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Impact of Foot and Mouth Disease on Oxidative Status and Ovarian Activity in Egyptian Buffaloes

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Abstract: The current investigations were carried out on 2205 heads of buffalo-cows raised at Lower Egypt in small holder private farms during 2004-2008 to throw lights on the effect of infection with Food and Mouth Disease Virus (FMDV) on oxidative status and ovarian activity. Animals were clinically examined and blood samples were collected for virological and biochemical investigations. FMDV was isolated from serum samples on Madin Darby Bovine Kidney (MDBK) cells, identified using Dot-ELISA test and serotyped by reverse transcriptase-polymerase chain reaction (RT-PCR). Concentration of Malondialdehyde (MDA), Nitric oxide (NO), Superoxide dismutase (SOD) and Total antioxidant capacity (TAC) were calorimetrically determined in serum as markers for oxidant/ antioxidant status. Serum progesterone level (ELISA) was monitored as a key for ovarian activity. Results showed that the isolated virus is FMDV serotype O. This virus was isolated from 18.73% of examined buffalo-cows. Obviously ($P<0.01$) high incidence of the disease was recorded during 2006 (21.23%) and during autumns (26.30%) and springs (24.73%). Significant ($P<0.01$) percent of infected animals showed no clinical signs of the disease (74.33), however, these signs were clear in only 25.67%. Depressed appetite, vesicles on mouth and feet and fever were the most observed clinical signs of the disease. Level of MDA increased, while SOD and TAC levels decreased ($P<0.01$) in serum of infected animals. Significantly ($P<0.01$) higher number (31.89%) of infected animals did not come in heat with very low serum progesterone level (<0.02ng/ml) and rectal palpation showed that these animals have smooth bilateral inactive ovaries during the breeding season. It was concluded that FMDV serotype O infection is associated with disturbed oxidative status and sub functional ovarian activity in buffalo-cows.

Key words: Buffaloes · FMD · ovary · progesterone · antioxidant · RT-PCR

INTRODUCTION

Buffaloes are the main source of good quality animal proteins and represent an integral part of the agricultural economy in many developing countries worldwide. Also, nowadays there is a great interest for buffaloes breeding owing to the recorded well known diseases resistance in this species [1].

Foot-and-mouth disease (FMD) is an economically, highly infectious viral disease of cloven-hoofed farm animals including cattle, sheep, goats and swine. The disease is caused by a small single-stranded, positive-sense RNA virus belongs to the genus aphthovirus within the family Picornaviridae. Based on serological relationship, globally FMDV is divided into seven distinct serotypes (O, A, C, Asia 1, SAT1, SAT2 and SAT3) and multiple subtypes [2, 3].

FMD infected animals experienced fever; vesicles in the mouth and on the muzzle, teats and feet [4]. This disease is endemic in many countries in Africa, Asia and South America, whereas an outbreak many cause high morbidity and mortality, especially in young animals [5, 6].

Oxidative stress results from the faster production of reactive forms of oxygen than its safely scavenging by the antioxidant mechanism and it has a negative effect on animal health and productivity as well as it has been implicated as a major initiator of tissue damage [7, 8].

Ovarian inactivity is among the most predominant causes of reproductive failure and economic losses in buffaloes as a result of fewer days in milk and fewer calves produced per year of life as well as high culling rate which is mainly due to failure of pregnancy. Currently a

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lot of factors were incriminated for induction of ovarian inactivity in farm animals [1].

FMD is probably one of the most contagious diseases affecting cattle; however, no enough data were available on buffaloes. The present work was carried out to investigate the effect of infection with FMD on oxidative status and ovarian activity in buffalo-cows under the prevailing Egyptian field conditions.

MATERIALS AND METHODS

The current investigations were carried out during a period of 4 consecutive years (2004-2008) as a part of the National Research Centre project No. 7120106.

Animals: A total number of 2205 heads of mature buffalo cows raised at areas deprived from general services at Lower Egypt was investigated. These animals were 4-9 years old, kept in small holder farms and fed on Egyptian clover during December to May and concentrate, crop residues and rice straw during other months of the year with no regular system of vaccination.

Experimental design: A complete case history and owner complain were recorded for each animal. Animals were clinically examined; special attention was given to the presence of salivation, fever as well as vesicles in the mouth and on the muzzle, teats and feet. Gynecological examination was carried out by palpating the internal genital organs through the rectal examination at least for 2 successive weeks. Animals which did not show estrous signs during the breeding season (September-May) and have small ovaries were considered to suffer from ovarian inactivity. The condition was confirmed later on by assaying serum progesterone level.

Samples collection: Samples of blood were collected from the jugular vein; serum was separated (3000 X g, 15minutes, 4ºC) for isolation of FMDV as well as for assaying the levels of some oxidant/antioxidant markers and progesterone.

Virus isolation and propagation: Serum samples were filtered using 0.22nm nitrocellulose filters and cultivated on MDBK cells (VACSERA, Egypt) and the suspected virus was titrated [9].

Virus identification and serotyping:

- Dot-ELISA technique was used for detection of FMDV [10].
- RT-PCR using special kit (Plexor® One-Step RT-PCR, Promega BioSciences Co., San Luis, CA, USA) was performed on positive Dot-ELISA serum samples as outlined by [11, 12] using the following primers:

-5’ TTCGAAACGGCACGGTCGGA 3’ 20bp

-5’ CACCGTGCCCACTTTGTCTG 5’ 20bp

A reference locally isolated FMDV strain (a positive control for RT-PCR)as well as hyperimmune serum against FMD (Dot-ELISA) were kindly obtained from the Veterinary Serum and Vaccine Research Institute, Abbassia, Egypt.

Oxidant/antioxidant markers: The concentrations of some oxidant/antioxidant markers including MDA [13], NO [14]), SOD [15]) and TAC [16] were determined in serum samples.

Progesterone level: Serum progesterone level was assayed by ELISA Microwell Technique using kits from Dima, Germany. The kit had a sensitivity of 0.02 ng/ml with inter-and intra-run precision coefficient of variations of 2.9 and 4.85%, respectively [17]

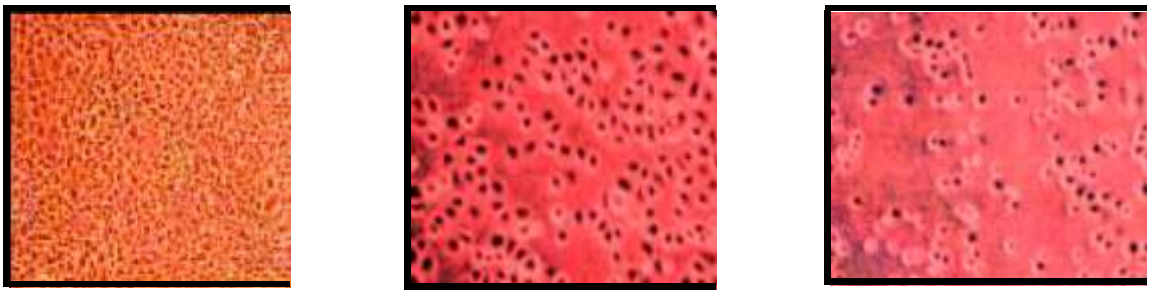
Statistical analysis: Data were computed and statistically analyzed using student "t" test and Chi square (χ^2) analyses [18].

RESULTS

Virus isolation: MDBK cells showed clear cytopathic effect after 24 hours post infection with consequent induction of different degrees of degradation and cell deterioration due to progression of the virus in these cells (Fig. 1).

Virus identification: Dot ELISA test revealed that 13.9% of serum samples collected from apparently healthy buffalo-cows (307 out of 2205) were positive for FMDV. However, 100% of the serum samples collected from animals showing the disease symptoms contained the virus.

Virus serotyping: RT-PCR product carried out on genomic RNA extracted from serum samples as well as positive controls gave the same band (Fig. 3) when the geneVP1(specific for O serotype) was amplified by the current used specific primers.



Normal MDBK cells MDBK cells 24 hours post infection MDBK cells 36 hours post infection

Fig. 1: Shows the degree of degradation and cell deterioration due to progression of the virus in MDBK cells (X=100)

Table 1: Overall incidence of FMD infection in buffalo-cows

	FMD positive animals	
Number of examined animals	Number	Percentage
2205	413	18.73

Table 2: Years variation in the incidence of FMD infection in buffalo-cows

		FMD positive animals	
Year	Number of examined animals	Number	Percentage
2004	250	48	19.20
2005	535	98	18.32
2006	405	86	21.23
2007	575	99	17.22
2008	440	82	18.64
χ^2value			1.02

Table 3: Seasonal variations in the incidence of FMD infection in buffalo-cows

		FMD positive animals	
Season of the year	Number of examined animals	Number	Percentage
Summer	536	88	16.42
Autumn	559	147	26.30
Winter	560	42	7.50
Spring	550	136	24.73
χ^2value			15.26**

**P<0.01

Table 4: Clinical signs of FMD infection in buffalo-cows

	FMD positive animals	
Clinical signs	Number	Percentage
Apparently normal	307	74.33
FMD signs	106	25.67
χ^2 value		23.78**

**P<0.01

Table 5: Clinical signs of FMD infection in buffalo-cows

	FMD positive animals	
Clinical signs	Number	Percentage
Salivation	9	8.49
Fever	11	10.38
Lameness	11	10.38
Off food	12	11.32
Vesicles	11	10.38
Ulcers on muzzle	8	7.55
Vesicles on feet	11	10.38
Vesicles on teat	3	2.83
More than one clinical signs	30	28.30
χ^2 value		34.73**

**P<0.01

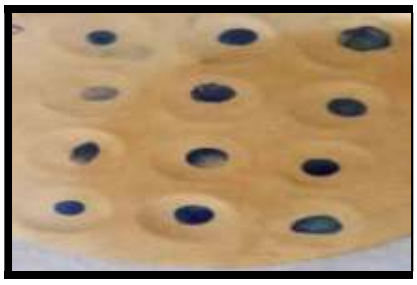


Fig. 2: Dot ELISA test in FMD positive animals with different intense color

Epidemiology: Out of 2205 examined buffalo-cows, 18.73% were positive for FMD (Table 1). The high incidence of the disease (21.23%) was recorded during 2006 (Table 2). Seasonal distribution indicated that the disease significantly (P<0.01) prevailed during autumns (26.30%) and springs (24.73%) as shown in Table 3.

Clinical signs: Infected animals showed clear clinical signs of the disease only in 25.67%, while a significant (P<0.01) percent (74.33) of these animals did not show any

Table 6: Some oxidant/antioxidant concentrations in relation to FMD infection in buffalo-cows

		FMD negative animals		FMD positive animals	
Marker	Parameter	Active ovary	Inactive ovary	Active ovary	Inactive ovary
Oxidants	MDA	1.80±0.06	3.89±0.07**	2.14±0.05	5.04±0.03**
	NO	31.44±2.37	30.99±1.17	32.19±3.03	28.99±2.07
Antioxidants	SOD	328.19±5.17	301.94±4.07**	318.99±670	289.84±5.27**
	TAC	1.44±0.08	0.48±0.06**	1.24±0.07	0.34±0.05**

**P<0.01, MDA=Malondialdehyde, NO = Nitric Oxide, SOD=Superoxide dismutase, TAC=total antioxidant capacity

Table 7: Ovarian activity in relation to FMD infection in buffalo-cows

		FMD positive animals	
Ovarian activity	Number of examined animals	Number	Percentage
Normal cyclic	1233	103	8.35
Inactive ovaries	972	310	31.89
χ^2 value			41.25**

**P<0.01

Table 8: Serum progesterone level in relation to FMD infection in buffalo-cows

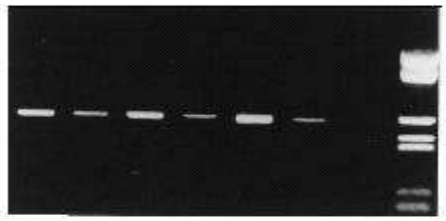
Ovaries	Phase	Control group	FMD infected group
Active	Follicular	0.53±0.06	0.64±0.05
	Luteal	2.74±0.07	3.19±0.09**
Inactive		<0.02	<0.02

**P<0.01

signs of FMD (Table 4). A significant(P<0.01) percent (28.30)of animals showed signs of FMD revealed more than one signs, while inferior appetite, vesicles on mouth and feet and fever were the most observed clinical signs in FMD infected animals (Table 5).

Effect of FMD infection on oxidative status: Concentrations of some oxidant/antioxidant markers are shown in Table 6. Significant (P<0.01) increase of MDA and decreases of SOD and TAC values were detected in the blood of animals suffering from ovarian inactivity either they are positive or negative to FMD as compared to normal cyclic animals.

Effect of FMD infection on ovarian activity: Gynecological examination confirmed by serum progesterone assaying indicated that a significant (P<0.01)high incidence of FMD infected animals (31.89%) did not come in heat as compared to healthy normal cyclic animals (8.35%) as shown in Table 7. Rectal palpation showed that these animals have smooth bilateral ovaries with no Graafian follicles or corpora lutea. In both positive and negative



300bp

Fig. 3: Agrose gel electrophoresis of RT-PCR of VP1 gene extracted from serum samples of FMD positive animals. Bands at 300bp showing that the virus is O strain. Lane 1: DNA marker, lane 2: negative control, lane 4: positive control and the rest are tested samples

FMD infected animals, serum progesterone level (Table 8) was very low (<0.02 ng/ml) as compared to the normal cyclic animals (>1 ng/ml during the luteal phase and 0.5-1ng/ml during the follicular phase of the estrous cycle).

DISCUSSION

This study was designed to evaluate the effect of infection with FMDV on oxidative function and ovarian activity in buffalo-cows reared at small holder farms under the local Egyptian field conditions as well as serotyping of the isolated virus. It is worthily to mention here that these animals were reared in services deprived areas and are not subjected to regular system of vaccination.

In the present investigations, FMDV serotype O was isolated from 18.73% of examined buffalo-cows. Also, in Egypt this virus was previously isolated by [19]. Importation of infected animals with this serotype from endemic areas could account for the appearance of new serotypes of FMD in Egypt [20, 21]. Moreover, the high error rates during genome replication of FMDV, like other RNA viruses that comprise a complex distribution of variant populations may be another cause for the appearance of new serotypes [22].

Dot-ELISA test was used here for detecting positive samples, while RT-PCR was used for typing virus isolates

using special primers for VP1 gene of serotype O of FMDV, whereas a band in the positive control and positive serum samples at 300 bp in length for VP1 was detected. Similar result was given by [11, 12].

FMDV was isolated from all animals showed the characteristic signs of FMD infection, while, it was isolated from 13.9% of apparently healthy animals. In this respect, [23] reported that a considerable percentage of the virus detected in serum is attributed to a carrier state or asymptomatic persistent infection which is common after infection of ruminants with FMD virus.

The current study revealed seasonal variations in the incidence of infection with FMDV, with obviously high incidences during autumns and springs. Similar trend were given by [12, 19, 20]. The condition could be attributed to climatic changes as well as increased animals importation during these seasons of the year.

Depressed appetite, vesicles on mouth and feet were the most observed clinical signs in FMD infected animals. The condition was related to feverial condition as well as the tropism of FMDV to the epithelium of the gastrointestinal tract [24].

In this study, serum concentrations of MDA increased, while SOD and TAC decreased significantly in infected animals. This implies that the affected animals are under stress condition. Oxidative stress has been implicated as major initiators of tissue damage and can affect enzymatic activity, signal transcription and gene expression, especially apoptotic gene [25]. In animal suffering from stressful conditions such as lead pollution, parasitism and retained placenta [26] found high values of SOD [27] showed low SOD with high level of MDA and [28] found an increase of SOD, respectively. The level of TAC decreased in non cyclic buffalo-cows in this study and was in agreement with the finding of [8] in buffalo cows suffering from impaired fertility [29] in postpartum anestrous dairy cows and [30] in mid lactating cows exposed to heat stress during summer.

In this study, significant higher percent (31.89) of infected animals were suffered from ovarian inactivity as monitored by the very low serum progesterone level and rectal palpation during the breeding season. No direct relation between FMDV and ovarian activity could be traced in the available literature, however, it was recently reported that this virus cause transplacental infection in ewes [31]. Also, it was concluded that contrary to theoretical predictions, replication of an RNA virus in a constant cellular environment may lead to expansion of cellular tropism, rather than to a more specialized infection of the cellular type to which the virus has been adapted [32]. However, ovarian inactivity in FMD infected buffaloes could be indirectly attributed to the poor body condition and negative energy balance that inhibits ovarian function following fever and depressed appetite with consequent decreased LH pulse frequency [33]. Moreover, 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 [33, 34].

It was concluded that FMDV serotype O infection is associated with sub functional ovarian activity and disturbed oxidative status in buffalo-cows. Proper vaccinal system depending upon molecular biology is recommended.

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