World Journal of Zoology 6 (2): 196-203, 2011
ISSN 1817-3098

Impact of Parasitic Infestation on Ovarian Activity in Buffaloes-Heifers with Emphasis on Ascariasis

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Abstract: Ovarian activity is the most important factor that determined productive efficiency in buffalo-heifers. The present study was designed to throw light on the impact of parasitic infestation on ovarian activity in buffalo-heifers, with special references to ascariasis. The study was conducted on 435 mature non pregnant buffalo-heifers reared at Lower Egypt and kept in smallholder farms. Animals were clinically and gynecologically examined, also blood and fecal samples were collected. Heifers were divided into two groups; the 1st group included 141 heifers with normal cyclic ovaries and the 2nd group included 204 heifers suffering from ovarian inactivity during the breeding season of buffaloes (September-March). Fecal samples were examined for determined internal parasites and blood smears were examined for blood parasites. The incidence of internal, external and blood parasites was recorded in both groups. Results revealed that, heifers suffering from ovarian inactivity had obviously higher incidence of different parasitic infestation as compared to the normal cyclic heifers. Heifers infested with ascariasis showed macrocytic hypochromic anemia, changed red cell parameters and decreased total leucocytic count, neutrophils and lymphocytes with oesinophilia. Decreased serum glucose, cholesterol, total proteins, albumin, globulins, calcium, inorganic phosphorus, zinc and copper concentrations were evident in heifers having inactive ovaries heifers with marked changes in oxidant/antioxidant parameters. It was concluded that parasitic infestation has adverse effect on ovarian activity of buffalo heifers, especially coccidiosis and ascariasis

Key words: Buffalo-heifers · Ovaries · Ascariasis · Blood constituents · Oxidant/antioxidant parameters

INTRODUCTION

Buffaloes represent an important part of the agricultural economy in Egypt and some other developing countries. The world buffalo's population is 166.4 million heads, according to the last survey [1]. In Egypt, there is 3.920 million heads producing 3,300,000 ton of milk and 270000 ton of meat [2]. This species is mostly reared in small holder farms under harsh socioeconomic conditions, this leads to low productive and reproductive performances.

Parasitic infestation represents an important cause of direct and indirect losses in farm animals [3]. It can affect the reproductive performance of farm animals through impaired growth rate of young stocks, increased age at puberty in heifers and prolonged the inter estrous intervals in mature animals [4]. Endoparasites cause significant economic losses and health problems in domestic animals [5]. Also, in animals fed on green fodder, natural infection with gastrointestinal nematodes are commonly observed, these internal parasites decreased average daily gain and retard sexual maturation [6]. An outbreak of haemoparasitoses affected animals of various ages Brown Swiss. The animals presented fever, severe anemia, jaundice, abortion or premature birth, loss of appetite, decrease milk production and accentuated weight loss in a short period of time [5]. Arthropod ectoparasites are the most ubiquitous life forms affecting ruminant animals and commonly affect the daily activity and health status of ruminants [7].

In buffaloes, a tight negative relationship was detected between parasitic infestation and oxidative status of the animals [5].

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The current investigation aimed to through light on the role of parasitic infestations, either by external or internal parasites on ovarian activity in mature buffalo heifers. Investigations of the changes in some relevant blood constituents following infestation (Taking *Ascaris* infestation as an example)especially of oxidant/antioxidant markers was another target.

MATERIALS AND METHODS

A total number of 345 mature non pregnant buffaloe-heifers was included in this investigation which was carried out during a period of 2 years. These animals were 2-3 years old, reared at small holder farms in Lower Egypt and were examined during the breeding season of buffaloes (September-March). Animals were mainly feed on green fodder and small amount of concentrates with and deprived from regular veterinary services. A complete case history was recorded and heifers were clinically examined for general body conditions as well as for the presence of external parasites.

Gynecological Examination: Gynecological examination was carried out by rectal palpation aided by ultrasonography (Pia Medical Falcs e`Saote, the Netherlands with an endorectal linear array 6-8 MHz transducer) to monitor ovarian status. Animals were divided into 2 main groups: The first group included 141 mature non pregnant normal cyclic animals. The second group included 204 heifers that suffering from ovarian inactivity.

Sampling: Blood samples with and without anticoagulant as well as fecal samples were collected. Blood samples were collected in tubes containing anticoagulant powder (EDTA) for performing complete blood picture, determination of glutathione reduced (GSH) value [8], as well as examination of smears for blood parasites [9]. The coagulated blood samples were used for separation of serum (x1500.g; 15 minutes; 4° C) and analysis of some blood constituents.

Analysis:

- Complete blood picture including erythrocytic count, haemoglobin concentration, packed cell volume (PCV %), red cell indices, total leuckocytic count and differential leuckocytic count were done according to standard techniques described by Feildman *et al.* [10].
- Glucose, total protein, albumin, cholesterol, calcium and inorganic phosphorous concentration were analyzed using diagnostic kits (Biodiagnostic, Egypt).
- Serum trace elements (Zn and Cu) were determined according to the method described by Fernandez and Kahn [11] using atomic absorption spectrophotometer.
- Oxidant/antioxidant markers including Malondialdhyde (MDA) [12], nitric oxide (NO) [13], catalase (CAT) [14], superoxide dismutase (SOD) [15], Ascorbic acid (ASCA) [16] glutathione-reduced (GSH) [17] and total antioxidant activity (TAA) [18] were analyzed using diagnostic kits (Biodiagnostic, Egypt).
- Fecal samples were examined physically for its color, presence of blood and mucous then examined microscopically using direct smear and floatation sedimentation technique for parasites according to Soulsby [11].

Statistical Analysis: All numerical data were statistically evaluated for the mean and standard error. The significance of the results was determined using Student "t" test [19].

RESULTS

Table (1) reveals that, parasitic infestation by the different types of parasites adversely affect the ovarian function in buffalo-heifers. The incidence of internal, blood and external parasites averaged 91.18 vs. 48.94, 16.18 vs. 9.22 and 31.37 vs.13.48% in buffalo-heifers with inactive and active ovaries, respectively. Coccidia and ascaris were the main prevailing internal parasites, while Babesia was the main prevailed blood parasites and lice was the main external parasites recorded in this study.

Ascariasis infested heifers revealed decreased ($P<0.01$) erythrocytic parameters (PCV, Hb concentration and RBCs count), with developed of macrocytic hypochromic anemia. In the same time, these animals revealed decrease ($P<0.001$) TLC, neutrophils and lymphocyte with eosinophilia (Table 2). Table (3) shows that, ascariasis induced decreased blood glucose, cholesterol concentrations, total protein, albumin, globulins concentration ($P<0.001$) as compared with healthy heifers. Ascaris infested heifers showed significant decrease in calcium ($P<0.001$) and inorganic phosphorous ($P<0.01$) concentrations.

Table 1: Effect of parasitic infestation on ovarian Aactivity in mature buffalo-heifers

Parasitic infestation	Normal cyclic heifers (N= 141)	Heifers having inactive ovaries (N= 204)
Internal parasites		
Ascaris	22 (15.60)	59 (28.92)
Coccidia	29 (20.56)	82 (40.20)
Ascaris and Coccidia	14 (9.93)	31(15.20)
Fasciola	4 (2.84)	14 (6.86)
Total	69 (48.94)	186 (91.18)
Blood parasites		
Babesia	5 (3.55)	15 (7.35)
Thieleria	4 (2.84)	9 (4.41)
Babesia and Thieleria	4 (2.84)	9 (4.41)
Total	13 (9.22)	33 (16.18)
External parasites		
Lice	11 (7.80)	44 (21.57)
Mange	8 (5.67)	18 (8.82)
Ticks	0 (00.0)	2 (0.98)
Total	19 (13.48)	64 (31.37)

Number of positive cases from the total number of the group (%)

Table 2: Effect of ascariasis infestation on hemogram of buffalo-heifers (Mean±SE)

Parameters	healthy heifers (N=10)	Ascaris infested heifers (N=10)
-RBCs (×10^6/µl)	5.86±0.07	5.08±0.16***
-Hb (g/dl)	11.74±0.11	10.07±0.09***
-PCV (%)	34.04±0.22	28.33±0.42***
-MCV (fl)	52.10±0.32	54.61±0.12*
-MCH (pg)	19.71±0.22	18.87±0.14*
-MCHC (%)	36.18±0.17	36.01±0.13
-WBCs (×103/µl)	8.13±0.16	6.11±0.15***
-Neutrophils (%)	32.24±0.76	28.10±0.12***
-Lymphocytes (%)	56.24±0.45	51.20±0.45***
-Monocytes (%)	6.42±0.11	6.87±0.09
-Eosinophils (%)	3.46±0.19	7.93±0.49***
-Basophils (%)	0.04±0.01	0.00

*Significant at P<0.05. *** Significant at P<0.001.

Table 3: Effect of ascariasis infestation on some serum biochemical constituents in buffalo-heifers (Mean± SE)

Parameter	Healthy heifers (N=10)	Ascaris infested heifers (N=10)
-Blood glucose (mg/dl)	65.59±1.23	51.61±2.18**
-Cholesterol (mg/dl)	152.26±4.79	124.07±2.36**
-Total proteins (g/dl)	5.33±0.09	4.78±0.11**
-Albumin (g/dl)	3.67±0.05	3.41±0.03**
-Globulins (g/dl)	1.67±0.09	1.19±0.09**
-A/G ratio	2.26±0.13	2.56±0.07

** Significant at P<0.01.

Table 4: Effect of ascariasis infestation on some mineral and trace element values in buffalo heifers (Mean±SE)

Parameter	Healthy heifers (N=10)	Ascaris infested heifers (N=10)
-Calcium (mg/dl)	10.23±0.21	9.07±0.10***
-Inorganic phosphorus (mg/dl)	4.85±0.30	3.65±0.08**
-Ca/P ratio	2.15±0.08	2.49±0.03**
-Zinc (Zn, µg/dl)	158.4±5.49	119.0±1.99***
-Copper (Cu, µg/dl)	185.7±2.60	93.3±3.12***

** Significant at P<0.01. *** Significant at P<0.001.

Table 5: Effect of ascariasis infestation on oxidant/antioxidant values in the serum of buffalo-heifers (Mean±SE)

	Healthy heifers (N=10)	(Ascaris infested heifers (N=10)
-Malondialdhyde (MDA, mmol/L)	1.89±0.09	4.98±0.17**
-Nitric oxide (NO, µmol/L)	21.22±0.34	35.342±0.75**
-Catalase (CAT, U/ml)	2.58±0.09	0.67±0.07**
-Superoxide dismutase (SOD,U/ml)	336.37±2.84	301.341±2.36**
-Ascorbic acid (ASCA, µgm/L)	139.26±1.11	109.11±0.91**
-Glutathione-reduced (GSH, mmol/L)#	7.24±0.32	3.16±0.18**
-Total antioxidant activity (TAA, mmol/L)	1.58±0.07	0.51±0.01**

*** Significant at P<0.01. # in whole blood

Those heifers shown significant increase in Ca/P ratio (P<0.01) compared with healthy heifers. Also, the infested heifers revealed a significant decrease in zinc and copper (P<0.001) concentrations compared with the healthy heifers (Table 4). Table (5) indicates that, ascaris infested heifers have significant increased (P<0.001) of MDA and NO with a significant decrease (P<0.01) of CAT, SOD, GSH and TAA.

DISCUSSION

In Egypt, buffaloes have a unique role in livestock production and the agricultural economy whereas, it produces about 65% of total consumable meat and milk [20, 21] However, the productivity of these animals is limited by poor reproductive efficiency, seasonality, reproductive disorders as long post-partum anoestrus and it is further hampered by several external environmental factors mainly; nutrition and parasitism [22]. C*ryptosporidium parvum* is recognized as one of the most important pathogens causing enteritis and severe diarrhea in calves up to 1 month of age and the infection may be responsible for some mortality. Its impact is mainly associated with the impairment of intestinal functions and lower performance of animals [23]. Also, coccidiosis, often caused by *Eimeria zuernii* infection, is an important diarrheal disease in calves [24]. Bangoura and Daugschies [25] reported that, acute sublethal *E. zuernii* coccidiosis causes distinct loss of fluid and blood via intestine. Klevar *et al.* [26] added that, *N. caninum* infection might cause abortion problems in high risk areas. Also, infection by theileria limits the movement of cattle between countries and can result in production losses and high mortality in susceptible animals. Because these diseases are most severe in recently introduced animals, they are a constraint on the importation of new breeds or improved stock. The two diseases with the greatest economic impact in cattle are East Coast fever (infection with *Theileria parva*) and tropical theileriosis (infection with *Theileria annulata*) [27]. Kamau *et al*. [28] reported that, *Theileria* cause a benign infection of cattle in parts of Australia whereas they are endemic, but have, in recent years, been suspected of being responsible for a number of outbreaks of disease in cattle near the coast of New South Wales. Also several outbreaks of anemia, jaundice, abortion and mortality in cattle in New South Wales were attributed to the intracellular parasite *Theileria buffeli* [29]. The intracellular protozoan parasite *Theileria annulata* causes a severe and often fatal, disease of pure and cross-bred cattle in tropical and subtropical countries [30]. Villi *et al.* [31] added that, clinical exploration of the animals infected with *Trypanosoma theileri* revealed fever, progressive weight loss, anemia and frequent recumbent position.

From the clinicopathological point of view, delayed puberty heifers revealed macrocytic hypochromic anemia with significant decrease in erythrocytic parameters (PCV, Hb content and RBCs count); these findings coincided with those given by Ahmed *et al*. [20] where they found that, blood picture of non cyclic buffalo-heifers revealed significant decreased of erythrocytic count, Hb content, PCV and MCHC together with increased MCV. Lotfollahzadeh *et al*. [32] concluded that the anaemia observed in cattle infected with *F. hepatica* is a normocytic, hypochromic anaemia and the most important aetiology of the anaemia is the chronic blood loss due to the blood-sucking activity of the adult flukes and leakage of blood from the bile duct to the intestine, which results in iron deficiency. The increased activities of serum enzymes indicated chronic hepatic and bile duct injuries associated with chronic infection with *F. hepatica.* Bangoura and Daugschies [25] reported that, acute sublethal *E. zuernii* coccidiosis causes distinct loss of fluid and blood via intestine. This dominates also the haematological picture of the disease, which is mainly characterized by haemoconcentration. Leukocyte concentration was depressed during the early patent period, whereas it increased markedly from day 24 after infection on. Also, Lotfollahzadeh *et al*. [32] discussed that, haematological analyses for whole blood and serum

of *Fasciola hepatica* infected cattle revealed decreased levels of packed cell volume (PCV), haemoglobin concentration, mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) compared with uninfected cattle, also decrease in the concentration of serum iron was observed. Culgovici *et al*. [33] added that anemia was the most important change found in Trypanosoma infected animals, which showed lower averages of PCV than parasitologically negative animals. Infected individuals showed lower averages of PCV than parasitologically negative animals, indicating higher anemia in the former compared with the latter group. Mostafa [3] mentioned that, the causes of decreasing erythrocytic paramerters may be attributed to the direct effect of under nutrition or the indirect effect associated with parasitic infestation as well as Cu deficiency. It was found that iron transport within the body is adversely affected and tended to accumulate in many tissues following Cu deficiency [34]. Moreover, Hb content in Cu deficient animals was significantly low [35]. Cu is essential for erythrocyte production [36]. Cardoso *et al.* [37] reported hypochromic anaemia is related to Cu deficiency.

Delayed pubertal heifers showed significant decrease in TLC, neutrophils and lymphocyte, these findings might be due to absence of estrogen which is responsible for normal cellular and humeral immune response in heifers [38]. Migration of leukocytes to be infiltrated in tissue of genital tracts may be another cause, especially in cases associated with bacterial infection. Also, it was reported that unsuitable agroclimatic conditions are associated with low leucocytic count [39]. On the other hand, the significant eosinophilia in the current delayed heifers was a normal consequence to parasitic infestation as demonstrated before [40].

In the present study, the concentrations of some serum biochemical parameters in delayed puberty heifers revealed significant decreased in calcium and inorganic-phosphorous concentrations with significant wide Ca/P ratio. Also, there was a significant decrease in total proteins, albumin, globulins, glucose and cholesterol concentrations as compared with normal animals. Similar findings were recorded by Abdoon *et al.* [41] who related these results to ration formulation, management and agroclimatic conditions. Also, it was found that the parasitic infestation has a detrimental effect on serum total protein, albumin, globulin, calcium and inorganic phosphorous concentrations [42]. Degar *et al.* [5] observed that, the activities of Cu-Zn in the *Dictyocaulus viviparus* infected cattle were low in comparison to the control group. Also, Saber *et al*. [43] reported that, cattle clinically infected with the blood parasite (*T. annulata*) had significantly lower serum total protein, calcium, cholesterol and triglycerides concentrations and significantly higher alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity, phosphorus, sodium, potassium, bilirubin (direct and indirect) and Blood Urea Nitrogen (BUN) concentrations than the healthy cattle.

Serum Cp concentration was significantly lower in delayed puberty heifers as compared with normal puberty heifers. Sharma *et al.* [36] observed a significant decreased of serum Cp in the Cu deficient animals. They advocated the use of Cp: Cu ratios in the diagnosis of induced Cu disorders in ruminants. Although serum Cp decreased by Cu deficiency, the relative concentration increased during infection and inflammation [44]. Cp also promoted the incorporation of iron into the storage protein, ferritin [45].

The present study clarified that the concentrations of some trace elements Zn and Cu were lowered in delayed heifers compared with the normal pubertal heifers. These findings agreed with those of Saxena *et al.* [46] who found a correlation between serum Cu and Zn concentrations and age at puberty in crossbred heifers, but Small *et al.* [47] did not find serum Zn, Cu, or Magnesium concentrations to be related to first-service conception rates in cattle. In heifers loss of appetite and reduced fertility are seen even in Cu deficiency, along with interference in the release of LH. It was suggested that the cause is the inhibitory effect of estradiol in the pituitary gland due to sex hormonal disturbances [48]. Cu absorption in ruminants is low (>1.0-10.0%) relative to values reported in non-ruminants [49].

The percentage of dietary Zn that is absorbed decreased as dietary Zn increased in ruminants. Zn requirements of ruminants appear to be affected by dietary factors based on the variable animal responses that were observed after Zn supplementation. However, dietary factors that affect Zn bioavailability in ruminants are not clearly defined [50].

It was found that Zn deficiency in the female lead to problems such as impaired synthesis/secretion of follicular stimulating hormone FSH and LH, abnormal ovarian development, disruption of the estrous cycle, frequent abortion, a prolonged gestation period, teratogenicity, stillbirths, difficulty in parturition, pre-eclampsia, toxemia and low birth weights of infants [51]. Ahmed *et al.* [52] noticed that heifers suffered from Zn and Cu deficiencies show delayed puberty, stunted

growth and infertility. It was reported that supplementation of deficient dairy cows with Zn improve reproductive performance via shorting interval (30 days) from calving to resumption of oestrus as compared to control (69 days) [53].

The present study highlighted association between oxidative stress and depleted antioxidant system on one hand and the occurrence of delayed puberty in buffalo-heifers on the other hand whereas, the concentrations of some oxidant/antioxidant markers revealed a significant increase of MDA and NO with a significant decrease of CAT, SOD, GSH and TAA in the delayed heifers compared with the normal pubertal heifers. Similar results reported by Ahmed *et al.* [20,21], whereas, they found a tight relationship between oxidative stress and ovarian inactivity in buffalo-heifers. This implies that the delayed pubertal heifers were under stress condition. Oxidative stress has been implicated as a major initiator of tissue damage and could affect enzymatic activity, signal transcription and gene expression, especially apoptotic gene [54]. Lotfollahzadeh *et al*. [32] discussed that, the increased activities of serum enzymes indicated chronic hepatic and bile duct injuries associated with chronic infection with *F. hepatica.* Also, Degar *et al.* [5] observed that, the concentration of MDA was high, but the activities of SOD and CAT and the concentration of GSH, vitamin C and beta-carotene were low (Cu-Zn-SOD, CAT, GSH, vitamin C and beta-carotene in the *Dictyocaulus viviparus* infected cattle in comparison to control animals. It was reported that oxidative stress plays a number of significant roles in female reproductive biology; mainly it influences ovarian function by affecting the growth of Graafian follicles and oocyte maturation [55]. Saleh [56] concluded that, *B. bigemina* infection in cattle is associated with a parasitic burden-dependent corpuscular oxidative damage as indicated by membrane lipid peroxidation and methaemoglobin formation, which are contributed to COF and intravascular haemolysis. Bangoura *et al*. [24] found that, *E. zuernii* infection impairs intestinal function and induces catabolic metabolism in affected calves. Bilirubin, urea and cholesterol concentration and creatine kinase activity were particularly affected indicating catabolism of protein and lipids. Degar *et al.* [5] added that, lipid peroxidation was observed and the activities and concentrations of antioxidants system were decreased in the lungs of cattle infected with a gastrointestinal nematode parasites (*Dictyocaulus viviparous*) in comparison to non infected animals. Lotfollahzadeh *et al*. [32] observed significant increases in AST, GGT and ALP activities in cattle infected with *F. hepatica* when compared with uninfected cattle. In theileriosis as the severity of disease increased the anaemia, MCF and LDH activity increased and SOD activity decreased at any parasitaemia [57]. Shimamura *et al.* [58] reported that ROS have anti-gonadotrophic and anti steroidgenic actions in rat luteal cells. Degar *et al*. [5] suggested that endoparasitic infection is among the major causes of oxidative stress.

It was concluded that in buffaloes, a tight negative direct or indirect relationship was detected between parasitic infestation and ovarian activity.

ACKNOWLEDGEMENT

The author is greatly indebted to all colleagues participating in the weekly field trips to Lower Egypt for their technical help, support and also to National Research Centre, Egypt.

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