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Plasma Cortisol and Thyroid Hormone Profile in *Theileria equi* Affected Horses

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Abstract

Equine theileriosis caused by *Theileria equi* is an endemic disease in most of the countries. The latently infected animals may exhibit low performance following physical, immunological or mental stress due to the flare-up of underlying parasitemia leading to clinical form of the disease. In the present study, 72 horses were screened for *T. equi* by using polymerase chain reaction based on equine merozoite antigen-5 and 6, which revealed 25 to be positive for theileriosis. Further study was conducted to assess the cortisol and thyroid hormone (T3 and T4) levels in healthy (n=47) and in *T. equi* infected (n=25) horses. In horses positive for *T. equi*, there was significant increase ($p<0.05$) in cortisol (42.80±0.69 vs. 24.44± 0.12 ng/ml) and T4 level (47.56±4.06 vs. 38.14±2.40 ng/ml) with significant reduction ($p<0.01$) in T3 level (0.44±0.13 vs. 1.20±0.19 ng/ml) when compared to the healthy group. This may be the first report revealing that theileriosis infected horses are under stress due to the involvement of endocrine system as well as metabolic pathways.

Introduction

Equine theileriosis caused by *T. equi* is endemic in Asia, Southern Europe, Latin America, and Africa. Thus about 90% of the world's equine population resides in areas where *T. equi* is endemic (Grause *et al*., 2012). Equine theileriosis is of worldwide importance due to the fact that the disease causing agents can be readily transmitted by carrier animals or by infected ticks. The disease commonly occurs secondary to other infections and even strenuous exercise may predispose horses to the clinical manifestation of the disease (Kutscha *et al.,* 2008). The economic importance of this disease to poor farmers is concerned with weakness and inability of horse to work, cost of treatment and deaths (Kumar *et al*., 2013; Salib *et al*., 2013).

Cortisol plays an important role in integral endocrine response to stress, and it is also used to determine pituitary pars intermedia dysfunction in horses, a progressive neuroendocrine dysfunction which may lead to the higher risk of developing metabolic disturbances, laminitis, secondary infections (Cordero *et al.,* 2012;

Suarez-Esquivel and Castro-Ramirez, 2016) and colic (Mair *et al.,* 2014). Eventhough information related to thyroid functions in horses is scarce, hypothyroidism was reported to be related with laminitis, chronic myositis and poor reproduction performance (Breuhaus *et al.,* 2006). Radio-immuno assay being the gold standard test for hormonal analysis is used for determination of cortisol, T3 and T4 level in horses. The aim of this study was to monitor the cortisol, iodothyronine (T3), thyroxine (T4) level in *T. equi* infected horses (confirmed by PCR) in comparison with the healthy group to determine the extent of involvement of metabolic pathways as well as endocrine system in equine theileriosis.

Materials and Methods

The study was carried out from September 2014 to May 2015. In all, 72 blood samples were collected in sterile K_3EDTA vials and serum clotting vials from horses managed under field and farm conditions. DNA of *T. equi* was extracted from whole blood by using universal polymerase chain reaction based on equine merozoite antigen-5 and 6. The stress hormone cortisol and metabolic hormones T3 and T4 were estimated in serum samples by employing RIA techniques.

Polymerase chain reaction

DNA extraction was carried out according to the instruction manual of QUIAamp DNA blood mini kit (Quiagen, Germany). The DNA was quantified by using nanodrop spectrophotometer (Eppendorf Thermofisher) and stored at -20°C until used for PCR. DNA of *Theileria equi* was amplified by using PCR where the primer sequences used were Forward (EMA-5) 5'-TCGACTTCCAGTTGGAGTCC-3' and Reverse (EMA-6) 5'-AGCTCGACCCACTTATCAC-3' (Batssetseg *et al.,* 2001). The amplification conditions for *T. equi* included 40 cycles with enzyme activation at 95°C for 10 min, denaturation at 94°C for 1 min, primer annealing at 60°C for 1 min and amplification at 72°C for 1 min followed by final extension at 72°C for 5 min. The final PCR products were subjected to electrophoresis in a 1.5% agarose gel with TBE buffer and DNA bands were visualized by using UV transilluminator or Gel documentation System. Positive samples for *T. equi* detected by using Giemsa stained blood smear technique were used as positive control.

Radio-immunoassay

Serum samples of 72 horses were used for estimation of cortisol and thyroid hormones by using the competitive Radio-immuno assay. The standard assay kits (Beckman Coulter, Cat. 1699 and Cat. 1447) containing monoclonal antibody-coated tubes, ^{125}I-labeled tracer, calibrators or control samples (lyophilized) were used for estimation of cortisol and thyroid hormones. Samples and calibrators were incubated with ^{125}I-labeled specific hormone to be tested as a tracer in antibody-coated tubes. After incubation, the liquid content of tubes was aspirated and the radioactivity was determined in gamma counter. A standard curve was prepared and unknown values were obtained from the curve by interpolation.

Data on endocrine profile was statistically analysed using student's "t" test as (Snedecor and Cochran, 1994), where the $p<0.05$ was considered as statistically significant and $p<0.01$ as statistically highly significant.

Results and Discussion

Out of 72 horses' blood samples screened for theileriosis by PCR using specific primers against equine merozoite antigen-5 and 6, twenty five (34.72%) horses were found positive. The rests 47 were considered as healthy ones. The cortisol level in *T. equi* infected horses was 42.80±0.69 ng/ml, while it was 24.40±0.12 ng/ml in healthy group. The cortisol level was found to be increased significantly ($p<0.05$) in *T. equi* infected horses compared to healthy horses. Cortisol, a physiological indicator of stress, is a steroid hormone produced by adrenal cortex stimulating gluconeogenesis, fatty acid mobilisation and protein breakdown. The increased cortisol levels may result from one of several mechanisms, such as destruction of a portion of the pituitary gland and overproduction of other hormones like adreno-corticotropic hormone (ACTH) or cortisol releasing hormone (CRH). Wang *et al.* (2014) reported that resting without any particular exercise can also increase the stress level of horses. The cortisol concentration studied in horses under different housing and work conditions by using

radioimmunoassay revealed that horses with abnormal cortisol circardian ratio (CCR) had 2.3 times more chance to have colic (Leal *et al*., 2011). Tapan (2013) reported breed-wise, age-wise and sex-wise cortisol level in healthy camels and alteration of the same in case of trypanosomisis.

The T3 level was 0.44±0.13 ng/ml in *T. equi* infected horses, while it was 1.20±0.19 ng/ml in healthy group. The T3 level was found to be decreased significantly ($p<0.01$) in *T. equi* infected horses compared to healthy horses. The T4 level was 47.56±4.06 ng/ml in *T. equi* infected horses, while it was 38.14±2.40 ng/ml in healthy group. Thus, the T4 level was found to be increased significantly ($p<0.05$) in *T. equi* infected horses compared to healthy horses. Thyroxine (T4) is the main secretory product of the normal thyroid gland and a considerable amount of T3 is derived from T4. Tri-iodothyronine (T3) is 3-5 times more potent than T4 and its activation is step-regulated individually by peripheral tissues. The deficiency of thyroid hormone affects the function of all organ systems. Most signs are directly related to slowing of metabolism, which results in lethargy, unwillingness or inability to exercise as was seen in the present study. It was also reported that thyroid hormones are affected in case of bovine theileriosis (Sangwan *et al.,* 2002; Badiei *et al.,* 2010). Tapan (2013) reported significant increase in both T3 and T4 level in case of trypanosomiasis in camels. Bayley *et al.* (1995) opined that thyroid status should be based on both T3 and T4 as T3 is being more stable than T4 and more reliable for monitoring and correlating with the disease conditions.

Conclusion

The increase in cortisol level may be due to the stress from diseases associated with underlying *T. equi* infection. The decrease in T3 and increase in T4 level indicates affection of thyroid gland. Thus, the cortisol and thyroid hormones may be used as indicator for equine theileriosis. This may be the first study reporting the alteration in cortisol and thyroid hormone levels in horses affected with theileriosis.

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Conflict of Interest:

All authors declare that they have no conflict of interest.

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