Egytptian J. Virol. 5 (1): 471- 485 (2008)

Some factors affecting immune response of sheep vaccinated with bivalent foot and mouth disease vaccine

M.A. Farag*, Abeer, E. Mansour* and S.M. Ziedan**

The effect of bovine viral diarrhea virus (BVDV) infection and Aflatoxine B1 (AFB_1) on the immune response of sheep vaccinated with bivalent Foot and mouth disease (FMD) vaccine were studied. Significant decrease in humoral immune response against the vaccine strains O1/3/93 and A/1/Egypt/2006 accompanied by protection percentage of 33% against challenged virus O1/3/93 with low values of ΔOD were recorded in sheep previously infected with BVDV one week before vaccination. Challenging the immunity of sheep both simultaneously infected with BVDV and vaccinated or infected one week post vaccination against O1/3/93 revealed protection percentages of 66%. Sheep fed on commercial ration treated with 40µg/kgm ration of prepared AFB_1 and challenged at three weeks post vaccination showed protection percentage of 33%, decrease in antibody titers and low values of ΔOD. The immunosuppressive effects of BVDV and AFB1 on the immune response of sheep vaccinated with bivalent FMD vaccine were discussed.

Keywords: Bivalent serotype A&O foot and mouth disease vaccine, BVDV infection, Aflatoxine B1 as immunosuppressive factors - sheep, Egypt.

---

* *Department of Foot and Mouth disease,*
** *Department of Rinder pest like diseases,*
Veterinary Serum and Vaccine Research Institute, Abassia, Cairo. P.O.Box 131.

(Received March 2008)
(Accepted May 2008)

INTRODUCTION

Foot and mouth disease (FMD) is a contagious viral disease of cloven-hoofed animals which has a great potential to cause sever economic losses. Due to the presence of complicated epizootiological field aspect, FMD is and will remain a serious economic problem and it is difficult to be eradicated from Egypt. In a country in which control of FMD relies predominately on vaccination the stability of the currently used vaccine in high potency is the only way to protect susceptible animals against FMD outbreaks **(Farag et al., 2005 & Abed EL-Rhaman et al., 2006)**. Vaccine failures among dairy and fattening farms have been recorded under field conditions. It may be attributed to different factors either pertinent to the vaccine incompetency to invoke specific protection or inability of the vaccinated animal to mount an adequate immune response. **Buxton et al., 1981, Sharp et al., 1982, Sharp and Langley, 1983** reported that inadequate immune response against FMD vaccination may be attributed to immuno-suppressive, such as some parasitic infection as Toxoplasmosis gondi, Trypanosoma congolense and Theileria annulata. **Abeer et al., 2003** found that BVDV had immune suppressive effects on sheep vaccinated with monovalent O1/3/93 inactivated FMD vaccine. BVDV is the most immunosuppressive viral disease; it causes significant suppression of specific and nonspecific defense mechanism against other organisms **(Zeidan, 1988). Thaxton et al., 1971., Michael et al., 1973, Reddy et al., 1984 and Raisuddin et al., 1990** reported that Aflatoxine B1 had immunosuppressive effect on humoral and cellular immune response. This paper aim to study the effects of BVDV and Aflatoxine B1 on the immune response of groups of sheep vaccinated with bivalent inactivated gel vaccine and either infected with BVDV or fed with commercial ration treated with Aflatoxine B1.

MATERIALS AND METHODS

Animals

Forty two male balady rams of one year old and weighting of 50 to 65 kg were used. The animals were apparently healthy, free from external and internal parasites and they were proved to be free from antibodies against bovine virus diarrhea virus (BVDV) and both serotypes O1/3/93 and A/1/Egypt/2006 of foot and mouth disease virus (FMDV).

Viruses

FMD virus

Serotypes O1/3/93 and A/1/Egypt/2006

Vaccine strains O1/3/93 and A/1/Egypt/2006 were used for neutralization test and production of bivalent FMD vaccine and tongue epithelium of serotype O1/3/93 was used for challenged test.

Bovine virus diarrhea virus (BVDV):

BVD virus (Kena strain):
BVD-MD (Kena strain) was used for experimental infection of animals according to **(Baz, 1982)**.

BVD (Iman strain):
BVD virus Iman strain was used for neutralization test according to **Baz (1975)**.

Foot and mouth disease vaccine

Bivalent serotypes O1/3/93 and A/1/Egypt/2006 of FMDV locally prepared and tested binary ethyleniemine inactivated gel and saponin adjuvanted FMD vaccine were used. Rams were vaccinated with 1 ml of bivalent FMD vaccine subcutaneously.

Samples

Blood samples were collected from all groups of animals for cell mediated studies at 3rd, 7th, 14th and 21st days post vaccination and 1st and 2nd week post challenges. Serum samples were collected from all group of rams at weekly interval in the first four weeks post vaccination then every two week up to 18 weeks post vaccination The sera were inactivated at 56°C for 30 minutes and stored at –20°C until used for monitoring the antibody against O1/3/93 and A/1/Egypt/2006 of FMD virus.

Rowell Park Memorial Institute, 1640 Medium (RPMI-1640):
RPMI-1640 without sodium bicarbonate was supplied by Sigma Pharmaceutical Company. It was prepared according to manufacture direction and used for lymphocyte transformation test.

Ficol solution:
It was supplied by Sigma Company in a liquid form of a density of

1.077 gm consisted of 57gm Ficol 400 and 9 gm diatizoote dissolved in 100ml distilled water.

Mitogens:

Concanavallin-A: It was supplied by Biochromk-1224, Berlin, Germany and used for the in vitro lymphocyte blastogenesis assay. According to manufacture direction, Concanavallin-A was diluted with RPMI-1640 complete medium.

4, 5 dimethyle thiazol-2-y1, 2,5-diphenyltetrazolium bromide (MTT)

MTT was supplied by Sigma Company and used to estimate the activity of the various dehydrogenase enzyme in active mitochondria of activated lymphocytes.

Sodium dodecyle sulphate (SDS): It was supplied by Sigma Company and used for lymphocyte transformation test.

Production of Aflatoxin by A. flavous

Aflatoxin was produced according to the method of **EL-Tahan, 1996.**

Infection of the commercial ration:

Commercial ration was infected with filtrate of A. flavus NRRL2999, which was grown on yeast extraction media. The filtrate was mixed thoroughly with ration in plastic bags. The final concentration of sAFB1 in infected ration was 40ug/kgm.

Serum neutralization test

The micro-neutralization test was carried out according to **OIE Manual (2000)** based on the method described by **Golding et al., (1976)** using BHK-21 monolayer cells and O1/3/93 and type O outbreak FMD virus isolate.

EXPERIMENT AND RESULTS

Out of forty two rams, thirty six rams were divided into six groups, six of each as follows.

Group 1: Vaccinated one week post experimentally infection with virulent strain of BVDV.

Group 2: Vaccinated one week pre-experimental infection with virulent strain of BVDV.

Group 3: Simultaneously infected with virulent strain of BVDV and vaccinated with bivalent serotypes O1/3/93 and A/1/Egypt/2006 of FMD virus.

Group 4: Vaccinated only with bivalent FMD vaccine.

Group 5: Fed on commercial ration treated with 40µg/gm ration of prepared Aflatoxin B_1

Group 6: Fed on commercial ration free from AflatoxinB_1.

Three animals from the above five groups were challenged against type O1/3/93 FMDV at three weeks post vaccination.
The last 6 rams divided into two groups three in each. They were used as control groups of challenge virus of Foot and mouth disease serotype O1/3/93 during challenged test.

Table 1 showed duration of neutralizing antibody titers against the two vaccine strains O/1/93 and A/1/Egypt/2006 of foot and mouth disease virus (FMDV) of different groups of rams vaccinated with bivalent FMD vaccine and infected with BVDV. Sharp drop in antibody titers detected in rams infected with BVDV one week before vaccination.

The duration of antibody titers detected in rams of group 5 fed on ration treated with Aflatoxin 1 with concentration of 40µg/kgm ration was illustrated in table 2. Lowering in antibody titers against the two vaccine strains O/1/93 and A/1/Egypt/2006 were detected in animals of group 5 in comparison with UN fed control group.

Table 3 represents the protection percentages of challenged vaccinated rams infected with BVDV. 33% protection against serotype O1/3/93 whereas, rams simultaneously vaccinated and infected or infected with BVDV one week post vaccination recorded protection percentage of 66%.

Rams fed on commercial ration treated with 40µg/kgm ration of prepared Aflatoxin B_1 and challenged at three weeks post vaccination showed protection percentage of 33% table 4.

Cellular immune response expressed as Delta optical density of rams vaccinated with bivalent FMD vaccine and infected with BVDV and challenged with serotype O1/3/93 of FMDV were tabulated in table 5.

Table 6 illustrated cellular immune response expressed as Delta optical density of rams fed on ration contains 40µg/kgm ration in comparison with UN fed control group.

Table 1: Neutralizing antibody titers expressed as log_{10} $TCID_{50}$ of different groups of rams vaccinated with bivalent FMD vaccine and infected with BVDV

Mean neutralizing antibody titers against serotype O1/3/93 and A/1/Egypt/2006 of FMDV strains expressed as log_{10} $TCID_{50}$																								
	Weeks post vaccination																							
Groups of rams	0		1		2		3		4		6		8		10		12		14		16		18	
	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A
Group 1	0.15	0.3	0.3	0.3	0.45	0.6	0.6	0.75	0.75	0.9	1.05	1.05	0.6	0.6	0.3	0.3	0.15	0.45	0.15	0.15	0.15	0.0	0.0	00
Group 2	0.3	0.3	0.45	0.6	0.6	0.75	0.9	1.05	1.2	1.2	1.35	1.35	1.05	1.05	0.75	0.6	0.3	0.3	0.15	0.15	0.0	0.0	0.0	0.0
Group 3	0.15	0.3	0.6	0.75	0.75	09	1.05	1.2	1.2	1.35	15	1.5	1.8	1.8	1.35	1.5	1.2	1.2	0.9	0.9	0.3	0.6	0.3	0.3
Control group (4)	0.3	0.45	0.75	0.9	0.9	1.05	1.2	1.35	1.5	1.65	1.8	1.95	2.25	2.4	2.1	2.1	1.8	1.8	1.5	1.5	1.2	1.2	0.6	0.75

Group 1 rams infected with BVDV one week before vaccination Group 2 rams infected with BVDV one week post vaccination

Group 3 rams simultaneously infected with BVDV and vaccinated with bivalent FMD vaccine

Group 4 rams only vaccinated with bivalent FMD vaccine

Table 2 Neutralizing antibody titers expressed as log10 TCID50 of vaccinated groups of rams fed on different rations.

Mean neutralizing antibody titers against serotype O1/3/93 and A/1/Egypt/2006 of FMDV strains expressed as log_{10} $TCID_{50}$																						
Weeks post vaccination																						
Groups of rams	0		1		2		3		4		6		8		10		12		14		16		18	
	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A
Group 5	0.3	0.15	0.45	0.3	0.6	0.6	0.75	0.9	1.05	1.2	1.35	1.5	1.35	1.35	0.75	0.9	0.6	0.75	0.6	0.6	0.3	0.3	0.15	0.3
Group 6	0.3	0.3	0.6	0.75	0.9	1.05	1.2	1.5	1.35	1.65	1.95	2.1	2.25	2.4	2.1	2.1	1.5	1.65	1.5	1.35	1.2	1.2	0.6	0.75

Group 5: group of rams fed on prepared AFB1 ration containing 40ug/kgm ration Group 6: group of rams fed on ration free from AFB1
Average of Group 1 = 1.142 Average of Group 2 = 1.563 There is a significant decrease between group 1 and the control group 2 (control)

Table 3: Protection percentage of groups of rams infected with BVDV one week before vaccination and challenged against O1/3/93 FMD virus

Groups	Primary and secondary lesions		Protection percentage
	Primary lesion	Secondary lesion	
Group 1	2/3	2/3	33%
Group 2	1/3	1/3	66%
Group 3	1/3	1/3	66%
Group 4	0/3	0/3	100%
Control group	3/3	3/3	0%

Group 1challenged rams infected with BVDV one week before vaccination.
Group 2 challenged rams infected with BVDV one week post vaccination.
Group 3 challenged rams simultaneously vaccinated with bivalent FMD vaccine and infected with BVDV.
Group 4 challenged rams vaccinated only with bivalent FMD vaccine as control vaccine.
Control group: rams infected only with serotype O1/3/93 as FMD virus control.

Table 4: Protection percentage of vaccinated groups of rams fed on different rations and challenged against O1/3/93 FMD virus

Groups	Primary and secondary lesions		Protection percentage
	Primary lesion	Secondary lesion	
Group 5	2/3	2/3	33%
Group 6	*1/3	0/3	100%
Control group	3/3	3/3	0%

Group 5 challenged vaccinated rams fed on AFB1 ration contains 40ug/kgm ration
Group 6 challenged vaccinated rams fed on ration free from AFB1n
Control group challenged non vaccinated rams fed on ration free from AFB1
*1 primary lesion at the site of inoculation

Table 5: Cellular immune response expressed as Delta optical density of rams vaccinated with bivalent FMD vaccine and infected with BVDV and challenged with serotype O1/3/93 of FMDV.

Group number	Mitogen and virus used	ΔOD of samples using different Mitogens					
		Days post vaccination				week post challenged	
		3rd day	7th day	14th day	21 days	1st week	2nd week
Group 1	PHA	0.150	0.169	0.181	0.200	0.230	0.205
	FMDV	0.160	0.180	0.193	0.215	0.248	0.269
Group 2	PHA	0.195	0.315	0.375	0.390	0.400	0.341
	FMDV	0.230	0.335	0.390	0.418	0.430	0.395
Group 3	PHA	0.190	0.310	0.370	0.385	0.399	0.350
	FMDV	0.220	0.343	0.400	0.419	0.439	0.400
Group 4	PHA	0.250	0.360	0.410	0.443	0.475	0.400
	FMDV	0.271	0.385	0.435	0.460	0.492	0.430

Group 1: Rams vaccinated with bivalent FMD vaccine and infected with BVDV one week pre-vaccination.
Group 2: Rams vaccinated with bivalent FMD vaccine and infected with BVDV one week post-vaccination.
Group 3: Rams simultaneously vaccinated with bivalent FMD vaccine and infected with BVDV.
Group: 4: Rams vaccinated only with bivalent FMD vaccine.

Table 6: Cellular immune response expressed as Delta optical density of rams fed on different rations

Group number	Mitogen and virus used	ΔOD of samples using different Mitogens					
		Days post vaccination				week post challenged	
		3rd day	7th day	14th day	21 days	1st week	2nd week
Group 5	PHA	0.200	0.180	0.190	0.195	0.215	0.187
	FMDV	0.145	0.159	0.195	0.219	0.215	0.200
Group 6 Control	PHA	0.225	0.256	0.291	0.345	0.356	0.301
	FMDV	0.215	0.230	0.327	0.370	0.385	0.325

Group 5: Rams vaccinated with bivalent FMD vaccine and fed on prepared AFB1ration containing 40ug/kgm ration
Group 6: Rams vaccinated with bivalent FMD vaccine and fed on commercial ration free from AFB1
FMDV: Foot and mouth disease virus PHA: Phyto Haem Agglutinin (non specific Mitogen).

DISCUSSION

Successful vaccination against viral disease comes in a part through controlling and reducing the incidence of the infectious diseases. Effective vaccination is due to its ability to stimulate a balanced immune response involving both humoral antibody and cell mediated immunity, long duration of immunity and induction of mucosal immunity (**Ada, 1996, and Pringle, 1996**).

Failure of effective vaccines has associated with many factors such as: 1) concurrent infection of the recipient animal with immunosuppressive viral infection as IBRV, parainfluenza-3 and BVDV (**Olsen and Krakowaka, 1984, Eskara and Splitter, 1997**). 2) Some parasitic infection as Toxoplasmosis Gondi, Trypanosoma Congolense and Theileria Annulata. 3) Mycotoxins through consumption of ration contain mouldy grain which constitutes a high source of mycotoxins that induce depressing of cell mediated immune function and reduce immunoglobulin and complement production (**Buxton et al., 1981, Sharp et al., 1982, Sharp and Langley, 1983, Koller, 1979**).

So far, an attempt to investigate the effect of bovine virus diarrhea virus (BVDV) infection and Aflatoxine B1 (AFB_1) on the immune response of groups of sheep vaccinated with bivalent vaccine of serotypes O1/3/93 and A/1/Egypt/2006 of foot and mouth disease (FMD) virus were studied.

Regarding to the effects of BVDV infection on humoral immune response of vaccinated rams, the present study demonstrated that sheep infected with BVDV one week before vaccination revealed low neutralizing antibody titers (0.3-1.05 and $\log_{10}$ $TCID_{50}$) against both serotypes A/1/Egypt/2006 and O1/3/93 and the titers remain under the protective level (1.2 $\log_{10}TCID_{50}$) at all time of the experiment, protection of 33% against challenged virus O1/3/93 was also recorded at three weeks post vaccination.

Rams simultaneously vaccinated and infected with BVDV or infected one week post vaccination detected slight decreased in neutralizing antibody titers during the first four weeks

post vaccination. Neutralizing antibody titers of protected levels against the two serotypes of the vaccine strains were recorded up to 12 weeks in simultaneously infected and vaccinated sheep; whereas sheep infected one week post vaccination detected protected neutralizing antibody titers up to 6-8 weeks post vaccination. 66% protection against challenged virus O1/3/93 was also recorded at three weeks post vaccination in both groups of rams, these results agree with **Kardiassis et al., 1964, Wisniewski,1962, Zeidan, 1988, Eskara and Splitter, 1997, Abeer et al., 2003** who found that vaccine failure has been associated with the concurrent infection of the recipient animal with BVDV.

The effects of BVDV infection on cellular immune response of rams vaccinated with bivalent FMD vaccine and infected with BVDV either one week pre or post vaccination and/or simultaneously infected and vaccinated revealed decreased in ΔOD started from the 3rd day up to 14 days post vaccination. Similar results were obtained by **Muscoplate et al., 1973, Iman Hassen 1993, Abeer et al., 2003** who found that the immuno suppressive effect of BVDV on vaccinated sheep either infected pre and or post vaccination is mainly due to the replication of BVDV in lymphocytes and monocytes of infected animals as the virus was recovered from their Buffy coat, the authors added that infect lymphocytes and monocytes with BVDV might impair their proliferate response and altered their function.

Regarding to the effects of Aflatoxin B1 on the immune response of rams vaccinated with bivalent FMD vaccine the present results revealed that rams fed on ration contains Aflatoxin B1 of 40ug/kgm ration detected neutralizing antibodies of low titers against the two vaccine strains with its short duration in comparison with the control group. These results could be explained the suppressive effect of Aflatoxine B1, and the explanation is supported by **Fernandez et al., 1997** who suggested that AFB1 causes a failure in acquired immunity system of lambs by decreasing antibody producing and altering serum profile proteins. **Tung et al., (1970)** reported that Aflatoxin1 had harmful effects on humoral immune response through specific activation of lysosomes which in turn act on

immunoglobulins and results in their bolishing. **Edwards et al., 1971** reported that Aflatoxin has a destructive effect on the immunoglobulins synthesis tissues by bursa and spleen.

The results of cellular immune response of rams fed on ration contains Aflatoxin1 of 40ug/kgm ration revealed that low values of ΔOD estimated at 3 weeks post vaccination and 1st and 2nd weeks post challenges. These results supported by **Soos and Tuboly 1983, Singh and Arora 1989, and Michael et al., 1973** who reported that Aflatoxin1 act on suppression antigen processing through its toxic effect on the cell of the reticuloendothelial system which are represented by macrophages. The authors added that Aflatoxin1 inhibits various functions of T-lymphocytes, impairing the immune responses of the host and affects various lymphoid cells including macrophages, which play an important role in the host resistance to foreign agents and immune responsiveness.

The final conclusion of this represented study is that 1): Avoid vaccination of animals with bivalent FMD vaccine if animals infected with BVDV. Control immunosuppressive effect of BVDV through applying national vaccination regime against BVDV. 2): Avoid consumption of a mouldy contaminated ration where the mycotoxins exceed the permissible limit induced immunosuppressive effect in foot and mouth disease vaccinated rams. So, we recommended that qualitative and quantitative mycotoxicological analysis of the ration must be applied before feeding of animals and the concentration of AFB1 should not be exceeded 10ug/kgm ration according to FDA, the ration should be treated. Those precautions are to avoid the vaccination failure under field condition.

REFERENCES

Abed EL- Rahman A. O., Farag M. A., Samira EL-Kilany, Eman M. A., Manal Abo Yazed and S. Zeidan (2006): isolation and Identification of Foot and Mouth Disease virus during an outbreak of 2006 in Egypt. Kafr EL-Sheikh Vet. Med. J. Vol. 4 . No. 1, 2006.

Abeer, E. Mansour., Halima, M. EL- watany., Ziedan, S. M., Samira EL- Kilany

and Daoud, A. M. (2003): Effects of Bovine Viral diarrhea (BVD) infected sheep on the iommune response of foot and mouth disease vaccine. J. Egypt. Vet. Med. Assoc. 63, No. 4: 183-193.

Ada, G. (1996): Over view of vaccines: Humana press, New Jersy.

Baz, T. I. (1982): " Isolation of BVD/MD virus from newborn calves suffering from intermittent diarrhea and severe unthrifttness". Agr. Res. Rev., 60(7):173-188.

Baz, T. I. (1975): "Isolation, characterization and serological studies on BVD-MD virus in Egypt". Ph.D. Thesis (Microbiology), Fac. Vet. Med. Cairo university.

Buxton, D., Reid,H.W. and Finalyson, J. (1981): Immunosuppression in toxoplasmosis: studies in sheep with vaccines. Forchlamdial abortion and louping ill virus. Vet. Rec., 109:559-561.

Edwards, G.S., Wogan, G.N., Sporn, M.B. and Pongm, R.S. (1971): Structure activity relationships in DNA binding and Nuclear effects of Aflatoxin and analoges. Cancer Res., 31:1043-1950.

EL-Tahan, F. H. (1996): Studies on toxicity and carcinogenicity of Aflatoxin in laboratory animals. Ph. D. Micro. Thesis, Fac. Vet. Med. Zagazig Univ.

Eskara and splitter (1997): Bovine herpes virus-1 infects activated CD4+ lymphocytes. J. Gen. Virol., 78: 2159-2166.

Farag, M. A., M. A. Aggour & A.M.Daoud (2005): ELISA as a rapid method for detecting the correlation between the field isolates of foot and mouth disease and the current used vaccine strain in Egypt. Vet. Med. J., Giza. Vol. 53, No.4: 949-955.

Fernandez, A., Hernandez, M., Sanz, M. C., Verde, M. T. and Ramos, J. J. (1997): Serological serum protein and responses to Brucella melitensis in lambs fed of aflatoxins. Vet. Human Toxico., 39(3):137-140.

Golding, S. M., Hedger, R. S. & Talbot, P. (1976): Radial immunodiffusion and serum neutralization techniques for the assay of antibodies to swine vesicular disease . Res. Vet. Sci. 20, pp.142- 147

Iman, K. A. Hassen (1993): Further studies on bovine virus diarrhea in calves" . Ph.D. Thesis (virology), Fac. Vet. Med., Cairo University.

Kardiassis, I., Papans, C., Brovas, D., Karvalakis, I. and Scimenis, A. (1964): "Antibody response in cattle after vaccination against FMD with a monovalent (O) vaccine prepared in Greece". Bull. Soc. Vet. Hell., 1:94-104.

Koller, L.d. (1979): Effect of environmental contaminants on the immune system. Adv. Vet. Sci., Comp. Med., 23: 267-295.

Michael, G. Y., Rao, P. V. and Yadgiri, B. (1984): Effects of selected levels of dietary aflatoxins on performance of broiler chicken. Ind. J. Anim. Sci., 54:68-75.

Muscoplat, C. C., Johnson, B. W. and Stvens, J. B. (1973): "Abnormalities in vitro lymphocytes response during during BVD-MD virus infection". Am. J. Vet. Res., 34:753.

OIE (2000): FMD, Chapter 2.1.1. In manual of Standard for Diagnostic Test and Vaccine, 4th ed., 2000, Paris, pp.77-92.

Olsen, and Kakowaka, S. (1984): Immune dysfunction associated with viral infections. Comp. cont. Ed., 6:422-430

Pringle, C. R. (1996): Temperature sensitive mutant vaccines. In vaccine protocols. Humana press, New Jersy, app. 17-32.

Raisuddin, K.P., Zaidi, S. I., Sazena, A. K. and Ra, P. K. (1990: Effects of Aflatoxin on lymphoid cells of weaning rat. J. Appl. Toxicol., 10: 245- 250.

Reddy, D. N., Rao, P. V. and Yadgiri, B. (1984): Effects of selective levels of dietary Aflatoxin on the performance of broiler chicken. Ind. J. Anim. Sci., 54:68-75.

Sharp, R. T. and Langley, A. M. (1983): "The effect of Theileria annulata infection on the immune response of cattle to foot and mouth disease" Brit. Vet. J., 139 (5): 378-385.

Sharp, R.T., Langley, A. M., Mowat, G.N., Magaskill, J.A. and Holmps, P,M. (1982): Immunosuppression in bovine trypanosomiasis, response of cattle infected with trypannosoma Congolese to Foot and mouth disease vaccination and subsequent live virus challenge. Res. Vet. Sci. 32: 289-293.

Singh, I. P. and Arora, A. (1989): Immune response of kids fed on Aflatoxin. Ann. Nutr. Aliment, 1990-31, 723-44.

Soos, T. and Tuboly, S. (1983): Study of the immunogenicity of three Aphthovirus strains. I. Cellular immunity. Mag. Allatrov. Lapia, 38: 341-344.

Thaxton, J.P. and Hamilton, P. B. (1971): Immunosuppression in Broilers by Aflatoxin. Poult. Sci., 50:1936.

Tung, H.T., Donaldson, W.E. and Hamilton, P. B. (1970): Effect of Aflatoxin on some marker enzymes of lysosomes. Biochim. Biophys. Acta, 222:665-667.

Winsniewski, J. (1962): Content of foot and mouth disease virus in organs and tissues of cattle after slaughter determined by a tissue culture method". Bull. Off. Int. Epiz., 57: 902-907.

Zeidan, S. (1988): Virus in or "clinico-pathological study on bovine virus diarrhea in sheep" Ph.D. Thesis (clinical pathology), Fac. Vet. Med. Cairo University.