

Original Research Article

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Haematological Alterations in Gastrointestinal Helminths Infected Horses at Anand District of Gujarat, India

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ABSTRACT

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A study was conducted to estimate the haematological alterations of gastrointestinal helminthic infection in horses of Anand district of Gujarat, India from March-2016 to February-2017. Based on qualitative faecal examination, a total number of 70 gastrointestinal helminths infected and 70 non-infected blood samples were collected during the clinicodiagnostic approach from horses for study of haematological parameters. The infected horses showed a significant reduction in the mean Hb (10.111 ± 0.13 g/dl), TEC ($6.504 \pm 0.1010^6/\mu\text{l}$), PCV (30.064 ± 0.42 %), lymphocyte (39.856 ± 0.26 %), monocytes (2.892 ± 0.06 %) and significant increase in TLC ($9.897 \pm 0.16 \times 10^3/\mu\text{l}$), neutrophil (52.914 ± 0.30 %), eosinophil (4.033 ± 0.09 %), basophil (0.302 ± 0.01 %), MCH (15.618 ± 0.13 pg) and MCHC (33.798 ± 0.16 g/dl). The MCV level increased non-significantly in infected horses and recorded as 46.289 ± 0.46 fl.

Introduction

Parasitic diseases are responsible for the poor health of equine due to their direct effects like irritation, annoyance, intoxication, mechanical obstruction, tissue destruction, competitive food uptake, anaemia, due to which their condition is lost and draught power is reduced. Gastrointestinal parasites, in heavy infection may bring alteration in the normal haematological values among affected animals (Pavord and Fisher, 1987) like

neutrophilia, eosinophilia and anaemia (Thamsborg *et al.*, 1998).

Materials and Methods

The study was done at Veterinary Clinical Complex (VCC), Department of Surgery and Radiology, College of Veterinary Science and A. H., AAU, Anand and the surrounding field areas. Animals were bled from the jugular vein into vacutainer tubes containing EDTA (Ethylene Diamine Tetraacetic Acid) for the

estimation of various haematological parameters. Haematological studies of the blood samples collected from horses were done by Automatic Whole Blood Analyzer (Mindray BC- 2800 Vet) at the Department of Animal Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, AAU, Anand. The haematological parameters viz. haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocytes count (TLC), differential leukocyte count (DLC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin

Concentration (MCHC) were studied from gastrointestinal helminths infected and non-infected horses.

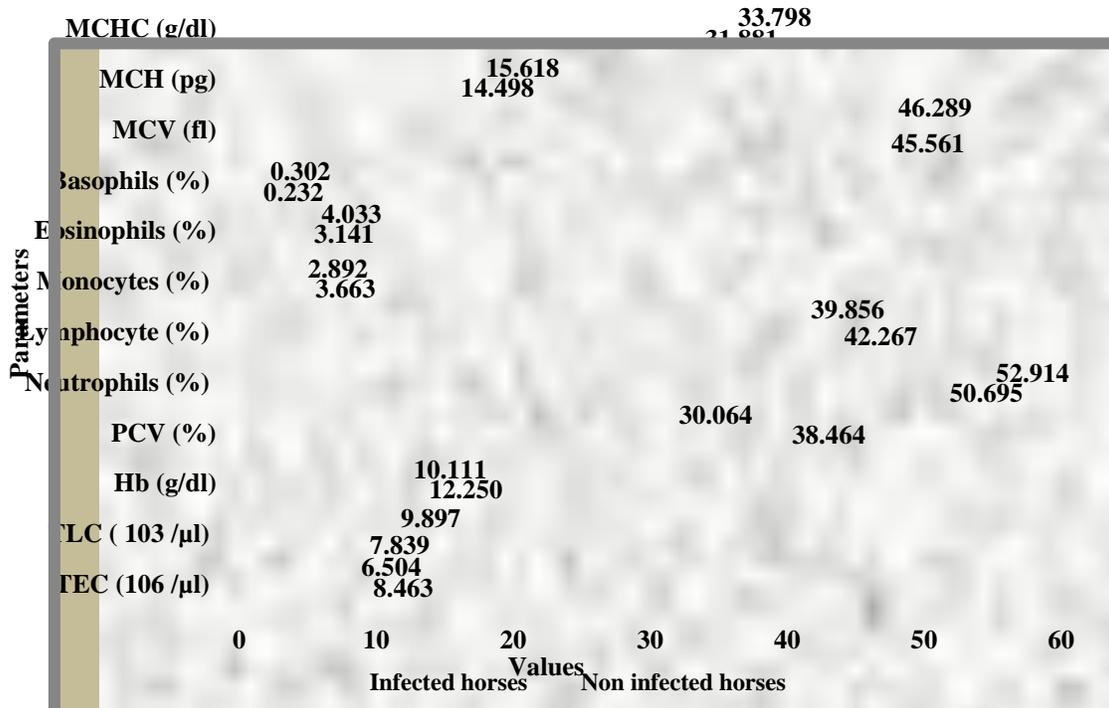
Results and Discussion

The infected horses showed a significant reduction in the mean Hb, TEC, PCV, lymphocytes, monocytes and significant increase in TLC, neutrophils, eosinophils, basophils, MCH and MCHC as compare to non infected horses. The MCV level increased non-significantly in infected horses. Haematological values were listed in table 1 and figure 1.

Table.1 Haematological values of gastrointestinal helminths infected and non- infected horses (Mean ± SE)

Sr. no.	Parameters	Non infected horses (n=70)	Infected horses (n=70)
1	Total Erythrocyte Count (TEC) 10 ⁶ /μl	8.463±0.18	6.504±0.10*
2	Total Leukocyte Count (TLC)10 ³ /μl	7.839±0.22	9.897±0.16*
3	Haemoglobin (Hb) g/dl	12.250±0.19	10.111±0.13*
4	Packed Cell Volume (PCV)%	38.464±0.63	30.064±0.42*
5	Neutrophils (%)	50.695±0.71	52.914±0.30*
6	Lymphocyte (%)	42.267±0.66	39.856±0.26*
7	Monocytes (%)	3.663±0.10	2.892±0.06*
8	Eosinophils (%)	3.141±0.09	4.033±0.09*
9	Basophils (%)	0.232±0.01	0.302±0.01*
10	Mean Corpuscular Volume (MCV) fl	45.561±0.60	46.289±0.46**
11	Mean Corpuscular Haemoglobin (MCH) pg	14.498±0.18	15.618±0.13*
12	Mean Corpuscular Haemoglobin Concentration (MCHC) g/dl	31.881±0.30	33.798±0.16*
(* = p< 0.05, ** = p> 0.05)			

Fig.1 Haematological values of gastrointestinal helminths infected and non- infected horses (Mean ± SE)



The decreased value of Hb, PCV and TEC recorded in the present study could be due to the nature of helminths, particularly of strongyles, which are well known voracious blood suckers which cause direct loss of whole blood (Souls by, 1982; Peal *et al.*, 1989 and Sohail, 1989). Decrease in TEC values might be a result of suppression of erythropoietic activity of bone marrow by parasites (Hayat *et al.*, 1999). Heavy worm loads (strongyles) generally lead to anaemia, caused by both migrating larvae and adult worms to the branches of the intestinal (mesenteric) arteries where they cause damage, irritation and parasitic aneurysm-verminous arteritis. The larva causes anaemia by inducing haemorrhagic tracts in the liver parenchyma during migration and also by producing nodules in the wall of caecum and colon. On rupture of these nodules considerable bleeding takes place. Similarly, the adults suck considerable amount of blood

causing anaemia (Radostits *et al.*, 2007). The mean value of total leukocyte count (TLC) was significantly increased ($p < 0.05$) from $(7.839 \pm 0.22t$ o $9.897 \pm 0.1610^3/\mu\text{l})$ in non-infected as compare to infected horses. These findings are in accordance with Kadyrov (1979); McCrow and Slocombe (1985); Esmat *et al.*, (1997); Sipra *et al.*, (1999); Lewa *et al.*, (1999); Hubert *et al.*, (2004); Francisco *et al.*, (2009); Bodecek *et al.*, (2010); Parsani *et al.*, (2011); Singh *et al.*, (2012); Kumar (2012); Kachhawa (2013); Mudgal (2013); Khan *et al.*, (2014) and Salem *et al.*, (2015). In the present study, increased values of total leukocyte count in gastrointestinal parasites infested horses could be due to localized helminths infection and secondary bacterial infection of gastrointestinal tract of horses as stated by Benjamin (1985). This may also attributed to larval migration of helminth parasites via liver and lung.

The mean value of neutrophils (%) was significantly increased ($p < 0.05$) from 50.695 ± 0.71 to 52.914 ± 0.30 % in non-infected and infected horses, respectively. These findings are in agreement with several workers *viz.* Murphy and Love (1997); Corning (2009); Kumar (2012); Kachhawa (2013); Mudgal (2013) and Khan *et al.*, (2014). Neutrophils are actively amoeboid and phagocytic. They engulf foreign particles and generally digest them. Neutrophils manufacture a trypsin-like enzyme with which they digest foreign particles and dead tissue. When the foreign particle enters in the body, the leukocytes pass out of the blood vessels and surround the threatened area. Neutrophils through their pseudopodial process engulf the foreign particle and destroy them. The phagocytic action of neutrophils may thus, be correlated with their increased number in the present study (Mudgal, 2013). Neutrophils when released into the circulation, has a bizarre, multilobed nucleus and numerous cytoplasmic granules that resembles lysosomes. The granules contain a host of hydrolytic, oxidative and proteolytic enzymes as well as two antibacterial substances, lysozyme and phagocytin. Their function is engulfing and destroying foreign material by phenomenon called as phagocytosis. For phagocytosis of helminth material particles opsonins are required. Opsonins represent the specific antibodies directed against helminthic material. Other functions of neutrophils are secretion of lytic substances to degrade helminth cuticular portions as pathogens (Jones and Hunt, 1983).

The mean value of lymphocytes (%) was significantly decreased ($p < 0.05$) from 42.267 ± 0.66 to 39.856 ± 0.26 % in non-infected and infected horses, respectively. These results are in conformity with the findings of Sipra *et al.*, (1999); Kumar (2012); Kachhawa (2013); Mudgal (2013); Khan *et al.*, (2014) and Salem *et al.*,

(2015). Decreased percentage of lymphocytes were suggestive of active participation of lymphocytes in immune mechanism to evade the helminth antigen. Hence their numbers gradually decreased.

The mean value of monocytes (%) was significantly decreased ($p < 0.05$) from 3.663 ± 0.10 to 2.892 ± 0.06 % in non-infected and infected horses, respectively. These findings were in accordance with several workers including Sipra *et al.*, (1999); Kachhawa (2013); Mudgal (2013); Waqas *et al.*, (2014) and Salem *et al.*, (2015). Monocytes are capable of phagocytizing and digesting the particulate matter, such as cellular debris. When foreign body enters in the body these monocytes leave the blood vessels through exocytosis and reach to the tissues and different organs and converted into the macrophages. They are responsible for processing helminth antigen thus enhancing the cell mediated and humoral immunity. These macrophages are capable of digesting and phagocytizing foreign material. Monocytes number is decreased in this study it may be due to their release from blood vessels in massive number and their conversion into macrophages.

The mean value of eosinophils (%) was significantly increased ($p < 0.05$) from 3.141 ± 0.09 to 4.033 ± 0.09 % in non-infected and infected horses, respectively. These findings are in line with the findings of Kadyrov (1979); McCraw and Slocombe (1985); Murphy and Love (1997); Sipra *et al.*, (1999); Bodecek *et al.*, (2010); Parsani *et al.*, (2011); Kumar (2012); Kachhawa (2013); Mudgal (2013) Khan *et al.*, (2014); Waqas *et al.*, (2014) and Salem *et al.*, (2015). In the present study, eosinophilia was noticed in the helminth infected horses. The suggested reason is constant irritation caused by the migration of the larvae through intestinal mucosa causing damage and inflammation

along the way (Leder and Weller, 2000). The increased eosinophil count could be due to local immune response in the gut for the nematodes, which results in circulating and tissue hypereosinophilia (Dawkins *et al.*, 1989). Eosinophils play a definite role in the development of immunity and phagocytosis of the antigen- antibody complex (Litt, 1964). Eosinophils play a significant role in engulfing foreign materials and helminths. Eosinophils are also thought to play a role in the immune response, possibly accepting antigen or “information” from macrophages that have engulfed antigen. In massive infections, cell counts may increase 10–30 times. Eosinophils have receptors that bind them to antibody and then kill the parasite, presumably through a unique component of the eosinophil granule, the eosinophil major basic protein (MBP). MBP has been shown to kill parasites *in vitro* (David *et al.*, 1980).

The mean value of basophils (%) was significantly increased ($p < 0.05$) from 0.232 ± 0.01 to 0.302 ± 0.01 % in non-infected and infected horses, respectively. These findings are in accordance with Kumar (2012); Kachhawa (2013); Mudgal (2013) and Khan *et al.*, (2014). Basophils and mast cells have similar functions. Basophils and mast cells have FcER receptor which acts as a binding site for IgE antibody in the cell. When helminthic infection takes place then helminth antigens along with mast cell bound IgE triggers mast cell degranulation and the release of vasoactive molecules and proteases. These molecules stimulate smooth muscle contraction and increase the vascular permeability. The violent contractions of the intestinal muscles and the increase in permeability of intestinal capillaries leading to an efflux of fluid into the intestinal lumen can result in dislodgment and expulsion of many worms (Tizard, 1996). Same way basophils play role against helminth infection.

MCH and MCHC values were increased significantly ($p < 0.05$) but there was non significant ($p > 0.05$) increase in MCV values in infected horses as compared to non infected horses. MCV, MCH and MCHC values were higher in infected horses as compared to non-infected horses but these values were within normal range according to the Sastry (1976). In this study haemoglobin (Hb), total erythrocyte count (TEC) and packed cell volume (PCV) values were reduced in infected horses as compared to non infected horses.

It is suggestive of normocytic normochromic anaemia. Gasser *et al.*, (2004) observed normocytic, normochromic anaemia in horses suffering from strongyloid nematodes.

There is compensatory mechanism in nature in providing the body with adequate amount of erythrocyte aeration surface. Even in the same species, a lower erythrocyte count is made up by an increase in the red cell diameter and hence there is increase in the MCV as well as increase in the MCH (Sastry, 1976).

In conclusion, haematological alterations resulted in normocytic normochromic anaemia, neutrophilia, eosinophilia and lymphocytopenia in infected horses as compare to non infected horses.

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Responsibilities

Prakritisinh contributed to study design and to data collection, analysis and interpretation. J. J. Hasnani and P. V. Patel contributed for data analysis and interpretation. Namrata

Singh and Adesh Kumar contributed for data analysis.

All the authors contributed to the preparation of paper and approved the final manuscript for the publication.

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