

Field Investigation on the Correlation Between Ovarian Activity and Fasciolosis in Buffalo-Cows

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Abstract: Reproductive disorders and parasitic infestation are the main problems that determine productivity in buffaloes and cause great economic losses. This study was designed to focus on the relationship between ovarian activity and Fasciolosis in buffalo-cows. A total number of 240 buffalo-cows reared at Lower Egypt was included in this study. Animals were clinically and gynecologically examined and blood and fecal samples were collected for carrying out some relevant analyses. Some treatment trials were carried out using anti Fasciola drugs. Results revealed that 37.5% of the examined buffalo cows suffered from ovarian inactivity. Fecal examination and ELISA test revealed that 13.75 and 18.33% of animals were positive for Fasciola, respectively. The prevalence of Fasciola was higher in buffalo-cows suffering from ovarian inactivity (28.9–38.9%) as compared to normal cyclic animals (4.7 – 6.0 %). Serum progesterone level was higher ($P < 0.001$) in Fasciola-positive as compared to normal healthy animals during the follicular phase of the estrous cycle. Serum nitric oxide (NO, ($P < 0.01$), catalase (CAT, $P < 0.05$), ascorbic acid (ASCA, $P < 0.01$), total antioxidant capacity (TAC, $P < 0.001$), copper (Cu, $P < 0.05$), iron (Fe, $P < 0.001$) and selenium (Se, $P < 0.001$) values were low in buffalo-cows suffering from fasciolosis as compared to healthy animals. Absence of clinical signs of Fasciolosis and negative fecal examination were shown in 71.43, 85.71 and 75.00% of infested animals following treatment with Ivomec Super, Fasciontel and Dectomax, respectively. It could be concluded that a tight relationship is existed between cessation of ovarian activity and Fasciolosis in buffalo-cows. ELISA detected more prevalence of Fasciolosis than routine fecal examination. Fasciontel is the drug of choice for treatment of fasciola infected buffalo-cows.

Key words: Buffaloes • Ovarian activity • Fasciolosis • Blood constituents • Treatment

INTRODUCTION

Buffaloes are the main source of good quality meat and milk in Egypt and some other developing countries, despite this species is mostly reared under harsh socioeconomic conditions and shows low productive and reproductive potentials.

Reproductive problems and parasitic infestation are the main problems that determine productivity in buffaloes and cause great economic losses. Ovarian inactivity is the main reproductive disorders facing buffalo production, especially in animals kept in small holder farms and exposed to a lot of stressful conditions such as parasitism, malnutrition, bad hygiene and pollution [1-3].

Fasciolosis is a hepatic parasitic infection caused by *F. hepatica* or *F. gigantica* that affects numerous

mammalian species, mainly ruminants and occasionally human, in several countries of Europe, Asia, America and Africa, particularly in Egypt. The economic significance of fasciolosis is mainly due to either direct losses following decreased growth rate, low milking capacity and the confiscation of altered livers in slaughterhouse [4] or the indirect losses due to the interference with the reproductive efficiency as well as retardation in the growth of young animals, biochemical and pathological conditions as anemia [5-7]. It was found that 58.4% of repeat breeder cows are seropositive to *F. hepatica* [8]. The clinical signs of fasciolosis were observed until the sixth week post-infection (PI). However, animals developed recognized symptoms of acute fasciolosis, comprising pyrexia, inappetance, anemia and poor weight gain, diarrhea and sub-mandibular and facial oedema from 5, 6, 8, 16 and 17 weeks PI, respectively.

These signs were intermittent in nature and are of variable duration [9].

Oxidative stress resulting from imbalance between oxygen reactive radicals and antioxidants has a negative effect on animal health and production [3,10] as it initiates tissue damage [11] and plays a number of significant roles in female biology [3,12,13]. Vitamins, trace elements and enzymes are among the antioxidants which can positively affect reproductive function in farm animals as administration of vitamin E and/or selenium (Se) improved fertility in cattle [3,14 -17]. Zinc, copper, iron and selenium have significant roles in reproductive biology [18] and it was recorded that decreased blood copper and zinc concentrations in growing animals result in general weakness, delayed puberty, stunted growth, macrocytic hypochromic anemia and infertility of heifers [19].

This study was designed to focus on the possible relationship between ovarian activity and fasciolosis in buffalo-cows. Also, Emphasis were stressed on the possible role of oxidative status, precise diagnosis and efficient treatment to manage this problem.

MATERIALS AND METHODS

The present study was carried out during the period extended from September 2004 – June 2007 as a part of the National Research Centre Project No. 7120106.

Animals: A total number of 240 buffalo – cows kept in small holder farms at villages of Lower Egypt was included in this study. Buffalo–cows were fed on Barseem, few amounts of concentrates, crop residues and rice straw. A full case history and owner complaint of each animal were recorded. General health condition was examined. Gynecological examination was carried out by inspecting the external genitalia and by palpating the internal genital organs through rectal examination for two successive weeks at least. Animals did not show estrous signs at least 6 months after calving during the breeding season (September – March) and have small non-functioning ovaries have been considered to suffer from ovarian inactivity. The condition was confirmed later on by monitoring progesterone level.

Samples collection

1-Blood samples: Samples of blood with and without EDTA were collected from all animals. Uncoagulated blood samples were used for performing complete blood picture as well as determination of reduced glutathione (R-GSH) and selenium values. Serum was separated from

coagulated blood samples by centrifugation ($\times 3000g$, 15 minutes at $4^{\circ}C$) and kept at $-20^{\circ}C$) until use for assaying progesterone level as well as some oxidant/antioxidant markers and for serodiagnosis of Fasciola using Enzyme-linked Immunosorbent assay (ELIZA).

2-Fecal samples: Fecal samples were collected from the rectum of animals in polyethylene bags and used for the detection of Fasciola eggs using sedimentation technique [20].

Antigen preparation: *F. gigantica* excretory secretory product (E/S) antigens were obtained as described by [21] with little modifications. In brief, mature flukes were obtained from the infected condemned livers of slaughtered buffaloes at Cairo abattoir. The flukes were washed several times with distilled water and then maintained in RPMI media- 1640 pH 7.3 containing 2% glucose 20 mM hepes and 25 mg gentamycin at $37^{\circ}C$ overnight. The culture media was collected and centrifuged at 10000 rpm for 1 hr. The protein content of supernatant (E/S) antigens was determined by the method of [22].

Analyses:

- Complete blood picture including erythrogram and leukogram were carried out and anemia indices were calculated as outlined by [23].
- Serum progesterone level was assayed by ELIZA microwell technique using kits from DIMA (Germany). The kit had sensitivity of 2.0 pg/ml with inter- and intra- run precision coefficient of variations of 2.9 and 4085, respectively [24].
- Oxidant/ antioxidant markers including malondialdehyde; MDA [25], nitric oxide; NO [26], catalase; CAT [27], superoxide dismutase; SOD [28], ascorbic acid; ASCA [29], reduced glutathione; R-GSH [30] and total antioxidant capacity; TAC [31] were calorimetrically assayed.
- Trace elements including zinc (Zn), iron (Fe) and copper (Cu) concentrations in diluted serum samples and selenium (Se) in whole blood samples were determined using atomic absorption spectrophotometer (Perkin Elmer, 2380) as outlined by [32].
- **Serodiagnosis:** The immuno assay was conducted using standard ELISA protocol [32] with some modifications. The optimal reaction condition in regards to sensitizing antigen concentration diluted

Table 1: Prevalence of Fasciolosis in relation to ovarian activity in buffalo-cows using fecal analysis and ELISA techniques (%).

Ovarian status	No. of examined animals	Fecal analysis				ELISA			
		Positive		Negative		Positive		Negative	
		No	%	No	%	No	%	No	%
Animal with normal cyclic ovaries	150	7	4.70	143	95.30	9	6.00	141	94.00
Animals with bilateral inactive ovaries	90	26	28.90	64	71.10	35	38.90	55	61.10
Total	240	33	13.75	207	86.25	44	18.33	196	81.66

serum and conjugate dilution were chosen for use with ELISA after preliminary checker board titration. In the present study, the optimal conditions were 10 mg/ ml coating buffer antigen concentration and 1: 100 serum dilutions. The cut off values of optical densities were calculated as described by [34].

Treatment trials: A total number of 45 buffalo-cows was selected to carry out some treatment trials. The animals were selected to have an average body size fits for breeding and divided into 4 groups as follows:

- The first group included 12 buffalo-cows that kept without treatment as a control group.
- The second group included 14 buffalo-cows, each was subcutaneously injected with Ivomec Super (ivermectin-clorsulon ; Merck Sharp and Dohme, the Netherlands) in a dose of 1ml / 50 Kg live body weight.
- The third group included 21 buffalo-cows, each was subcutaneously injected with Fascintel 5% (Closantel; Tornel Laboratories, Mexico) in dose of 1ml/ 15 Kg.
- The fourth group included 8 buffalo-cows, each was intramuscularly injected with Dectomex (doramectin; Pfizer, Egypt) in a dose of 1ml / 50 Kg live body weight.

Animal were treated using the recommended doses following instructions of the producing companies and checked weekly then after for absence of clinical signs of fasciolosis and negative fecal examination.

Data were computed and statistically analyzed [35].

RESULTS

1-Incidence of ovarian inactivity in buffalo-cows: Out of 240 examined buffaloes, 90 buffalo-cows (37.5%) showed bilateral smooth inactive ovaries (BSO). These animals showed no heat signs after calving for at least 6 months

during the breeding season (September – March). Rectal examination revealed small size genital organs with no palpable physiological structures (Graafian follicles – corpora lutea) on their ovarian surface.

2-Prevalence of Fasciolosis in buffalo-cows:

Examination of 240 buffalo-cows for Fasciolosis using fecal examination and ELISA revealed that 33(13.75%) and 44(18.33%) animals were positive. The prevalence of Fasciola by the two methods was higher in buffalo-cows suffering from ovarian inactivity (28.9 and 38.9%, respectively) as compared to normal cyclic (4.70 and 6.0%, respectively) animals (Table 1).

3-Effect of fasciolosis on blood constituents in buffalo-cows

3.1-Hemogram: Blood picture of Fasciola positive buffalo-cows revealed decreases of the erythrocytic count (P<0.01), Hb (P<0.001), PCV (P<0.001) and MCHC (P<0.001) together with increased MCV(P<0.001) indicating macrocytic hypochromic anemia as compared to normal healthy animals. On the other hand, leukogram of Fasciola-infected buffalo-cows showed obvious changes in lymphocytes (P<0.001), eosinophils (P<0.001) and basophils (P<0.05) as compared to healthy animals (Table 2).

3.2-Serum progesterone level: Serum progesterone level during the follicular phase of the estrous cycle was higher (P<0.001) in Fasciola-positive animals compared to normal healthy animals with no marked changes during the luteal phase (Table3).

3.3--Oxidant / antioxidant markers: Concentrations of some oxidant / antioxidant markers in the blood of buffalo-cows in relation to fasciolosis are shown in Table 4. Decreases of NO (P<0.01), CAT (P<0.05), ASCA (P<0.01) and TAC (P<0.001) were detected in the blood of buffalo-cows suffering from fasciolosis as compared to healthy animals.

Table 2: Effect of fasciolosis on the blood picture in buffalo-cows (Mean±SE)

Hemogram	Parameters	Healthy animals	Fasciola-infected animals
Erythrogram	Red cell count (10 ⁶ /μL ³)	5.57±0.11	5.21±0.05**
	Hemoglobin content (g/dl)	14.90±0.34	11.69±0.48***
	Packed cell volume (%)	36.22±0.18	36.66±0.39***
	Mean corpuscular volume(Fi)	36.60±1.93	53.74±1.18***
	Mean corpuscular Hemoglobin concentration(%)	35.91±0.10	33.55±0.52***
Leukogram	Total cell count (10 ³ /μL ³)	6.07±0.36	7.47±0.78
	Lymphocytes (%)	60.16±1.68	52.83±0.44***
	Neutrophils (%)	39.55±2.18	41.35±0.58
	Esinophils (%)	1.25±0.56	2.87±0.34***
	Basophils (%)	0.24±0.14	0.67±0.12*
	Monocytes (%)	1.25±0.60	2.28±0.34

*P<0.05 ** P<0.01 *** P<0.001

Table 3: Serum progesterone level (ng/ml) in Fasciola positive buffalo-cows during the different phases of ovarian activity (Mean±SE)

Phase	Healthy animals	Fasciola-infected animals
Follicular	0.44±0.13	0.59±0.13***
Luteal	2.89±0.17	2.88±0.63
Inactive ovary	<0.02	<0.02

*** P< 0.001

Table 4: Effect of Fasciolosis on oxidant /antioxidant markers in the blood of buffalo-cows (Mean±SE)

Parameters		Healthy animals	Fasciola-infected animals
Oxidants	MDA (mmol/ml)	1.98±0.09	1.99±0.85
	NO (mmol/L)	25.55±1.58	19.49±0.29**
Antioxidants	CAT (U/ml)	2.28±0.04	0.96±0.56*
	ASCA (μg/dl)	132.17±5.12	95.56±1.47**
	SOD (U/ml)	338.16±7.11	232.0±64.04
	GSH-R (mmol/L)	6.38±0.11	3.57±0.16
	TAC (mmol/L)	1.43±0.08	0.88±0.09***

* P<0.05, ** P< 0.01 *** P< 0.001

(MDA= Malondialdehyde, NO=Nitric oxide,CAT= Catalase, ASCA= Ascorbic acid, SOD= Superoxide dismutase, GSH-R= Glutathione reduced, TAC= Total antioxidant capacity)

Table 5: Effect of fasciolosis on some trace elements concentration in blood of buffalo- cows (Mean±SE)

Elements	Healthy animals	Infected animals
Zinc (μg/dl)	139.11±2.17	136.53±6.01
Copper (μg/dl)	78.65±4.13	59.50±6.46*
Iron (μg/dl)	168.40±4.17	111.25±1.33**
Selenium (μg/dl)	144.85±0.43	134.85±1.41***

* P<0.05, P< 0.01 *** P< 0.001

Table 6: Use of some antifasciola- drugs to treat fasciolosis in buffalo-cows

Drug	No. of Animals	Response*	
		No	%
No drug (Control)		00	00.0
Ivomec super	14	10	71.43
Fasciontel	21	18	85.71
Dectomax	8	6	75.00

*As shown by absence of clinical signs and negative fecal examination

3.4-Trace elements: Table (5) showed marked decreases of Cu (P<0.05), Fe (P<0.001) and Se (P<0.001) in Fasciola infected buffalo-cows as compared to healthy animals.

3-Treatment trials: Table (6) recorded the response of the infected animals following the application of some treatment trials. Data indicated that 71.43, 85.71 and 75.00% of Ivomec super, Fasciontel and Dectomax – treated showed absence of clinical signs of fasciolosis and negative fecal examination.

DISCUSSION

Poor reproductive efficiency and parasitic infestation are the most important obstacles for increasing buffalo productivity and cause great economic losses [1-3].

The current investigation was carried out to throws light on the most important problem that hinder sound reproduction in buffalo-cows ; ovarian inactivity and its possible relation to one of the most important parasitic infection; fasciolosis.

In this study, 37.5% of the examined buffalo-cows during the breeding season showed bilateral smooth ovary with 28.9 and 38.9% were found positive to Fasciola using parasitological examination and ELISA, respectively. A recent study [36] demonstrated a significant delay of 39 days in the onset of first estrus in fluke-infected heifers. Also, [37] concluded that fluke-infected heifers had markedly lower levels of progesterone than did uninfected animals. They suggested that liver flukes somehow alter normal metabolism and/or balance of sex hormones. In the same time, [8] reported that 58.4% of cows with a history of repeat breeding are seropositive to *F. hepatica*. In this respect, it was reported that the cessation of ovarian function following parasitic infection is a usual sequence to the decline in general health, failing of appetite and eventually loss of weight and anemia [2]. Moreover, the altered intestinal function markedly affects nutrients and water intake from the gut and induced obvious immune deficiency following lack of adequate

macronutrients or selected micronutrients, especially Zn, Se, Fe and the antioxidant vitamins after infection and complicates the condition [38]. The effects of *F. gigantica* infection on body weight gain is mainly due to the reduction in dry matter intake following inappetence [39].

In the current investigation, ELISA test detected more prevalence of fasciolosis in buffalows compared to fecal examination (18.33% vs. 13.75%). This finding coincides with those of [40] in bovines who detected a prevalence of 11.4 % using the sedimentation test and 24.4% for the indirect ELISA. This condition could be explained in light that ELISA can detect antibodies to E/S products as early as 2 weeks post infection [41-43].

In this work, fasciolosis caused obvious changes in the hemogram of infected animals in the form of hypochromic macrocytic anemia and clear changes in lymphocytes, eosinophiles and basophiles. These results were in agreement with the finding of [44] Also, [45] noted a decrease in Hb, PCV, total erythrocyte counts and appearance of reticulocytes in the blood of the infected buffaloes suggesting the occurrence of regenerative anemia. It was found that red blood cell counts and PCV were inversely related to worm burden, but animals compensated for reduced numbers of red blood cells by increasing red cell Hb content [46]. Also, [19] added that animals showed general weakness, stunted growth and infertility, mostly suffering from hypochromic macrocytic anemia.

Oxidative stress and the occurrence of fasciolosis were investigated in buffalows herein. In this respect, [47] reported dramatic increases in the number of eosinophils present in the peritoneal cavities of primary infected and challenged rats which produce free radicals in response to adult fluke crude antigen. NO and CAT, ASCA, SOD, R-GSH- and TAC were low in Fasciola-infected animals. Also, [48] found significant decreases in concentrations of reduced glutathione, vitamins C, E and A, particularly during the migratory phase of fasciolosis (at 4 weeks PI). They concluded that fasciolosis is associated with enhanced oxidative reactions and reduced antioxidant defense capability of rat serum. Marked improvement of SOD and GPX activities and in lipid peroxide levels after vitamins supplementation as compared to their corresponding values after treatment of *F. hepatica* patients with triclabendazole alone could be explained on the basis of the potent action of these vitamins in protection against oxidative damage. [49].

In this study, serum Zn, Cu, Fe and Se concentrations decreased in Fasciola- infected as

compared to healthy animals. It was reported that animals have Cu or Zn deficiency are usually suffering from general weakness, stunted growth and infertility [15,17,19]. Moreover, in Cu-deficient animals, increased susceptibility to infection and growth retardation is the main causes for infertility [50]. A positive association between increased pre-partum blood Se concentration and decreased incidence of mastitis, ovarian cysts and anoestrus/silent estrus during the post-partum period was reported [51].

Some treatment trials were carried to overcome the problem of fasciolosis in buffalows under investigation. Considerable percentage of buffalows treated with Ivomec super (71 %), Fasciontel (85.71 %) and Dectomax (75%) responded to treatment and showed absence of clinical signs of fasciolosis and negative fecal examination. In this respect, [51] compared effects of an injectable doramectin preparation with those of an injectable ivermectin-clorsulon preparation on control of liver flukes and growth performance in cattle. Average daily gain of the doramectin-treated group was significantly greater than that of ivermectin-clorsulon group. In the same time, it was reported that Fasciontel is a wide-spectrum parasitocidal drug (Closantel) with long-lasting effect and it works against infestations caused by mature and immature forms of *F. hepatica* and *F. gigantica*. Also, [52] documented high efficacy of an experimental fasciocide (Closantel) in treating cattle naturally infected with *F. hepatica* as compared to the efficacy of clorsulon depending on fecal egg counts examination.

It could be concluded that a tight relationship existed between cessation of ovarian activity and fasciolosis in buffalows. Infected animals suffer from anemia, poor weight, enhanced oxidative reactions and reduced antioxidant defense capability of serum. Detection of fasciolosis by ELISA is more efficient than routine fecal examination. Fasciontel is the drug of choice for fasciolosis in buffaloes.

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