OF GOATS BRED IN BRAZIL AND NATURALLY INFECTED BY CAPRINE ARTHRITIS ENCEPHALITIS VIRUS

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ABSTRACT

The aim of the present study was to evaluate variations in proteinogram occurring during the estrus cycle of animals infected or not by caprine arthritis encephalitis. Forty blood samples were collected from female goats in different phases of the estrus cycle (estrus, proestrus, metaestrus and diestrus). Samples were classified as positive (n = 5) and negative (n = 5), according to the results of the survey of antibodies against caprine arthritis encephalitis as performed by immunodiffusion in agar gel. Samples were also used in the determination of albumin, total protein and alpha, beta and gammaglobulin by means of electrophoresis and biuret method, respectively. Electrophoresis showed that estrus, proestrus, metaestrus and diestrus positive animals presented total protein mean equal to 7.96 ± 0.69, 7.67 ± 1.14, 7.77 ± 0.36, 7.48 ± 0.83; mean albumin equal to 3.22 ± 0.41, 3.11 ± 0.68, 3.30 ± 0.67, 3.28 ± 0.57; mean alphaglobulin equal to 0.70 ± 0.09, 0.70 ± 0.12, 0.69 ± 0.12, 0.61 ± 0.11; mean betaglobulin 1 equal to 0.85 ± 0.04, 0.87 ± 0.04, 0.90 ± 0.12, 0.83 ± 0.11; mean betaglobulin 2 equal to 0.77 ± 0.18, 0.68 ± 0.17, 0.60 ± 0.15, 0.54 ± 0.09; and mean gammaglobulin equal to 2.43 ± 0.47, 2.31 ± 0.54, 2.28 ± 0.49, 2.21 ± 0.54. Negative animals presented mean total protein equal 7.76 ± 1.31, 8.03 ± 1.10, 7.50 ± 0.51, 6.49 ± 1.14; mean albumin equal to 3.06 ± 0.46, 3.19 ± 0.46, 3.03 ± 0.62, 2.93 ± 0.96; mean alphaglobulin equal to 0.69 ± 0.19, 0.75 ± 0.18, 0.70 ± 0.24, 0.58 ± 0.20; mean betaglobulin 1 equal to 0.79 ± 0.03, 0.79 ± 0.23, 0.80 ± 0.15, 0.66 ± 0.16; mean betaglobulin 2 equal to 0.67 ± 0.20, 0.95 ± 0.37, 0.81 ± 0.40, 0.73 ± 0.41; and mean gammaglobulin equal to 2.35 ± 1.42; 2.42 ± 1.30, 2.23 ± 1.03, 1.92 ± 0.70, respectively. Variance analysis did not show any statistically significant differences. Animals infected by caprine arthritis encephalitis (CAE) did not present any changes in proteinogram, regardless of the phase of the estrus cycle they were in.

KEY WORDS: Goats, estrus cycle, CAEV, serum protein.

RESUMO

DINÂMICAS DA PROTEÍNA SÉRICA DURANTE O CICLO ESTRAL DE CABRAS CRIADAS NO BRASIL E NATURALMENTE INFECTADOS PELA ARTRITE ENCEFÁLICA CAPRINA. O presente trabalho teve com objetivo avaliar variações ocorridas durante o ciclo estral no proteinograma em animais infectados pela artrite encefalite caprina. Foram coletadas 40 amostras de sangue de cabras, nas diferentes fases do ciclo estral (estro, proestro, metaestro e diestro), as quais foram classificadas como positivas (n = 5) e negativas (n = 5), de acordo com o resultado da pesquisa de anticorpos contra o vírus da artrite encefalite caprina realizada pela prova de imunodifusão em gel de ágar. Nas amostras foram determinadas as concentrações de albumina, alfaglobulina, betaglobulina e gamaglobulina e dosagem de proteínas totais pelos métodos de eletroforese e biureto respectivamente. As análises eletroforéticas, realizadas, estro, proestro, metaestro e no diestro, os animais positivos apresentaram média de 7,96 ± 0,69, 7,67 ± 1,14, 7,77 ± 0,36, 7,48 ± 0,83 de proteína total, média de 3,22 ± 0,41, 3,11 ± 0,68, 3,30 ± 0,67, 3,28 ± 0,57 de albumina, média de 0,70 ± 0,09, 0,70 ± 0,12, 0,69 ± 0,12, 0,61 ± 0,11 de alfaglobulina, média de 0,85 ± 0,04, 0,87 ± 0,04, 0,90 ± 0,12, 0,83 ± 0,11 de betaglobulina1, média de 0,77 ± 0,18, 0,68 ± 0,17, 0,60 ± 0,15; 0,54 ± 0,09 de betaglobulina2 e média de 2,43 ± 0,47, 2,31 ± 0,54, 2,28 ± 0,49, 2,21 ± 0,54 de gamaglobulina e os animais negativos apresentaram média de 7,76 ± 1,31, 8,03 ± 1,10, 7,50 ± 0,51, 6,49 ± 1,14 de proteína total; média de 3,06 ± 0,46; 3,19±0,46; 3,03±0,62; 2,93±0,96 de albumina; média de 0,69 ± 0,19, 0,75 ± 0,18, 0,70 ± 0,24, 0,58 ± 0,20 de alfaglobulina; média de 0,79 ± 0,03, 0,79 ± 0,23, 0,80 ± 0,15, 0,66 ± 0,16 de betaglobulina1; média de 0,67 ± 0,20, 0,95 ± 0,37, 0,81 ± 0,40, 0,73 ± 0,41 de betaglobulina2; e média de 2,35 ± 1,42, 2,42 ± 1,30, 2,23 ± 1,03, 1,92 ± 0,70 de gamaglobulina, respectivamente. Não foram encontradas diferenças estatísticas significantes na análise de variância. Os animais infectados pela artrite

encefalite caprina (CAE) não apresentam alterações no proteinograma independentemente da fase do ciclo estral em que se encontravam.

PALAVRA-CHAVE: Caprinos, cliclo estral, CAE, proteína sérica.

INTRODUCTION

Caprine arthritis encephalitis (CAEV) is caused by a virus of the family *Retroviridae*, subfamily *Orthoretrovirinae*, genus *Lentivirus* (Buchen-Osmond, 2004). It is a single-stranded virus that replicates by means of the production of deoxyribonucleic acid (DNA), and depends on intermediate reverse transcriptase. This kind of virus may insert its genetic material in the genome of the host (Pugh, 2002).

The virus enters monocytes and macrophages, inducing persistent infection. Restricted replication enables the virus to remain latent in monocytes, undetected by the host immune system, leading to many debilitating, severe, progressive diseases. The virus quickly replicates in the beginning of the infection, activating the immune system, which limits, but does not eliminate the virus. Infected macrophages express viral proteins similar to the antigens of the main histocompatibility complex, which are recognized by T lymphocytes and stimulate the production of cytokines. These macrophages may be more susceptible to activation, inducing lymphocyte and macrophage proliferation. Lymphocyte proliferation determines the pathological changes characteristic of CAE infection. Transmission routes reported are the ingestion of milk or colostrum from an infected dam, and horizontal transmission after a long period of contact. The virus preferentially affects joints, mammary gland, lungs and brain, where the immune system of the host tends to manifest chronic inflammation (Bohland & D'Angelino, 1999; Pugh, 2002).

CAE is one of the diseases most responsible for the limitation to the development of productive goat breeding in São Paulo and in Brazil. In 2003, the Brazilian herd was constituted of 9,581,653 head (Instituto Brasileiro de Geografia e Estatistica, 2004). Prevalence rates of the disease show that the agent is disseminated throughout the country, as an enzootic disease that is present in most of the geo-economic regions and herds. CAE leads to important losses, due to the discard of premature kids, mortality and decrease in productivity (Bohland, 1998).

Some organic changes or even the intensity of the inflammatory response may be influenced by the phase of the estrus cycle in which the animal is (Haenlein, 2002). The hypothesis that this phenomenon is even greater influenced by the occurrence of chronic diseases such as CAE should be further studied.

The estrus cycle of female goat lasts 21 days, in average. Its phases, proestrus, estrus, metaestrus and diestrus, last, respectively, 2 to 3 days, 30 to 40 hours, 2 to 3 days and 13 to 15 days (Raposo, et al., 1999). These phases occur in a cyclic and sequential manner, except for anestrus, which has to be considered in this species, for goats are seasonal polyestrous animals.

Due to the biological meaning (Kaneko,1997) and multiple functions of proteins in the body (such as being part of the cell structure, tissues, organs, hormones, enzymatic catalysts, antibodies, and others), the evaluation of serum levels of total proteins and their fractions (albumin, alphaglobulin, betagalobulin 1, betaglobulin 2 and gammaglobulin), as detected by electrophoresis, and the influence caused by the estrus cycle and / or chronic, multisystemic diseases (Birgel Junior et al., 2001; Garcia et al., 2002), such as caprine arthritis encephalitis, is an important tool aiding clinical diagnosis.

The objective of the present study was to evaluate the dynamics of serum proteins during the estrus cycle of female goats affected and unaffected by caprine arthritis encephalitis.

MATERIAL AND METHOD

This study used 10 female Saanen and Toggenburg female goats bred in an intensive system and fed goat food, hay and mineral supplement.

These animals were separated into two groups according to their status as determined by immunodiffusion in agar gel antibodies against caprine arthritis encephalitis virus (Cutlip et al., 1977).

For the evaluation of serum protein dynamics, blood samples were collected by a puncture in the external jugular vein, with 10 mL Vacutainer tubes. They were centrifuged for 15 minutes and serum was separated and fractionated in two aliquots stored at -20º C until they were to be analyzed.

Total proteins were quantified in an automatic biochemical analyzer using the biuret method (Kaneko, 1997). Electrophoresis fractioning of serum proteins was performed in cellulose acetate strips, according to the techniques described by Friedman (1961).

Data on the determination of total protein and its fractions by means of electrophoresis (albumin, alphaglobulin, betaglobulin 1, betaglobulin 2 and gammaglobulin) were submitted to variance analysis (ANOVA) and to the Student Newman-Keuls test to

compare pair of means at a 5% significance level (α = 0.05).

Statistical analyses were performed by means of the Graphpad Instat software.

RESULTS

Values observed for the different protein fractions of adult goats positive or negative for caprine arthritis encephalitis were inside the normal range expected for the species and age (BIRGEL & ARAÚJO, 1968).

Results obtained in the present study are presented in Table 1 and no influence of the period of estrus cycle was detected.

DISCUSSION

Interference of retrovirosis in humoral immune responses have been studied in other species (BIRGEL JUNIOR et al., 2001; GARCIA et al., 2002), mainly in relation to enzootic bovine leucosis (EBL). These authors studied the dynamics of serum proteins not only as markers of acute inflammatory process, but also as an indirect way to evaluate humoral response.

BOHLAND & D'ANGELINO (1999) described the clinical and epidemiological aspects of caprine arthritis encephalitis and its importance to Brazilian goat breeding. They did not identify any information on the variations observed with the estrus cycle.

The phenomenon observed in the present study has been partially described in bovines affected by EBL, which is also a multisystemic retrovirosis of chronic evolution, in which proteinogram presented no differences between positive and negative animals (GARCIA et al., 2002), although the interference of the estrus cycle was not been evaluated by those authors. No differences were observed in the dynamics of proteins and fractions attributable to the estrus cycle and / or caprine arthritis encephalitis.

Table 1 - Average values, standard deviation and contrast between averages obtained in the goats' serum with or without falling CAEV in cycle estral different phases. State of São Paulo, 2004.

Phases of the estrous cycle	Total protein g / dll		albumin g / dll		alphaglobulin g / dll		betagalobulin 1 g / dll		betaglobulin 2 g / dll		gammaglobulin g / dll	
	CAEV-	CAEV +	CAEV -	CAEV +	CAEV -	CAEV +	CAEV -	CAEV +	CAEV -	CAEV +	CAEV -	CAEV +
Proestrous	7.76±1.31Aa	7.96±0.69Aa	3.06±0.46Aa	3.22±0.41Aa	0.69±0.19Aa	0.70±0.09Aa	0.79±0.03Aa	0.85±0.04Aa	0.67±0.20Aa	0.77±0.18Aa	2.35±1.42Aa	2.43±0.47Aa
Estrous	8.03±1.10Aa	7.67±1.14Aa	3.19±0.46Aa	3.11±0.68Aa	0.75±0.18Aa	0.70±0.12Aa	0.79±0.23Aa	0.87±0.04Aa	0.95±0.37Aa	0.68±0.17Aa	2.42±1.30Aa	2.31±0.54Aa
Metaestrous	7.50±0.51Aa	7.77±0.36Aa	3.03±0.62Aa	3.30±0.67Aa	0.70±0.24Aa	0.69±0.12Aa	0.80±0.15Aa	0.90±0.12Aa	0.81±0.40Aa	0.60±0.15Aa	2.23±1.03Aa	2.28±0.49Aa
Diestrous	6.49±1.14Aa	7.48±0.83Aa	2.93±0.96Aa	3.28±0.57Aa	0.58±0.20Aa	0.61±0.11Aa	0.66±0.16Aa	0.83±0.11Aa	0.73±0.41Aa	0.54±0.09Aa	1.92±0.70Aa	2.21±0.54Aa

A: horizontal contrast between averages (α = 0.05)
a: vertical contrast between averages (α = 0.05)

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