INFLUENCE OF AFLATOXIN AND ZEARALENONE ON BIOCHEMICAL ASSAY AND IMMUNE RESPONSE ON CATTLE NATURALLY INFECTED WITH BRUCELLOSIS AND EXPERIMENTALLY VACCINATED GUINEA PIGS WITH S19

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Abstract

One hundred cases of aborted cows from different Governorates in Egypt were tested to investigate the role of fungi and their mycotoxins namely, aflatoxin and zearalenon in enhancing abortion in Brucella-infected animals. These effects were also explored in experimentally vaccinated guinea pigs with *Brucella abortus* strain 19 vaccine and fed with contaminated ration containing mycotic toxins.

Serological testes including Rose Bengal plate, buffered acidified plate antigen, tube agglutination and complement fixation tests were used to identify the brucella infected animals and isolation of brucella organisms from internal organs of aborted foaeti and animals. The obtained serological and bacteriological results were correlated with mycological examination, detection of toxins (aflatoxin and zearalenone) in serum, feeds and internal organs of aborted foeti and immunological electrophoretic changes in serum protein fractions in addition to kidney and liver function tests.

The results indicated that the infection by moulds and their toxins had a direct effect on decreasing the humoral immune response in naturally infected cattle with Brucella, and it may play a role in enhancing abortion. The same findings were obtained in experimentally vaccinated guinea pigs fed on a contaminated ration containing mycotoxins.

INTRODUCTION

Brucellosis is a contagious bacterial disease that infects different species of animals and transmitted to man. In animals the disease is characterized by abortion and retained placenta. It causes serious economic losses in animal production in addition to its effects on human health. The first step of any successful eradication

control program is the diagnosis of the disease, which depends mainly on the detection of the elicited antibody titer.

Mycotoxins play an important role in food born diseases of human and animals (Hassan , 1994). A number of investigations have been directed towards determining the effect of aflatoxin and zearalenone on antibody response and the serum proteins in animals against brucellosis. The rational of these reports was that aflatoxin inhibits protein synthesis, and therefore could inhibit antibody formation. (Zaghloul and Shehata, 1991).

On the other hand, the mycotoxin zearalenon causes an increase of the agglutinating and later of the complement fixing activity probably caused by the enhancing of antibody avidity against brucellosis and increasing production and persistence of IgM when zearalenon administered in rabbits immunized with anti-brucella abortus vaccine (Ciuchini *et al.*, 1988).

The aflatoxin potential of inhibiting antibody formation through inhibition of protein synthesis has drown the attention of investigation interested on the effect of mycotoxin on the immunresponses.

Therefore, the aim of the present work was to find out the relationship between fungi and mycotoxins (aflatoxin and zearalenon) and the brucellosis in cattle., in addition to study the immunological effect of mycotxoins on resistance against brucellosis in experimentally vaccinated animals.

MATERIALS AND METHODS

Sampling

1. Blood samples from 100 cases of aborted cows mostly due to brucellosis infection were collected from different farms in Kafr El-Shiekh, Monofya, Sharkia, Behyra and Sohag Governorates.
2. The internal organs of 40 aborted foeti (including, liver, spleen, kidney, lymph nodes and intestinal contents) were collected for isolation of brucella and moulds as well as measurements of mycotoxins.

3. A total of fifty feed samples used by infected animals (hay, wheat, straw, tibn and yellow corn) were examined for mould contaminations and detection of mycotoxins.

Laboratory animals

A total of twenty-four female Guinea pigs vaccinated with S.19 vaccine and have an average weight of 300-400 g were fed commercially prepared food and supplied with water *ad libitum* throughout the experiment.

Serological diagnosis of brucellosis

The sera of cows and Guinea pigs were examined by buffered acidified plate antigen, Rose-Bengal plate, tube agglutination and Complement fixation tests (Morgan, 1969 and Alton *et al.*, 1988).

Isolation of Brucella organisms

The samples of intestinal contents, liver, kidney and lymph nodes of aborted foeti were subjected to bacteriological examination aiming to isolate brucella organisms. This was carried out according to Alton *et al.* (1975) and Alton *et al.* (1988).

Isolation and identification of fungi

Isolation and identification of moulds from feeds and internal organs of aborted foetei were carried out according to ICMSF (1978) and Refai (1979) .

Detection of mycotoxins

Detection of mycotoxins in serum, organs of aborted foetei of infected animals and feed stuffs were applied as described by Basil *et al.* (1981) and Hansen, (1993).

Biochemical changes in blood parameters

Serum ALT and AST enzymatic activities were estimated according to Reitman and Francle (1957). Serum urea was estimated according to Coulombe and Favreau (1963). Serum protein electrophoretic pattern was assayed according to Ornstein (1964) and Davis (1964) and total proteins were determined using biuret reagents (Sonnenwirth and Jarete, 1980).

Statistical analysis

Samples were simultaneously analyzed using t-student test according to Petrie and Watson (1999).

Experimental design for treatment of Guinea pigs with aflatoxin (AF), zearalenon (Z) and brucellosis

The used 24 Guinea pigs were vaccinated subcutaneously with strain 19 vaccine at a dose of 3-9 X 10^8 (1/10 of cattle dose) (R) and distributed randomly into 3 groups (A B and C) each of 8 animals. Group A is kept as control, while Group B was given Aflatoxin prepared according to El-Bazza *et al.* (1983), and Group C was given zearalenon prepared according to Caldwell *et al.* (1970). The doses used for administration of aflatoxins and zearalenon were as recommended by Thurstn *et al.*, (1974).

The design of experiment is illustrated in Tabl 1.

Table 1. Experimental design for treatment of strain 19 vaccinated Guinea pigs with aflatoxin, zearalenon

Days of treatment	Group A Sterilized normal saline	Group B aflatoxin (ppm)	Group C Zearalenon (ppm)
1st day	0.5 ml	0.3	0.5
4th day	0.5 ml	0.3	0.5
1st week	0.5 ml	0.3	0.5
2nd week	0.5 ml	0.3	0.5
3rd week	0.5 ml	0.3	0.5
4th week	0.5 ml	0.3	0.5
6th week	0.5 ml	0.3	0.5
8th week	0.5 ml	0.3	0.5
10th week	0.5 ml	0.5	0,5

RESULTS AND DISCUSSION

Brucellosis is one of the most dangerous bacterial diseases that has a direct impact on the animal wealth economy. Therefore, many investigations are directed to find out the all relating factors to this problem. In the present study, 100 aborted cattle cases from different Governorates were serologically tested for brucellosis by buffered acidified plate antigen (BAPA), Rose-Bengal plate (RBPT), Tube agglutination (TAT) and Complement fixation tests (CFT) (Table 2). The results obtained revealed that the percentages of infection are 70 %, 61 %, 65 % and 63 %, respectively by the used tests. From the obtained results, it was clear that the BAPAT was the most sensitive test to detect brucellosis infection serologically in comparing with the other used tests, while the CFT is the most specific one . These results were confirmed by the bacteriological examination of forty aborted foeti which revealed isolation of 9 strains of brucella organisms which were typed as *Brucella melitensis* biovar 3 . The results are in harmony with the results of many workers including El Bauomy (1993) , El-Gibally *et al.* (1995), Montasser *et al.* (2001) and Montasser *et al.* (2002).

The sera of some tested cattle revealed the presence of detectable levels of mycotoxins with immunological changes in the protein fraction contents (Table 3). Serum aflatoxin, and zearalenon were detected in 44% and 40% of tested cattle with the mean levels of 4.74, and 52.0 ppm, respectively. The tricothecene mycotoxin (T-2) was not detected. These results indicated that serum aflatoxin was prevailed more than the other mycotoxins in the serum of cattle in Egypt. The tricothecene mycotoxin (T-2) could not be detected. Toxins in association with infection in animals in Egypt were previously revealed by Hassan (1994).

The electrophoretic study of serum protein of the same animals revealed a significant decrease in total protein content, albumin, $\alpha 1$, $\gamma 1$, $\beta 1$, $\beta 2$ globulin, but the levels of $\alpha 2$, $\gamma 1$ and $\gamma 2$ globulin were significantly increased . These findings were previously detected by many workers (Richard *et al.*, 1975, Ciuchini *et al.*, 1988), who reported that the mycotoxins (aflatoxin and zearalenon) caused failure of the acquired immunity system of animal by decreased antibody production and altering serum profile protein.

In the current study we revealed that in cases of brucellosis infection in field which was associated with mycotxoicosis (aflatoxin and zearalenone), the acquired immunity of animals and electrophoretic parameters of serum protein were significantly altered (Table 4). In addition to the biochemical analysis of serum of these animals, hepatic and renal dysfunctions were indicated by increased levels of urea, cretainine, AST and ALT (Table 5). All these findings were also observed by Ray *et al.* (1986), Diekman and Green (1992)and Thurstn *et al.* (1974) .

For further investigation the forty case aborted foeti were subjected for fungal isolation and detection of their toxins including intestinal contents, liver, kidney, spleen and lymph nodes. Nine genera and nine species of moulds were isolated from internal organs. It is of interesting to report here that the different mycotoxins could be detected in these organs of aborted faeti particularly in liver, lymph nodes and spleen, but the mycotoxins were not detected at all in kidney, and this could be regarded to that the excretion system did not become well formed (Table 5). Different members of aflatoxins M1, B1, B2 G1 and G2 were detected in livers of aborted faeti in 20%, 20%, 12.5%, 7.5% and 0% of cases with mean levels of 7, 6, 3.5, 5 and 0 ppb, whereas in lymph nodes it was found in 5%, 20%, 10%, 5% and 5% of cases at mean levels of 5, 6.3, 7.5, 5 and 2 ppb, respectively. Ochratoxin and zearalenon were present in livers of 5% and 12.5% of cases with mean levels of 10 and 8 ppb, respectively. Other mycotoxins in other organs were not frequently detected (Table 5).

Eight genera and three members of aspergilli were isolated from feed used by aborted animals (Hay, wheat, straw, tibn and yellow corn). All samples of feeds used by cases of abortion gained detectable levels of different mycotoxins The aflatoxins were detected in 68% of hay samples, 72% of wheat and straw, 36% of tibn and 48% of yellow corn samples with mean levels of 30, 20, 25, 24 and 35 ppm, respectively. Zearalenone extracted from 24% of hay, 36% of wheat, 56% of straw and 20% of yellow corn samples. The levels of this toxin were 50, 30, 40, 0 and 22 ppm, respectively. Other mycotoxins (ochratoxin) were present in most of samples, whereas T2 was present only in wheat and starw samples. These results were in accordance with the results of Maryamma *et al.* (1990).

The experimental evaluation of frequent administrations of aflatoxin and zearlenone to Guinea pigs vaccinated by S.19 vaccine according to the design of the experiment revealed a significant decrease in total proteins, albumin and α1a, α1b, α2, γ1 and γ2 globulin, but the levels of β1 and β2 globulins were significantly increased in comparison to control group of Guinea pigs (Table 7). These results agreed with those obtained by Thurstn *et al.* (1974) and Ciuchini *et al.* (1988) who observed an increase of serum γ globulin, decrease in α2 globulin and frequent decrease in total protein concentrations in Guinea pigs daily dosed with aflatoxin or zearlenone.

The biochemical parameters of livers and kidneys dysfunction which were observed in clinical cases of aborted cattle due to mycotoxicosis were also reported in serum of Guinea pigs experimentally administered with doses of aflatoxins, zearlenone and *B, abortus* S19 vaccine (Table 8). These findings coincided with those reported by Ray *et al.* (1988) and Thurstn *et al.* (1972).

On the other hand, the follow-up of the humeral antibody titer of vaccinated animals of the control group (A) was increased gradually until the end of experiment with classical manner as reported by many authors (El-Bauomy *et al.* (1993) and El-Gibally *et al.* (1995). On the other hand, in group B injected by aflatoxin and goup C which was injected with zeraleonon the antibody titer levels were less than the control group. This may be due to increase of serum γ globulin, decrease in α2 globulin and frequent decrease in total protein concentrations.

In conclusion, the diagnosis of brucellosis must include all relating factors affecting the health of animal and its environment. This study throws light on the factors that could hamper the eradication and control program of brucellosis in farm animals. Among these factors are the fungal and mycotoxin affections, since we found that the toxins had a direct effect on the immune response and antibodies production as an immunosuppressive factors, and thus, affecting the efficiency of the vaccination program. We found also that moulds and mycotoxins increased the rate of abortion in infected animals. Therefore, maintaining good health conditions for vaccinated animals, besides, feeding vaccinated animals on feed free from moulds and mycotoxins will aid in the success of control program of brucellosis.

Table 2. Results of serological tests for brucellosis of aborted cows from different Governorate.

(Governorates)	No. of Animals.	Serological tests												
		BAPAT			RBT			TAT				CFT		
		+ve	-ve.	%	+ve	-ve	%.	+ve	±ve.	-ve	%	+ve	-ve	%
Sharkya	12	8	4	66.66	7	5	58.33	4	1	7	41.66	6	6	50
Monofya	40	22	18	55	19	21	47.5	20	1	19	52.5	21	19	52.5
Kafr El-Shikh	18	14	4	77.77	13	5	72.22	10	4	4	77.77	13	5	72.22
Behyra	20	16	4	88	14	6	70	14	2	4	80	15	5	75
Sohag	10	10	0	100	8	2	80	8	1	1	90	8	2	80
Total	100	70	30	70	61	39	61	56	9	35	65	63	37	63

BAPAT ; Buffered acidified plate antigen RBPT Rose Bengal plate test

TAT : Tube agglutination test CFT complement fixation test

Table 3. Determination of mycotxoins and biochemical alteration in serum protein due to brucellosis in cows.

Cases	No. of cases	Number of Brucella reactors		Mycotoxins in serum				T.protein (gm / dl.)	Albumin (gm / dl.)	Globulin (gm / dl.)					
				Aflatoxin B1 *		Zearalenone									
		+ve.	%	+ve.	%	+ve.	%			α1	α2	β1	β2	γ1	γ2
Infected cows	100	70	70%	44	44	40	40	7.18± 0.35	1.77± 0.10	0.86± 0.12	0.77± 0.12	0.44± 0.10	0.33± 0.06	2.51± 0.16	0.50± 0.06
Control	5	-	-	-	-	-	-	7.83± 0.09	2.45± 0.09	0.94± 0.01	0.61± 0.02	0.75± 0.03	0.39± 0.03	2.30± 0.07	0.41± 0.06

Aflatoxin B1 Max 9.2 Min 2.0 with mean 4.74 ppm Zearlenone Max 60.7 Min 23.0 with mean 52.0 ppm

ppm part per million

Table 4. Biochemical changes in kidney and liver function due to brucellosis infection in cows and mycotoxicosis (aflatoxin + zearalenone).

	Brucella infection	Aflatoxin and zeralenone in serum	Urea (mg/dl)	Creatinine (mg/dl)	GPT (u/l)	GOT (u/l)
Infected cattle	+	+	53.29 ± 3.75	0.94 ± 0.15	24.88 ± 2.77	28.35 ± 1.83
Control cattle	-	-	41.83 ± 1.73	0.78 ± 0.22	17.32 ± 3.18	19.72 ± 2.45

Table 5. Levels of mycotoxins in the internal organs of forty aborted foeti.

Organ		Liver			Kidney			Lymph node			Spleen		
		+ve sample		Mean	+ve sample		Mean	+ve sample		Mean	+ve sample		Mean
Mycotoxins		No.	%	ppb	No.	%	ppb	No.	%	ppb	No.	%	ppb
Aflatoxin	M1	8	20	7	0	0	0	2	5	5	2	5	10
	B1	8	20	6	0	0	0	8	20	6.3	1	2,5	2
	B2	5	12.5	3.5	0	0	0	4	10	7.5	0	0	0
	G1	3	7.5	5	0	0	0	2	5	5	0	0	0
	G2	0	0	0	0	0	0	2	5	2	0	0	0
Ochratoxin		2	5	10	0	0	0	0	5	10	0	0	0
T2		0	0	0	0	0	0	0	0	0	0	0	0
Zearalenone		5	12.5	8	0	0	0	5	12.5	9	3	7.5	8

ppb part per billion

Table 6. Mycotoxins in feeds used by aborted animals.

Mycotoxin	Aflatoxin (AF)			Ochratoxins			Zearlenone			T-2		
	Positive		Mean	Positive		Mean ppb	Positive		Mean ppb	Positive		Mean
Feeds	No.	%	ppb	No.	%		No.	%		No.	%	ppb
Hay	34	68	30	24	40	10	12	24	50	0	0	0
Wheat	36	72	20	4	8	15	18	36	30	6	12	18
Straw	36	72	25	30	60	8	28	56	40	22	44	0
Tibn	18	36	24	21	42	5	0	0	0	0	0	0
Yellow corn	24	48	35	5	10	8	10	20	22	0	0	0

Table 7. Protein electrophoresis of Guinea pig serum experimentally vaccinated with S19 vaccine and injected by aflatoxins and zearalenon (g / dl.)

	Total protein	Albumin	Globulin						
			α1a	α1b	α2	β1	β2	γ1	γ2
Treated animal	2.07 ± 0.72	2.09 ± 0.22	0.84 ± 0.10	0.87 ± 0.08	0.48 ± 0.12	0.50 ± 0.07	0.85 ± 0.18	0.89 ± 0.16	0.85 ± 0.21
Control	8.48 ± 0.08	2.70 ± 0.08	1.27 ± 0.07	1.02 ± 0.08	0.53 ± 0.13	0.48 ± 0.03	0.65 ± 0.05	1.13 ± 0.07	0.70 ± 0.05

Table 8. Biochemical changes in kidney and liver functions of guinea pigs due to experimental mixed infection with brucellosis and mycotoxicosis (aflatoxin and ochratoxin).

	Vaccine Reaction	Aflatoxin and zeralenone in serum	Urea (mg/dl)	Creatinine (mg/dl)	GPT (u/l)	GOT (u/l)
Vaccine groups	+	+	43.72 ± 3.40	0.79 ± 0.13	55.28 ± 2.70	93.72 ± 4.18
Control group	-	-	38.45 ± 4.18	0.72 ± 0.10	41.75 ± 4.12	58.43 ± 3.27

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تأثير الافلاتوكسين والزيرالينون على الخواص البيوكيميائية ورد الفعل المناعي على الأبقار المصابة طبيعيا بالبروسيلا وعلى خنازير غينيا المحصنة تجريبيا بلقاح العترة ١٩

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تم إجراء هذا البحث على ١٠٠ حيوان من الأبقار المجهضة من محافظات مختلفة من مصر لدراسة تأثير دور الفطريات وسمومها وخصوصا الافلاتوكسين والزيرالينون على الإجهاض في الحيوانات المصابة بالبر وسيلا وتأثير السموم السابقة على خنازير غينيا المحصنة بلقاح البر وسيلا ابورتس العترة ١٩ مع تغذيتها بعليقه تحتوى على السموم الفطرية ثم إجراء الأختبارات السيرولوجية وتشمل الروزبنجال والانتيجن الشريحى المخمد المحمض واختبار التلزن الأنبوبي واختبار المثبت المكمل لمعرفة الحيوانات المصابة بالبروسيلا وكذلك تم إجراء الزرع البكتيري لعزل ميكروب البروسيلا من الأحشاء الداخلية للأجنة المجهضة ومن البان الحيوانات .

تم مناقشة النتائج السيرولوجية والبكتريولوجية ومطابقتها بالفحص الفطري وتحديد السموم (الافلاتوكسين والزيرالينون) في السيرم والأغذية وكذلك الأحشاء الداخلية للأجنة المجهضة. وتم دراسة التغيرات المناعية للبروتين بالإضافة إلى عمل وظائف الكبد والكلى للحيوانات ومن النتائج اتضح أن العدوى بالفطريات وسمومها لها تأثير مباشر على تقليل رد الفعل المناعي للحيوانات المصابة طبيعيا ويلعب دور رئيسي في زيادة حالات الإجهاض . ونفس النتائج والمشاهدات وجدت أيضا تجريبيا في خنازير غينيا المحصنة بلقاح العترة ١٩ والمغذاة على عليقه تحتوى على السموم الفطرية .