

Original article

## EFFECT OF PARASITIC INFESTATION ON CARPAL SYNOVIAL CONSTITUENTS IN DONKEYS (*EQUUS ASINUS*)

## M. A. H. ABDELHAKIEM<sup>1</sup>, G. I. SOLIMAN<sup>2</sup>, M. RUSHDI<sup>3</sup> & H. K. ELSAYED<sup>4</sup>

<sup>1</sup>Department of Surgery, Anesthesiology and Radiology, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt; <sup>2</sup>Postgraduate researcher, Elkoseyia City, Assiut Governorate, Egypt; <sup>3</sup>Clinical Laboratory Diagnosis, Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University, Egypt; <sup>4</sup>Internal Medicine, Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University, Egypt.

## Summary

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The present study was carried out to examine the physical, microscopical and biochemical parameters of the synovial fluid from the carpal joints of donkeys suffering from *Strongylus* spp. and *Parascaris equorum* infestation. Forty-five donkeys were selected out from a total of sixty animals based on faecal analysis. Animals were divided into 3 groups, which included *Strongylus* spp. (n=23), mixed infestation (n=17, *Strongylus* spp. and *Parascaris equorum*) and control (n=5) groups. The aspirated synovial fluid from all animals (n=45) was examined physically and microscopically. Then, the total protein, albumin, globulins, calcium, phosphorus, magnesium, glucose levels, and gamma glutamyl transferase (GGT) and alkaline phosphatase (ALP) activities were measured in the serum and synovial fluid of all animals. The results revealed no changes of the physical and microscopical features of the synovial fluid of the infested and control animals but there were significant decreases in total protein, albumin, globulin, glucose and magnesium levels in the serum of *Strongylus* and mixed infection groups. The synovial fluid analysis exhibited an increase of calcium and phosphorus levels, and ALP activity, and decreased levels of total protein, glucose and GGT in *Strongylus* and mixed groups. The results of this study suggested significant changes in some biochemical parameters in both serum and synovial fluids in donkeys suffering from parasitic infestation.

Key words: analysis, carpus, donkeys, parasite, serum, synovial fluid

#### INTRODUCTION

Synovial fluid (SF) fills the cavity of the diarthroidal joints. It has several functions such as nourishment and maintenance of

the articular cartilage, removal of metabolites and lubrication of the joint. SF contains mediators which influence articular cartilage metabolism, such as cytokines, free radicals and hormones (Van Pelt, 1974; O'Farrel & Costello, 1980; Todhunter, 1996). It is a clear, transparent straw coloured viscid fluid but during joint affection, some changes of its cellular, chemical and physical characters may occur. The changes of the SF may indicate the severity of joint affection and metabolic derangement within the joints, but it does not refer to specific disease (Tyagi & Krishnamurthy, 1974; Jani *et al.*, 1994; Pal *et al.*, 1994; Barvalia *et al.*, 1995).

Although donkeys in the developing countries are considered very important draft farming animals, they do not get great care from their owners either in their nutrition or treatment. Helminthic parasites, particularly strongyle nematodes are common inhabitants of the gastrointestinal tract of equine species and can cause hazards with clinical signs varying from inappetence and weakness to sudden death (lewa *et al.*, 1999; Umur & Acici, 2009).

In the human literature, there is a debate about the impact of helminths on the joints. Some studies reported reactive arthritis due to helminths infestation (Vigliani & Campaille, 1977; Weinberger et al., 1979; Akoglu et al., 1984), while the others recorded the modulating effect of helminths in case of joint inflammation. Some of these studies proved that the arthritic animals experimentally infected with helminths had less severe inflammatory changes in the joints compared to animals that had arthritis and were free from parasitic infestation (Pearson & Taylor, 1975; Osada et al., 2009; Salinas-Carmona et al., 2009; He et al., 2010; Matisz et al., 2011).

Undoubtedly, the internal parasitism causes detrimental effects of the body systems including the joints. The synovial fluid is ultrafiltrate of the plasma (Van Pelt, 1974; Todhunter, 1996), so the changes of plasma in case of internal parasitic infestation may be followed by changes in SF. So the authors hypothesised that the parasitic infestation in donkeys may affect the synovial constituents and or joint components leading to joint disease.

To the authors' knowledge, this is considered the first study investigating the changes of the SF of the carpal joints in donkeys naturally infected with helminth parasites (*Strongylus spp.* and *Parascaris equorum*).

#### MATERIALS AND METHODS

#### Animals

A total of sixty were selected from onehundred donkeys presented to the Veterinary Teaching Hospital (Assiut University, Egypt) for different purposes. The selected animals had normal parameters (temperature, heart rate, and respiratory rate), but some of them were emaciated, had pale mucous membranes, decrease of feed intake or decrease the body gain with a normal appetite. All of the donkeys were free from any apparent external lesions.

These donkeys (n= 60) were subjected to faecal analysis detecting that 40 out of 60 animals were infested with *Strongylus* spp. and *Parascaris equorum*. The remaining 20 animals included 15 animals (excluded from the study) infested with parasites other than the 2 previous species (*Strongylus* spp. and *Parascaris equorum*), and 5 animals free from parasitic infestation. The latter 5 animals were used as control animals in this study.

According to the faecal analysis, donkeys (n=45) were classified into three major groups: *Strongylus* spp. (n=23), mixed infestation (n=17, *Strongylus* spp. and *Parascaris equorum*) and control (n=5) groups.

Animals were 25 males and 20 females, their age ranged from 1-5 years (mean 3 years) and their weights ranged from 80-120 kg (mean 100 kg).

### Samples

Faecal samples were collected from the rectum in clean and dry plastic cups and analysed directly after collection according to Soulsby (1982).

Blood samples (whole blood and serum) were collected from the jugular vein from the 45 animals according to Coles (1986). Whole blood samples were collected on Vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant and used for white blood cells count by means of a haemocytometer directly after collection. Serum samples were harvested and stored at -20 °C till biochemical analyses.

#### Synoviocentesis (joint tap)

The carpal joints were selected for synoviocentesis. It was performed according to Southwood (2008). They were free from any apparent lesion such as synovial effusion, peri-articular oedema or cellulitis, heat, redness and pain on palpation, extension or flexion. Also, there were no wounds in close proximity to joints. The donkeys were restrained by holding the limbs and head without sedation. The hair of synoviocentesis site was clipped. It was lavaged then scrubbed using 3.5% iodine tincture several times before the aspiration of synovia.

Synovial fluid collection was carried out under strictly aseptic conditions. The cranial approach for synoviocentesis was used. The carpus was flexed and the sterile needle (22 gauge, 1.5 inch) was inserted perpendicularly in the palpable space between the carpal bones either medial or lateral to the extensor carpi radialis tendon. The radiocarpal and carpometacarpal joints were easily detected and aspirated provided that the carpus was moderately flexed. The SF samples (3 mL) were collected from the carpal joint of the 45 animals and then divided into two parts; one part was stored at 4 °C for 2 days to test clot formation and the other part was kept in tube containing heparin and mixed properly to prevent coagulation and used for physical, microscopical and biochemical analysis

#### Physical examination of synovia

Physical examination included colour, viscosity, coagulation, odour, transparency, mucin test and clot formation. Samples for testing clot formation were kept in refrigerator for two days. Mucin test was performed according to the method described by Chauhan & Agarwal (2008).

#### Microscopical examination of synovia

Total synovial leukocytes count was measured in the SF using haemocytometer method, and then the SF samples were centrifuged; the supernatant fluid was collected in Eppendorf tubes and kept for biochemical analysis. A drop from the sediment was applied on a microscopic slide and examined for number of leukocytes/ high power field (leukocytes/HPF) according to the method described by Chauhan & Agarwal (2008).

# Biochemical analysis of serum and synovial fluid

The following biochemical constituents were measured in serum and SF samples: total protein, albumin, globulins, calcium, phosphorus, magnesium, glucose, gamma glutamyl transferase (GGT) and alkaline phosphatase (ALP) using commercial kits (Spectrum Diagnostics, Cairo, Egypt), and UV spectrophotometer (Optizen 3220 UV, Mecasys Co. Ltd, Korea).

#### Statistical analysis

Statistical analysis of the obtained data was done by using Statistical Package for the Social Sciences for Windows (SPSS, version 21, Chicago, IL, USA). Data were expressed as mean and standard deviation. Data from infested donkeys were compared with control group using one-way ANOVA. Statistically significant differences were determined at P $\leq$ 0.01 (highly significant) and P<0.05 (significant).

#### RESULTS

Forty-five donkeys were included in this study. According to the faecal analysis, donkeys were classified into three major groups: *Strongylus* spp. (n=23), mixed infestation (n=17, *Strongylus* spp. and *Parascaris equorum*) and control (n=5) groups.

*Strongylus* spp. group was re-classified according to the faecal egg count into mild (0–500 epg), moderate (500–1000 epg) and severe (>1000 epg) infesta-

tion groups (Soulsby, 1982). The mixed infestation group was classified according to the faecal egg count – mild (0–500 epg), moderate (500-1000 epg) and severe (>1000 epg) into five sub-groups as followed:

- Mixed mild (mild Strongylus spp. and mild Parascaris equorum);
- Mixed moderate (moderate *Strongylus* spp. and moderate *Parascaris equo-rum*);
- Moderate mild (moderate Strongylus spp. and mild Parascaris equorum);
- Severe mild (severe *Strongylus* spp. and mild *Parascaris equorum*);
- Severe moderate (severe *Strongylus* spp. and moderate *Parascaris equo-rum*).

Synovial fluids from donkeys infested with intestinal parasites were colourless, transparent, and viscous. No coagulation was detected in SF stored at 4 °C for 2 days. Results from the mucin test were the same in different groups and appeared as a tight ropy clump in a clear solution.

There were no significant changes in total WBCs count and in WBCs/HPF in all donkeys infested with parasites in comparison to the control group.

 Table 1. Effect of different degrees of Strongylus spp. infestation on serum biochemical constituents in donkeys. Data are expressed as mean±SD

Demonster	Control	S	trongylus spp. infestat	ion
Parameter	(n=5)	Mild (n=7)	Moderate (n=13)	Severe (n=3)
Total proteins (g/L)	72.7±6.2	62.5±13.8	70.1±15.5	50.5±4.5**
Albumin (g/L)	26.0±0.7	22.0±4.2	23.1±11.8	23.0±4.0
Globulins (g/L)	46.7±5.6	40.5±17.8	46.9±17.6	27.5±8.5**
Calcium (mmol/L)	2.06±0.22	2.32±0.67	2.27±0.67	2.73±1.0
Phosphorus (mmol/L)	1.43±0.46	1.44±0.31	$1.47 \pm 0.49$	1.36±0.37
Magnesium (mmol/L)	1.18±0.15	0.95±0.18*	0.90±0.24**	0.79±0.0.06**
Glucose (mmol/L)	4.48±0.17	4.21±1.1	3.88±1.23	3.19±0.35**
GGT (U/L)	16.11±3.91	11.93±4.45	11.65±6.09	8.46±6.11
ALP (U/L)	137.77±46.92	170.84±92.65	155.24±51.31	154.96±38.66

GGT: gamma glutamyltransferase; ALP: alkaline phosphatase; \*P<0.05, \*\* P $\leq$ 0.01 compared with control group.

 Table 2. Effect of different degrees of *Strongylus* spp. infestation on synovial biochemical constituents in donkeys. Data are expressed as mean±SD

Parameter	Control	St	rongylus spp. infestat	ion
Parameter	(n=5)	Mild (n=7)	Moderate (n=13)	Severe (n=3)
Total proteins (g/L)	25.1±6.1	21.1±4.3	23.2±8.6	19.8±10.4
Albumin (g/L)	8.4±5.0	9.3±5.9	8.5±2.7	12.3±8.1
Globulins (g/L)	16.7±10.5	11.8±7.9	14.6±8.3	7.5±6.2
Calcium (mmol/L)	$1.42 \pm 0.31$	1.77±0.56	1.89±0.49*	1.96±0.32*
Phosphorus (mmol/L)	1.19±0.08	1.3±0.38	1.51±0.71	2.13±.89*
Magnesium (mmol/L)	$1.00 \pm .30$	0.87±0.25	0.83±0.15	1.12±0.10
Glucose (mmol/L)	4.74±0.78	3.83±1.17	3.2±0.61**	3.06±0.47**
GGT (U/L)	$7.09 \pm .54$	4.02±1.47**	4.10±1.56**	4.40±.00**
ALP (U/L)	26.94±6.82	73.17±44.83*	49.51±17.32**	64.66±14.91**

GGT: gamma glutamyltransferase; ALP: alkaline phosphatase; \*P<0.05, \*\* P $\leq 0.01$  compared with control group.

The results of serum and synovial biochemical analysis of *Strongylus* spp. infested groups were summarised in Tables 1 & 2 and data about serum and synovial biochemical analysis of mixed infested groups were tabulated in Tables 3 & 4.

### DISCUSSION

The parasitic infestation especially with *Strongylus* spp. causes gastrointestinal tract (GIT) troubles in equine animals (Bliss, 1999). The other systems may be influenced due to decrease GIT absorption and decrease of perfusion of the main nutrients into the cells. Thereby, the present study was conducted to determine the changes of physical, microscopical and biochemical constituents of the SF in donkeys confirmed to have intestinal parasites.

The present study investigated the effect of two species of parasites inhabiting the gastrointestinal tract in donkeys. *Strongylus* spp. and *Parascaris equorum* are considered the commonest parasites that affect donkeys as reported by Gul *et al.* (2003); Shrikhande *et al.* (2009); Matthews & Burden (2013).

In the present study, the different degrees of parasitic infestation in donkeys did not cause any significant changes of the physical characters of the SF. It is worthy to mention that the mucin test is considered an indicator for the viscous property and quality of hyaluronic acid present in the SF (O'Farrell & Costello, 1980; McIlwraith, 1996). Hyaluronan is one of the components of the synovial fluid. It has anti-inflammatory effect (Punzi et al., 1989; Frisbie et al., 2009). So, the parasitic infestation did not affect hyaluronan level within the synovial fluid. Further, it referred to the integrity of the articular cartilage of the carpal joint. Any changes of the joints especially the articular cartilage will reduce the polymerisation of hyaluronic acid molecule. Eventually, a poor quality clot will appear (Palmer & Bertone, 1994; Smith et al., 2002). The decrease of viscosity of SF may result from either simple effusion due to trauma of the joint and plasma influx into the joint or synovitis which leads to decrease of hyaluronic acid synthesis (Fournier et al., 1969). In the present work, absence of clot formation within the SF indicates the normality of samples and

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ParameterControlMixed mildMixed moderateModerate mildSevTotal proteins $(g/L)$ $(n=5)$ $(n=5)$ $(n=5)$ $(n=5)$ $(n=3)$ $(n=3)$ Total proteins $(g/L)$ $72.7\pm 6.2$ $62.5\pm 13.8$ $61.0\pm 10.1$ $61.7\pm 6.2 \pm$ $47.5\pm 7.5\pm 7.5\pm 7.5\pm 7.5\pm 7.5\pm 7.5\pm 7.5\pm $	ParameterControl $(n=5)$ Total proteins $(g/L)$ $72.7\pm 6.2$ $12.7\pm 6.2$ Albumin $(g/L)$ $26.0\pm 0.07$ $26.0\pm 0.07$ Globulins $(g/L)$ $2.6.0\pm 0.07$ $46.7\pm 0.56$ Calcium $(mmol/L)$ $2.06\pm 0.22$ $1.43\pm 0.46$ Phosphorus $(mmol/L)$ $1.43\pm 0.46$ $1.3\pm 0.15$ GGT (UL) $1.43\pm 0.15$ $16.11\pm 3.91$ GGT (U/L) $1.37.77\pm 46.92$ GGT: gamma glutamyltransferase; ALP: alka in donkeys. Data are expressed as mean $\pm$ SD	Mixed mild (n=5) $62.5\pm13.8$ $22.0\pm4.2$ $40.5\pm17.8$ $2.15\pm0.64$ $1.38\pm0.2$ $0.9\pm0.23*$ $5.22\pm1.8$ $1.7.82\pm8.14$ $118.52\pm18.20$ dine phosphatase; *I parasitic infestation	ParameterControlMixed mildMixed moderateModerate mildSevere mildSevere mildSevere mildSevere moderateTotal proteins $(g/L)$ $(n=5)$ $(n=5)$ $(n=5)$ $(n=3)$ $(n=3)$ $(n=3)$ $(n=3)$ Total proteins $(g/L)$ $2.0\pm 0.07$ $2.2.0\pm 4.2$ $61.0\pm 10.1$ $61.7\pm 6.2*$ $47.5\pm 2.4**$ $19.4\pm 7.3**$ Albumin $(g/L)$ $2.6.0\pm 0.07$ $22.0\pm 4.2$ $20.2\pm 7.8$ $19.0\pm 1.2**$ $15.5\pm 3.4**$ $19.4\pm 7.3**$ Albumin $(g/L)$ $2.6.0\pm 0.07$ $22.0\pm 4.2$ $20.2\pm 7.8$ $19.0\pm 1.2**$ $15.5\pm 3.4**$ $19.4\pm 7.3**$ Albumin $(g/L)$ $2.6.0\pm 0.07$ $22.0\pm 0.22$ $2.15\pm 0.64$ $2.15\pm 0.64$ $2.15\pm 0.64$ $2.7\pm 5.1$ $32.0\pm 5.1**$ $19.4\pm 7.3**$ Albumin $(g/L)$ $1.43\pm 0.46$ $1.38\pm 0.2$ $1.99\pm 0.37$ $2.22\pm 0.63$ $2.22\pm 9.6.63$ $3.8\pm 0.2$ $1.92\pm 0.63$ Magnesium (mmo/L) $1.43\pm 0.17$ $2.06\pm 0.22$ $2.15\pm 0.64$ $2.15\pm 0.68$ $0.78\pm 0.15$ $1.42\pm 0.53$ $1.42\pm 0.38$ Magnesium (mmo/L) $1.43\pm 0.17$ $5.22\pm 1.8$ $2.64\pm 0.14**$ $3.02\pm 1.53*$ $5.07\pm 2.38$ $3.18\pm 0.54**$ GGT $(U/L)$ $16.11\pm 3.91$ $17.82\pm 18.20$ $22.4\pm 2.5\pm 3.44.25*$ $9.76\pm 1.51*$ $17.68\pm 0.50$ ALP $(U/L)$ $13.77\pm 46.92$ $118.52\pm 18.20$ $224.25\pm 344.25*$ $9.76\pm 1.51*$ $174.25\pm 0.50$ GGT camma glutamyltransferase; ALP: alkaline phosphatase; *P<0.05, ** P \le 0.01 compared with control group. $141.23\pm 62.76$ $144.26\pm 41.15$	Moderate mild (n=3) $61.7\pm6.2*$ $19.0\pm1.2**$ $42.7\pm5.1$ $2.27\pm0.63$ $2.23\pm0.48$ $0.78\pm0.15**$ $3.02\pm1.53*$ $5.96\pm2.75**$ $2.19.86\pm70.09$ mpared with control	Severe mild (n=3) 47.5±2.5** 15.5±3.4** 32.0±5.1** 2.21±0.91 1.42±0.53 1.08±0.3 5.07±2.38 9.76±1.51* 141.23±62.76 1 group.	Severe moderate (n=3) 46.6±15.1** 19.4±7.3** 27.2±10.0** 1.93±0.68 1.93±0.68 1.42±0.38 1.01±0.09 3.18±0.54** 17.68±0.50 144.26±41.15 144.26±41.15
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Albumin (g/L) $26.0\pm0.0$ Globulins (g/L) $46.7\pm0.0$ Calcium (mmol/L) $46.7\pm0.0$ Phosphorus (mmol/L) $1.43\pm0.0$ Magnesium (mmol/L) $1.43\pm0.0$ Magnesium (mmol/L) $1.43\pm0.0$ GGT (U/L) $1.18\pm0.0$ ALP (U/L) $1.6.11\pm3.0$ GGT (U/L) $137.77\pm0.0$ GGT: gamma glutamyltransferase;	07 56 22 46 15 17 .91 .91 .91 .51 ALP: alka s of mixed mean±SD	22.0±4.2 40.5±17.8 2.15±0.64 1.38±0.2 0.9±0.23* 5.22±1.8 17.82±8.14 118.52±18.20 dine phosphatase; *F	20.2±7.8 40.7±2.8 2.15±0.15 1.99±0.37 1.50±0.68 2.64±0.14** 8.30±0.40** 224.25±34.25* ><0.05, ** P≤0.01 co	19.0±1.2** 42.7±5.1 2.27±0.63 2.23±0.48 0.78±0.15** 3.02±1.53* 5.96±2.75** 219.86±70.09 mpared with contro <i>Parascaris equoru</i>	15.5±3.4** 32.0±5.1** 2.21±0.91 1.42±0.53 1.08±0.3 5.07±2.38 9.76±1.51* 141.23±62.76 1 group. m) on synovial bioc	19,4±7.3** 27.2±10.0** 1.93±0.68 1.42±0.38 1.01±0.09 3.18±0.54** 17.68±0.50 144.26±41.15 hemical constituents
$\begin{array}{llllllllllllllllllllllllllllllllllll$	56 22 46 15 17 .91 .46.92 ALP: alka s of mixed mean±SD	40.5±17.8 2.15±0.64 1.38±0.2 0.9±0.23* 5.22±1.8 17.82±8.14 118.52±18.20 line phosphatase; *I parasitic infestation	40.7±2.8 2.15±0.15 1.99±0.37 1.50±0.68 2.64±0.14** 8.30±0.40** 224.25±34.25* ><0.05, ** P≤0.01 co	42.7±5.1 2.27±0.63 2.23±0.48 0.78±0.15** 3.02±1.53* 5.96±2.75** 219.86±70.09 mpared with contro <i>Parascaris equoru</i>	32.0±5.1** 2.21±0.91 1.42±0.53 1.08±0.3 5.07±2.38 9.76±1.51* 141.23±62.76 1 group. <i>m</i> ) on synovial bioc	27.2±10.0** 1.93±0.68 1.42±0.38 1.01±0.09 3.18±0.54** 17.68±0.50 144.26±41.15 hemical constituents
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Magnesium (mmol/L)         1.18±0.           Glucose (mmol/L)         4.48±0.           GGT (U/L)         16.11±3           ALP (U/L)         137.77±           GGT: gamma glutamyltransferase;	15 17 .91 .46.92 ALP: alka s of mixed nean±SD	0.9±0.23* 5.22±1.8 17.82±8.14 118.52±18.20 line phosphatase; *I parasitic infestation	1.50±0.68 2.64±0.14** 8.30±0.40** 224.25±34.25* ><0.05, ** P≤0.01 co. (Strongylus spp. and	0.78±0.15** 3.02±1.53* 5.96±2.75** 219.86±70.09 mpared with contro <i>Parascaris equoru</i> .	1:08±0.3 5:07±2.38 9.76±1.51* 141.23±62.76 l group. <i>m</i> ) on synovial bioc	1.01±0.09 3.18±0.54** 17.68±0.50 144.26±41.15 hemical constituents
Glucose (mmol/L) 4.48±0. GGT (U/L) 16.11±3 ALP (U/L) 137.77± GGT: gamma glutamyltransferase;	17 .91 .46.92 ALP: alka s of mixed mean±SD	5.22±1.8 17.82±8.14 118.52±18.20 dline phosphatase; *I parasitic infestation	2.64±0.14** 8.30±0.40** 224.25±34.25* ><0.05, ** P≤0.01 co	3.02±1.53* 5.96±2.75** <u>219.86±70.09</u> mpared with contro <i>Parascaris equoru</i> .	5.07±2.38 9.76±1.51* 141.23±62.76 l group. m) on synovial bioc	3.18±0.54** 17.68±0.50 144.26±41.15 hemical constituents
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ALP (U/L) 137.77± GGT: gamma glutamyltransferase;	46.92 ALP: alka s of mixed mean±SD	118.52±18.20 lline phosphatase; *F	224.25±34.25* ><0.05, ** P≤0.01 co ( <i>Strongylus</i> spp. and	219.86±70.09 mpared with contro Parascaris equoru.	141.23±62.76 l group. m) on synovial bioc	144.26±41.15 hemical constituents
GGT: gamma glutamyltransferase;	ALP: alka s of mixed mean±SD	lline phosphatase; *I parasitic infestation	<0.05, ** P≤0.01 co. (Strongylus spp. and	mpared with contro Parascaris equoru.	l group. m) on synovial bioc	hemical constituents
Parameter	ol	Mixed mild	Mixed moderate	Moderate mild	Severe mild	Severe moderate
1 au anneus (n=5)	()	(n=5)	(n=3)	(n=3)	(n=3)	(n=3)
(g/L)	6.1	15.5±6.5*	$18.2\pm 2.2$	$16.0 \pm 7.0$	$10.2\pm 2.4^{**}$	$20.4 \pm 4.0$
Albumin (g/L) 8.4±5.0	0.	$8.1 \pm 2.1$	$12.6 \pm 6.3$	$12.6\pm 8.3$	$5.4 \pm 1.9$	7.9±4.6
Globulins (g/L) 16.7±10.5	0.5	7.4±5.0	$5.6 \pm 4.1$	3.3±1.4	4.8±3.7	$13.4 \pm 3.1$
Calcium (mmol/L) 1.42±0.31	.31	$1.62 \pm 0.34$	$1.52 \pm 0.25$	$1.45 \pm 0.83$	$1.43 \pm 0.21$	$1.99 \pm 0.58$
Phosphorus (mmol/L) $1.19\pm0.08$	.08	$1.72 \pm 0.63$	$1.29 \pm 0.16$	$1.21 \pm 1.01$	$1.13 \pm 0.16$	$1.67 \pm 0.92$
Magnesium 1.00±0.30 (mmol/L)	.30	1.34±0.47	$1.27 \pm 0.00$	$1.50 \pm 0.52$	$0.96 \pm 0.16$	$1.06 \pm 0.18$
Glucose (mmol/L) 4.74±0.78	.78	$4.69 \pm 0.78$	$1.68\pm0.21^{**}$	$5.24\pm1.63$	$3.56 \pm 0.8$	$3.02 \pm 0.87 *$
GGT (U/L) 7.09±0.54	.54	$4.15\pm0.72^{**}$	$4.35\pm1.15**$	4.4±1.55**	$1.86\pm0.83^{**}$	$5.19\pm 1.26^{*}$
ALP (U/L) 26.94±6.82	6.82	55.42±23.09**	74.55±47.55*	50.20±17.52*	51.63±9.46**	54.20±21.23*

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that they are free from fibrinogen or other large molecules of plasma (Sonowa *et al.,* 2010).

Generally, the WBCs are low in the SF of normal joint and its elevation indicates synovitis, while their presence in high number indicates infection (Tew & Hotchkiss, 1981). In the present study, there were no significant changes in synovial total WBCs count and in number of WBCs/HPF in all donkeys infested with parasites in comparison to control animals. Also, the total blood WBCs count showed no significant changes in all groups of donkeys infested with parasites compared to the control group; these findings are in accordance with the data of Abd-El-Salam (1998). On the other side, total synovial WBCs count in all groups was lower than that reported by Abd Ellah et al. (2012), who found that WBCs count in the SF from normal donkeys was 127.60±42.6/mm<sup>3</sup>. Normal synovial WBC count in studied groups indicated absence of inflammatory changes in the joint, which is supported by normal physical characters of the SF.

This study revealed the significant decrease of serum total protein and globulin in case of severe Strongvlus infestation, and significant decrease of total protein and albumin in case of moderate mild, severe mild and severe moderate mixed parasitic infestations (Table 1, 3). These results were consistent with the results of the previous studies (Jasko & Roth, 1984; Giles et al., 1985; Esmat et al., 1997; Murphy & Love, 1997; Thamsborg et al., 1998; Smets et al., 1999; Corning, 2009; Bodecek et al., 2010). However, at the same time some of these studies (Jasko & Roth, 1984; Giles et al., 1985; Murphy & Love 1997; Thamsborg et al., 1998) recorded an increase of serum globulins in horses and ponies affected with few

*Strongylus*. On the other hand, Ju *et al.*, (1993) and Guzel *et al.* (2014) found no changes in the total protein, albumin and globulin of horses suffering from internal parasitism.

It was reported that the decrease of serum protein may resulted from decreased feed intake, decrease of synthesis of protein by the liver or increase of losses (Sharkey & Radin, 2010). The Strongylus spp. are causing enetropathy which is considered one of the reasons beyond the protein losses (Jasko & Roth, 1984; Giles et al., 1985). According to the aforementioned reasons, the gastrointestinal lesions due to parasites are possibly the main causes behind the severe losses in serum total proteins. This assumption is verified by the associated decrease of globulin and magnesium. Furthermore, the present study showed significant hypoglycaemia which may occur due to poor nutrition or decreased benefit from ingested food due to the intestinal parasitism. In addition, the necrosis and desquamation of the intestinal epithelium and villi due to Strongylus infestation (Parsani et al., 2011) may lead to decreased intestinal surface area for absorption and in turn reduction of total protein, albumin, globulin and glucose in the blood. The parasitic infestation leads to a series of signs such as inappetence, anorexia, diarrhoea, decrease of body gain and emaciation (Galdhar & Roy, 2004; Peregrine et al., 2006). Moreover, the adverse effect of the internal parasites within the GIT was recorded in the previous studies (Jasko & Roth, 1984; Giles et al., 1985; Murphy & Love, 1997; Parsani et al., 2011). Thereby, the decrease of feed intake, protein losing enteropathy and decrease of protein absorption from GIT are considered the main factors beyond the hypoprotenaemia, hypoalbuminaemia and hypoglobulinaemia in donkeys with *Parascaris equorum* and/or *Strongylus* infestations. It was noticed from the results of this study that the effect of mixed infestation (*Strongylus* and *Parascaris equorum*) was more severe than the *Strongylus* infestation alone. This may be attributed to the double harmful effect of mixed infestation on the animals and their GIT which resulted in the obvious decrease of serum biochemical constituents of mixed infested group than in the *Strongylus* only group.

The results of this study revealed that hypoalbuminaemia was in both groups of parasitic infestation unlike the hypoglobulinaemia noticed in the mixed group only. This may be attributed to the fact that albumin is small molecule compared to the globulin, so it could be easily lost with fluids in case of enteropathy (Dobson, 1965; Holmes *et al.*, 1968). The damage of the intestinal crypts and mucosa is expected to be high in the case of the mixed parasitic infestations. Therefore, loss of both albumin and globulin is occurring.

The significant decrease in synovial total protein level in case of mixed mild (P<0.05) and severe mild (P<0.01) infestation may be attributed to hypoproteinaemia in the infested groups and decrease of the filtration of proteins with higher molecular size through the synovial membrane (Liberg *et al.*, 1977; Tulamo *et al.*, 1989).

It was reported that the SF is the ultrafiltrate of plasma, and the concentrations of glucose and electrolytes in the SF are nearly similar to those in plasma or serum (Steel, 2008). Therefore, the increase or decrease of electrolytes or glucose in the serum or plasma leads to their increase or decrease in the SF. In this study, the levels of all measured blood variables were higher in blood than in SF, because synovia derives its constituents from blood plasma (Liberg *et al.*, 1977). The present work revealed a significant decrease in glucose SF level which was consistent with hypoglycaemia (decreased glucose level in the serum). Glucose content tends to be similar in different joints, resembling levels in serum (van Pelt, 1974). In the present investigation, reduction in serum and synovial glucose level in donkeys infested with parasites might be due to the fact that adult worms depend on the carbohydrates which are available in GIT, consequently, the host glucose level will decrease. Jatkar & Singh (1974) recorded an inverse relationship between the glucose level and the number of parasites within the GI tract of the infested host. The aforementioned causes may contribute to the decrease of glucose level in the blood of the affected donkeys (Scofield, 1974).

Although serum calcium, phosphorus and ALP were not changed, their concentrations within the SF increased significantly. It is postulated that the increase of these parameters in SF might be attributed to subtle changes in the joints especially in the young and growing animals (Sharkey & Radin 2010). The obtained results revealed significant hypomagnesaemia in Strongvlus spp. infested donkeys, which may be ascribed to malabsorption of magnesium from the intestine as suggested by Parsani et al., (2011). However, the severe mixed infestation was not associated with significant changes in the serum magnesium level. This could not be fully elucidated but hypomagnesaemia in the Strongylus spp. group might be related to both decreased magnesium intake and the increase of its renal elimination or cellular translocation unlike the donkeys that had a mixed infestation (Sharkey & Radin, 2010).

The non-significant changes of serum enzymes especially ALP and GGT may rule out the hepatocellular damage as a cause of hypoprotenaemia and hypoglycemia and support the poor nutrition and hazard effect of intestinal parasites. Enzyme profiles are one of main biochemical parameters used in equine medicine to assess muscle and liver functions. While serum level of GGT is usually measured to evaluate liver function (Kaneko et al., 1997). The results of the present study revealed that the serum ALP was significantly increased (P<0.05) in case of mixed moderate infested group. A significant increase in synovial ALP was observed in case of mild (P<0.05), moderate (P<0.01) and severe (P<0.01) Strongylus spp. infestation group. These results are consistent with the results of the previous studies (van Pelt, 1974; Liberg et al., 1977). ALP is released from a variety of sources including liver, intestine and bone (Sharkey & Radin 2010). Therefore, the increase of ALP indicated the intestinal damage due to the parasitic infestation.

In the present study, synovial GGT activity was significantly decreased (P<0.01) in donkeys infested with Strongylus spp.  $\pm$ Parascaris equorum. Synovial GGT decreased significantly in case of mild, moderate and severe Strongvlus spp. infestation. There was a correlation between the severity of parasitic infestation and decrease of GGT. The decrease of GGT due to Strongylus spp. infestation could not be fully understood. There were two previous studies which recorded the increase of GGT and glutathione peroxidase in case of arthritis (Rambabu et al., 1990; Ostalowska et al., 2006). It is well known that glutamate is one of the three amino acids which form the glutathione. The latter form the different enzymes such as glutathione peroxidase and reductase which are anti-oxidants. According to the aforementioned studies, the increase of GGT in SF indicates arthritis or even any

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joint lesion, however the GGT was low than normal in case of *Strongylus* spp. in the present study. So, the arthritis and joint lesion in donkeys suffering from mild, moderate and severe *Strongylus* infestation could be ruled out. Also, the decrease of synovial GGT, despite being insignificant, correlated with its decrease in serum.

A limitation of this study was the use of a small number of animals (5) as a control group. Increased number of healthy (parasitic free) donkeys is required to get accurate values for the serum and synovial parameters to be used as reference values.

#### CONCLUSION

According to the results of the present study, *Strongylus* spp. and *Parascaris equorum* did not cause significant physical or microscopical changes of the SF but resulted in significant decreases of total proteins, glucose, GGT, and significant increases of calcium, phosphorus and ALP in the carpal joint of donkeys. Further studies using advanced diagnostic tools and techniques are required for evaluation of the joints in case of parasitic infestation.

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## Correspondence:

Mohammed Ahmed Hamdy Abdelhakiem Department of Surgery, Anesthesiology and Radiology, Faculty of Veterinary Medicine, Assiut University, Assiut, 71526, Egypt, phone: +01094024645/+20882333938, fax: +20882366503, email: hamdysurgery@yahoo.com, https://orcid.org/0000-0002-8888-5274