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Concentrations of serum amyloid A, haptoglobin, tumour necrosis factor and interleukin-1 and -6 in Anatolian buffaloes naturally infected with dermatophytosis

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ABSTRACT: Dermatophytosis is most frequently found in ruminants, in which non-pruritic periocular lesions are most typical, though generalised skin disease may develop. Accordingly, the infection causes major economic losses. The aim of this study was to measure the inflammatory status of Anatolian buffaloes with dermatophytosis by determining the serum concentrations of serum amyloid A (SAA), haptoglobin (Hp), tumour necrosis factor (TNF-α) and interleukin-1 (IL-1) and -6 (IL-6). Anatolian buffaloes (n = 26), aged three to 11 month, were divided into two groups: 11 animals served as the clinically healthy control group and 15 animals clinically and microbiologically diagnosed with dermatophytosis formed the experimental group. Concentrations of tested proteins were measured using commercially available ELISA kits. In all cases, concentrations of measured proteins were significantly higher ($P < 0.05$) in infected animals when compared to healthy controls: SAA: 41.05 ± 0.01 vs. 7.43 ± 0.11μg/ml; Hp: 96.21 ± 0.18 vs. 8.49 ± 0.79 μg/ml; TNF-α: 0.90 ± 0.99 vs. 0.10 ± 0.26 ng/ml; IL-1α: 186.22 ± 0.22 vs. 74.04 ± 0.90 pg/ml; and IL-6: 55.94 ± 0.50 vs. 32.45 ± 0.20 pg/ml. It was concluded that the elevated values of variables under study were a result of the inflammatory response to dermatophytosis; thus, these markers may serve as an additional diagnostic tool.

Keywords: dermatophytosis; anatolian buffaloes; serum amyloid A; haptoglobin; cytokines

Dermatophytosis is a group of external infections of keratinised tissues such as skin, hair, feather and nails in humans and animals (Gudding and Lund 1995; Ozkanlar et al. 2009; Papini et al. 2009; Aslan et al. 2010). Dermatophytosis, widely known as ringworm, is mostly caused by *Trichophyton verrucosum* and *Trichophyton mentagrophytes* (Scott 1994). Contamination usually occurs via contact with infected and susceptible animals or contaminated matrices like bedding or walls (Scott 1994). All domestic animals are susceptible to infection. Moreover, dermatophytosis is also a contagious and zoonotic infection (Papini et al. 2009; Yilmazer and Aslan 2010), which results in major economic losses because infection spreads from one animal to another very swiftly once it reaches the herd. Spores can survive for two to three years (Gudding and Lund 1995). As a result, young, weak and immunosuppressed animals are more likely to be infected.

Acute phase proteins (APPs) and cytokines are a group of blood proteins whose concentration may change in animals subjected to external or internal challenges such as infection, inflammation, surgical trauma, or stress (Murata et al. 2004). Our hypothesis was that their concentrations will react to mycotic infections, too. Therefore, this study was aimed at determining serum amyloid A (SAA), haptoglobin (Hp), tumour necrosis factor (TNF-α) and interleukin-1 (IL-1) and -6 (IL-6) levels in Anatolian buffaloes with dermatophytosis.

MATERIAL AND METHODS

In this study the material consisted of 15 Anatolian buffaloes clinically and microbiologically diagnosed with dermatophytosis and 11 healthy Anatolian buffaloes all of which were three to 11 months

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old. In clinical examinations of animals suffering from dermatophytosis, scurfy, local alopecic, circle-shaped, thick, white, asbestos-like plaques and erythematous lesions were observed all around the body, especially in the head and neck regions. No skin lesions were observed in the healthy animals. Serum was separated from the blood obtained from the *vena jugularis*. On the serum obtained from blood sample, SAA (Tridelta Development LTD, Ireland), Hp (Life Diagnostics Inc. West Chester, PA) IL-1β (Cusabio Biotech Co. Ltd, China), IL-6 (Cusabio Biotech Co. Ltd, China) and TNF-α (Cusabio Biotech Co. Ltd, China) concentrations were measured using commercial ELISA kits as recommended by the manufacturer. Skin and hair samples collected from the dermatophytic lesions were cultured in Saboraud Dextrose Agar with 0.5% Chloramphenicol (Oxoid CM0041). Cultures were incubated at 20 °C for seven days. At the end of incubation, colonies were taken on a loop and lam, and stained with malachite green. After examination under a light microscope, the preparations were typed according to their hyphae structures and colony morphology (Koneman and Roberts 1985).

PASW Statistics (version 18.0; SPSS, Chicago, IL) were used for data analyses. The Mann-Whitney *U*-test was used to compare mean differences between groups. The Wilcoxon signed-rank test was performed after a Friedman test to determine where significance occurred within group variables. A significance level of $P \leq 0.05$ was used. To avoid type 1 alpha errors, Bonferroni correction was used for the Wilcoxon signed-rank test.

RESULTS

Skin and hair sample isolation showed that Anatolian buffaloes with dermatophytosis were infested with *Trichophyton verrucosum*. Collected serum samples demonstrated that SAA (41.05 ± 0.01 μg/ml), Hp (96.21 ± 0.18 μg/ml), TNF-α (0.90 ± 0.99 ng/ml), IL-1 (186.22 ± 0.22 pg/ml) and IL-6 (55.94 ± 0.50 pg/ml) concentrations of dermatophytic Anatolian buffaloes were higher ($P < 0.05$) compared to the control group (SAA 7.43 ± 0.11; Hp 8.49 ± 0.79; TNF-α 0.10 ± 0.26; IL-1 74.04 ± 0.90; IL-6 32.45 ± 0.20) (Table 1).

DISCUSSION

Dermatophytosis usually causes enzootic infections in young and immunosuppressed animals (Ozkanlar et al. 2009). APPs and cytokines are considered as reliable indicators with respect to the detection, prognosis and monitoring of infections in animals and of animal welfare (Gonzalez et al. 2008). Although there is a growing trend towards the development of APPs as health indicators (Eckersall and Bell 2010; Ceciliani et al. 2012; Tothova et al. 2014), the literature reveals no previous study considering the possible changes that may occur in APPs during dermatophytosis in Anatolian buffaloes. Thus, this study was aimed at evaluating the concentrations of SAA, Hp and cytokines in Anatolian buffaloes with dermatophytosis in comparison to values from healthy animals.

In a study conducted on healthy and bronchopneumonic buffalo calves, El-Bahr and El-Deeb (2013) determined higher rates of SAA, Hp and cytokines in bronchopneumonic buffalo calves in comparison to the healthy ones. They explained these findings as the outcome of tissue damage resulting from infection or inflammation. In our study, SAA, Hp and cytokine levels were determined to be higher in Anatolian buffaloes with dermatophytosis than in the control group. We assume that these observations are due to the inflammation caused by dermatophytosis.

Table 1. The concentrations of SAA, Hp, TNF-α, IL-1 and IL-6 in the serum of control and Anatolian buffaloes (mean ± SD)

Parameters	Control	Dermatophytosis	*P*
Serum amyloid A (μg/ml)	7.43 ± 0.11[a]	41.05 ± 0.01[b]	0.000
Haptoglobin (μg/ml)	8.49 ± 0.79[a]	96.21 ± 0.18[b]	0.000
TNF-α (ng/ml)	0.10 ± 0.26[a]	0.90 ± 0.99[b]	0.000
IL-1 (pg/ml)	74.04 ± 0.90[a]	186.22 ± 0.22[b]	0.000
IL-6 (pg/ml)	32.45 ± 0.20[a]	55.94 ± 0.50[b]	0.006

$P < 0.05$; the variations between the groups are indicated with letters (a and b)

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In another study by Molina (2005) on buffaloes infected with *Fasciola gigantica* it was determined that serum IL-6 and IL-8 levels were elevated. The author concluded that the increased serum IL-6 and IL-8 levels in infected buffaloes suggest that these cytokines may play a role in the immune reaction during liver fluke infection in some species. Kumar et al. (2011) examined IL-8 levels in Murrah buffaloes during the peripartum period and reported that IL-8 was high in the postpartum period. They explained that the rise in IL-8 levels in buffaloes is a cumulative immune response not only to the physiological stress of parturition and uterine involution during the postpartum period, but also to the high thermal stress from the environment. In our study, concentrations were also found to be higher ($P < 0.05$) in Anatolian buffaloes with dermatophytosis than in the control group. These observations are likely to be a result of the inflammatory response to dermatophytosis.

CONCLUSION

Dermatophytosis increased the levels of serum amyloid A, haptoglobin, tumour necrosis factor and interleukins-1 and -6 in Anatolian buffaloes. We postulate that APP and cytokine levels might have increased as a result of the inflammation caused by dermatophytosis. We suggest that APPs and cytokines can be effectively used in the diagnosis and treatment of dermatophytosis in buffaloes.

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