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Seasonal variations of phagocytic response, immunoglobulin G (IgG) and plasma cortisol levels in Dhofari goats

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ABSTRACT

Unpublished observations in Oman revealed that both the morbidity and mortality figures for a wide variety of diseases in goats are higher during the winter than during the summer season. The present study therefore, attempted to ascertain whether there were any measurable seasonal differences in the phagocytic response, levels of circulating immunoglobulin G, blood cells or plasma cortisol in six adult male Dhofari goats investigated over a 1-year period. Interestingly, we observed that during the summer, goats had significantly higher levels of circulating lymphocytes, increased phagocytic activity against the bacterial target, *Staphylococcus aureus* and lower levels of plasma cortisol. It was postulated that the decreased phagocytic activity observed during the winter season might be related to reduced levels of opsonins, early neutrophil exhaustion and/or suppression of neutrophil activity as a result of higher cortisol levels and reduced periods of exposure to daylight. These results might also explain in part why the incidence of a variety of diseases in goats is commonly higher during the winter season.

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1. Introduction

Epidemiological surveys of goats undertaken by the Central Diagnostic Laboratory in the Sultanate of Oman have shown that there is an apparent increase in morbidity and mortality associated with a wide variety of diseases during the cooler (winter) months when compared to the hot summer months (unpublished data). This observation is supported by findings from a study in Northeast Brazil that showed an increased incidence of a number of bacterial, parasitic and viral diseases in goats during the rainy (winter) season compared to the hot summer months (Johnson et al., 1995). These researchers also presented evidence that the total level of serum immunoglobulins were significantly lower during the winter than during the summer months.

The ambient temperature in Oman reaches a low of 13.5 °C in winter and a high of 45.5 °C in summer. The effect of these temperature variations on the immune system of goats has not been studied. The present study was therefore undertaken to investigate the relationship between seasonal variations on two parameters of the immune system, namely phagocytic activity of polymorphonuclear granulocytes and levels of circulating serum immunoglobulin G, as well as levels of cortisol and blood cell counts of Omani goats.

2. Materials and methods

2.1. Animals

Six clinically healthy male Dhofari goats, 1 year of age, weighing between 45 and 50 kg were maintained together in a shaded and partially closed pen at the Agriculture Experimental Station at Sultan Qaboos University. The goats were fed chopped Rhodesgrass hay (*Chloris gayana*) and water *ad libitum*. They were also provided with 10% of their body weight with a commercially prepared ruminant concentrate (General Ruminant Concentrate, Oman Flour Mill, Oman) containing DM, 91.1%; CP, 16%; EE,

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3.7%; ADF, 15.8%; NDF, 34.9%; ash, 7.6%; and GE, 20.1 MJ/kg DM. The animals were de-wormed bi-annually with ivermectin (0.5 g).

2.2. Climatological measurements

An automated weather station (Casella Ltd., UK) monitored solar radiation, air temperature, relative humidity (RH), rainfall and wind speed. Daily maximum temperature (T_{max}, °C), minimum temperature (T_{min}, °C) and relative humidity data were used to calculate the temperature/humidity index (THI) using the formula THI = drybulb (°C) − 0.55 (7 − relative humidity) (drybulb − 14.4) with the relative humidity expressed as a decimal (Ravagnolo and Misztal, 2000).

2.3. Hematology

Blood samples were collected monthly for hematological, serological and cortisol analysis, as well as for the preparation of neutrophil-enriched cell preparations. Complete blood cell counts and differentials were determined using a CELL-DYN 3700 automated blood analyzer (Abbot Laboratory, Diagnostic Division, Abbott Park, IL 60064, USA). Quantification of total serum protein was performed using a standard refractometer (Attago, Chemlab Scientific Products Ltd., Japan).

2.4. Neutrophil-enriched cell preparation

Neutrophil-enriched cell preparations were isolated from heparin whole blood by density centrifugation as previously described (Johnson et al., 1991). Briefly, in conical siliconized 15 ml centrifuge tubes, 7 ml of blood were layered on top of 3 ml of Ficol-Histopaque and centrifuged at 1500 × *g* for 30 min. The plasma and buffy coats, as well as the red blood cell layer extending to immediately above the granulocyte pellets were removed. Contaminating erythrocytes were destroyed by hypotonic lysis. The neutrophil-enriched cell preparations were washed three times in Hanks Balanced Salts Solution (HBSS) without calcium and magnesium, and finally resuspended in HBSS with 0.1% gelatin to give a total count of 6×10^6 cells/ml. Differential counts were performed using Wright-Giemsa stain and viability was determined by trypan blue exclusion. Only samples with ≥95% viability and having ≥95% neutrophils were used in the study.

2.5. Preparation of bacterial suspensions

Staphylococcus aureus (strain no. ATCC 29213, Difco, USA) was cultured overnight on blood agar media (Difco, USA) at 37 °C under aerobic conditions. Subsequently, an isolated colony was emulsified in 5 ml of brain heart infusion broth and incubated overnight. The tube was centrifuged at 1500 × *g* for 30 min and the supernatant removed. The bacterial pellet was resuspended in HBSS with 0.1% gelatin. The density of the bacterial suspension was measured using a spectrophotometer to give an absorbency reading of 0.6 at 620 nm. The bacterial suspension (0.2 ml) was mixed with 9.8 ml of HBSS with 0.1% gelatin (1:50 dilution). This dilution was determined in preliminary studies to be equivalent to approximately 6×10^7 bacteria/ml. The suspension was left on ice until used in the phagocytic assay.

2.6. Phagocytic assay

The phagocytic assay was performed according to the method of Quie et al. (1967). Briefly, serum samples from the six goats were collected and pooled prior to each assay and diluted 1:5 with HBSS. The phagocytic assays were performed in siliconized test tubes. Each tube contained 0.1 ml of the bacterial suspension (6×10^6 cells), 0.4 ml of 20% pooled serum and 0.5 ml of neutrophils suspension (3×10^6 cells). The tubes were incubated at 37 °C with end-to-end rotation. The total viable bacteria at 0, 30, 60 and 120 min during the incubation period was determined by taking 2 μl of the reactant and mixing with 1 ml of sterile distilled water for neutrophil lysis to achieve a 1:500 dilution. 200 μl of the lysing preparation was plated on tryptic soy agar. The plates were incubated at 37 °C under aerobic conditions overnight. At the end of 120 min, tubes containing the phagocytic mixtures were centrifuged at 1500 × *g* for 5 min. Samples (2 μl) from the supernatant fraction were taken and diluted 1:500 for the determination of extracellular bacterial (ECB). The supernatants were then removed and discarded and the leukocyte pellets were washed twice with HBSS–1% gelatin and centrifuged at a speed of 400 × *g* for 5 min. The supernatants were discarded and the pellets were resuspended in 1 ml of sterile distilled water and thoroughly mixed for 5 min until complete lysis of the leukocytes occurred. 100 μl of the reactant were withdrawn and mixed with 900 μl of sterile distilled water. 200 μl of the mixture were plated onto tryptic soy agar to determine the number of cell-associated bacteria (CAB). All the inoculated plates were incubated over night at 37 °C and the colonies were counted using a colony counter.

2.7. Serum immunoglobulin G levels

IgG was quantified using an enzyme-linked immuno-sorbent assay (ELISA). Wells of the microtiter plates were coated with 100 μl of monoclonal anti-goat/sheep IgG Clone GT-34 (mouse ascites fluid, Sigma No. G-2904). The optimal antibody dilution (1:1250) was obtained by checkerboard titration. After overnight incubation at 4 °C, the plates were washed three times with phosphate buffered saline (PBS), containing Tween 20. Subsequently, 200 μl of fresh 3% bovine serum albumin (BSA-3%) was dispensed in each well and incubated for 30 min at 37 °C. The plates were washed with PBS Tween 20, three times. 100 μl of the diluted goat serum (1:10) was dispensed in each well and incubated at 37 °C for 1 h (each sample was run in duplicate). Thereafter, the plates were washed three times with PBS containing Tween 20. 100 μl of the conjugate enzyme (monoclonal anti-goat/sheep IgG (Clone GT-34, No. A8062, Sigma)) was dispensed in each well (1:50,000 dilution) and incubated at 37 °C for 1 h. After washing three times, 50 μl of alkaline phosphatase substrate (Sigma fast *p*-nitrophenyle phosphate (Sigma, No. N-2770)) was added and kept at 37 °C for 30 min. The reaction was stopped by the addition of 25 μl of 3N-NaOH and measured at 405 nm in an ELISA plate reader.

2.8. Cortisol assay

Blood samples were obtained from each animal between 08:00 and 09:30 at the end of each month for the determination of the plasma cortisol concentration. Plasma cortisol concentrations were measured using an immunoassay system (Beckman Coulter, Access2, immunoassay system), according to the manufacturer's instruction.

2.9. Statistical analysis

Data were subjected to analysis of variance procedure (Ott, 1993) to evaluate the effects of season on immune system parameters using GLM procedures of with month as a class on the proc. GLM statement. Correlation coefficients were performed to define the relationship between climatic changes (Temp., RH and THI) and IgG, total protein, cortisol, ECB, CAB, and PMNs activity. Significant differences between immune system parameter means were assessed using the least significant difference procedures. Duncan's multiple range tests was used for mean comparisons.

3. Results

3.1. Seasonal variations in temperature, humidity and photoperiod

The average daily temperature during the winter season (October–March) was 24 ± 0.4 °C with a range between 22 and 30 °C. During the summer season (April–September) the average daily temperature was 34 °C with a range between 32 and 36 °C. The relative humidity was higher during the winter (58 ± 0.8%) than during the summer (53 ± 1.3%) (p <0.05). The temperature humidity index (THI) was higher during the summer (83 ± 1.0%) than during winter (71 ± 2.0%). The average duration of daylight was 9.6 ± 0.5 h during the winter and 11.3 ± 0.8 h during the summer ($p < 0.05$).

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