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IMMUNOSUPPRESSIVE EFFECT OF BABESIA BOVIS IMMUNOGENS ON IMMUNIZATION AGAINST FOOT AND MOUTH DISEASE

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SUMMARY

The impact of natural infection with *Babesia bovis* on the immune response to FMD vaccine in calves was studied. Three groups of calves, group (1) were *Babesia* bovis free and vaccinated with inactivated FMD vaccine, group (2) naturally *Babesia bovis* infected and vaccinated with inactivated FMD vaccine,while group (3) was kept as non infection non vaccinated control.

The humoral immune response of each group was measured by serum neutralization test (SNT) for a period of 16 weeks. Babesia (*B.)bovis* naturally infected group of calves (gp. 2) showed tendency of lower antibody responses in comparison with uninfected (gp. 1) post vaccination with Foot and Mouth Disease (FMD) vaccine.

Parasitaemia of *B.bovis* infected calves were ranged from 1.5% to 2.5% at time of vaccination accompanied with reduction in packed cell volumes (PCV) up to 23% less than control group. The parasite persisted in the blood of infected calves as carriers with low parasitaemia.Three isolates of *B.bovis* have been identified.

Protein characterization of the three isolates of *B.bovis* immunogens resulting in immunosuppressive effect was investigated. The isolates identified and propagated in cell culture using microaerophilus stationary phase and characterized by sodium dedocyle sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Western immunoblot. The molecular weights of antigens were varied from12 to 165 Kilo Daltons (KDa). The isolates showed totally 30 polypeptide antigens. Nineteen antigens were detected as homologous and common between the isolates, their molecular weights were 160, 152, 132, 110, 85, 77, 70, 67, 60, 49, 45, 40, 39, 35, 33, 30, 23, 18 and 12 KDa. While the other 11 antigenic bands were detected

as heterologous and differ between the isolates. their molecular weights were 165, 155, 153, 144. 140, 120, 115, 112, 62, 37 and 20 KDa. Isolates no. 1, 2 and 3 contained 24, 28 and 24 out of the 30 immunogens respectively. The immune suppressive effect by reduction in serum neutralizing antibody titers of *B.bovis* infected calves might be due to one or more of *B.bovis* common antigens.

INTRODUCTION

Heamoprotozoan infection (babesiosis and theileriosis) were found to be immunosuppressive agent against viral vaccine as reported by Farah et. al.. (1997 & 2005); Samir et. al., (2001); Kassem et. al.. (2003) and Daoud et. Al., (2004).Bovine babesiosis existed as an endemic disease among cattle and buffaloes in Egypt. The prevalence of *Babesia species* in cattle and buffaloes at different governorates of Upper Egypt and delta varied from 3.6% to 40.5% as detected by indirect fluorescent antibody technique (IFAT) EL-Ghaysh (1993) and from 22% to 32.13% using dot ELISA Abd EL-Gwad (1993).

Foot and mouth disease is a viral vesicular disease of cloven – hoofed mammals that generally causes sever economic losses due to its highly contagious nature and economic importance, FMD is included in list A of the Office International des Epizooties (OIE) Mattion et. al ., (2004) .

Vaccination constitutes an important control policy for foot and mouth disease in affected areas with advanced eradication programs, as well as, in free regions that decide to use immunization as control measure after recent introduction of the disease Bergmann et. al., (2003).

Both of the veterinary authorities and animals owners directed their attention to provide good protection for their animals through successful immunization using safe and potent vaccine However, on the other hand, parasitic diseases such as babesiosis were found to be immunosuppressive agents facing animal vaccination against many viral vaccines as reported by Moua et. al., (1997); Daoud et. al., (2004) and Farah e al., (2005)

Therefore, the objective of this work was t study:-

* The impact of natural infection with Babes bovis on the immune response to FMD vacci in calves.
* Molecular characterization of *B. bovis* antige (immunogens) using SDS-PAGE and Weste blot.

MATERIALS AND METHODS

1- Calves: Eleven 6-months old calves, six them were free from haemo-parasite infecti while the others were naturally *B.bovis* infe ed. The calves were proven to be free fr

FMD antibodies through screening their sera against strain O1/93/Aga of FMDV using SNT Ferreira, (1976).

2- **Foot and Mouth Disease Vaccine:** Locally prepared and tested monovalent binary ethyleneimine inactivated FMD vaccine type O1/93/Aga (Egypt strain) with alumenium hydroxide gel 2% and saponine was employed, the calves were inoculated with 2 ml vaccine subcutaneously.

3- **Serum Neutralization test (SNT):** It was performed using the microtechnique described by Ferreira, (1976) in which serial dilutions of the sera were mixed with 10^4 $TCID_{50}$ of FMD virus . The neutralization titers of the tested sera were expressed as the log_{10} of reciprocal of serum dilution 50% end point according to the Reed and Muench (1938).

4-**Hematology:** Blood samples were collected weekly. PCV was measured using the microhaematocrit method. Also Geimsa stained blood smears were examined for the presence of piroplasms and detection of parasitaemia as mentioned by Romany et. al.,(2000).

5-**Isolation of *B.bovis*:** Three isolates were identified microscopically according to their size using Geimsa stained blood smears and tested by IFAT Leeflang and Peri, (1972) using specific anti B. bovis hyper immune serum.

6- ***B.bovis* positive serum:** was collected from cattle vaccinated with . exoantigens and challenged with 1X 10^9 infected *B.bovis* RBCs (Makram et. al., (2001).

7- **Propagation of isolates:** Each isolate was invitro cultivated and propagated using microaerophilus stationary phase according to method of Levy and Ristic (1980) with the modification of Holman et.al., (1993). The culture was continued for 5-10 passages until the parasitaemia exceeded 10%.

8- **Preparation of *B.bovis* antigens according to Goodger, et. al.,(1985):** The infected RBCs were exposed to per-coll density distribution to separate infected RBCs than uninfected. Infected RBCs were washed 3 times in phosphate buffer solution (PBS) and sediment by centrifugation. The cells pellet were frozen and thawed three successive times to raptures membranes and release haemoglobin. The washing pellet was repeated 3 -5 times in DPBS and sediment by centrifugation at 30000 g for 30 minutes. The pellet was sonicated at 100 watts for 3 minutes intermitted in ice path. The protein conc. was measured using the procedure of Lowery et al. (1952) micro assay and were stored at -20°C until used.

9- Characterization of the prepared antigens of *B. bovis* by SDS-PAGE: Forty micrograms of each antigen was electrophorezed by SDS-PAGE according to the method described by Laemmli, (1970) using 7 – 17% gradient separating gel and 4% stacking gel. Standard known molecular weight markers were loaded to estimate the molecular weights of different antigens. Two gels were applied, one for silver staining and the other transferred to nitrocellulose membrane.

10- Characterization of the prepared antigens of *B. bovis* by Western blots: The separated antigens of SDS-PAGE were electro transferred to nitrocellulose membrane according to method of Towbin et.al., (1979) . The membranes were incubated in 3% gelatin dissolved in Tris buffer solution containing 0.05% tween 80 (TBST) for 30 min. to block other active sites of membrane. The membranes were washed with Tris buffer solution (TBS) then incubated with 1: 200 diluted *B.bovis* positive hyper immune serum in TBST. The membranes were washed three times and incubated with 1 : 7500 secondary antibody (peroxidase conjugated rabbit antibovine IgG diluted in TBST. The colour of bands was developed by adding a solution of 60 mg of 4 chloronaphthol dissolved in 20 ml absolute methanol and 100 ul H_2O_2 to the membranes immersed in 100 ml TBS with shaking.

Experimental design

The calves were divided into 3 groups. In group 1, 3 calves were *B.bovis* free and vaccinated with FMD vaccine. Group 2 was consisted of 5 calves naturally *B.bovis* infected and vaccinated with FMD vaccine. While group 3, was kept as control. The calves were monitored for serum neutralizing antibody titers as indicator immune response for FMD vaccine, blood parasitaemia and PCV.

RESULTS AND DISCUSSION

Parasitic infections constitute one of the majo problems to cattle and buffaloes industries b causing anemia, loss body weight, retardation o growth, lowering their viability resulting in in crease financial losses McCoosker, (1981).

On the other hand, control of FMD in Egypt achieved by immunization campaign Daoud e al., (1988). The success of these programes greatly affected by immunosuppressive diseas such as theileriosis and babesiosis which exerts profoundly depressive impact on immunologic responses and predisposes the animals to serio outbreaks Soulsby, (1987). For this reason, it w one of necessaries to study the immune respons of naturally *B.bovis* infected calves to inactivat FMD vaccine and characterize different antige of *B.bovis* isolates.

Results in table(1) showed that *B.bovis* infected group was suffering from mild to moderate signs of babesiosis at time of vaccination with FMD represented in slight fever, reduction in PCV and 1.5 -2.5% parasitaemia. Prior to vaccination, temperature of *B.bovis* infected calves (gp. 2) were les than 40°C . Reduction of PCV was 10 -23% less than non infected calves (gp. 3). Parasitaemia as detected by Geimsa stained smears were 1.5 – 2.5% in blood of (gp. 2) and persisted as carriers as in table (1) . Similar results were obtained by Romany et al. (2000) who reported that *B. bovis* infected calves suffered from fall in PCV accompanied with parasitaemia. The author considered babesiosis as one of main anemic disease of cattle.

Specific humoral immune response of calves immunized with FMD vaccine illustrated in table (2). Mean SN titers in *B.bovis* infected calves (gp.2) were consistently lower than in non infected group (gp.1) throughout the experimental period. The mean SN titer was 1.9 $\log_{10}$ in the non infected group while it was 1.4 in *B. bovis* infected (gp.2) 4-weeks post vaccination. At 8 weeks post vaccination, the serum antibody titers of *B.bovis* infected calves were 1.2 $\log_{10}$ and the animals became susceptible to infection with FMD virus. These results are agreed with Kardiasis et. al., (1964), Wisniewski et. al., (1972) and Bengelsdroff ,(1989) who found that more than 95% of the vaccinated cattle with SN titers of greater than 1.2 were protected from generalized FMD while cattle with SN titers less than or equal 1.2 were not protected and developed generalized infection.

This important observation parallel with that of Farah et al, (1997) who noticed a lower antibody response to FMD vaccine in *Theileria annulata* naturally infected cattle. The authors added that antibody reductions were varied from significant to non significant values depend on the level of parasitaemia.

Consequently, it was necessary to have details of antigenic structure of *B.bovis* (immunogens) isolated from infected calves. After propagation of the parasites in cell culture (fig. 1 a, b and c) and separation of infected RBCs using density percoll distribution (fig. 1d). *B.bovis* antigens were characterized using SDS-PAGE and Western blot. *B. bovis* positive serum showed reactivity with 30 immunogens (antigenic bands). The molecular weights of those polypeptides were ranged from 12 to 165 KDa. Isolate no. 2 contained the majority of different immunogens (28 out of 30) and it was recommended for vaccine against babesiosis. Isolates no. 1 and 2 were more homologous as they contained similar 25 bands and differ in 4 antigens (165, 140, 120 and 62 KDa. Isolates no. 3 was more heterologous from no. 2 as it differ in 8 antigenic bands (155, 153, 144, 140, 115, 112, 37 and 20 KDa) respectively (table 3 and Fig.3) Similar results were obtained by McElawin et al

(1988) in USA who mentioned that *Babesia species* contained some isolate common antigens in addition to some isolate specific antigens.Also, Stephen et al.,(1989) recognized the 120-85-55 and 42 KDa.surface protein antigens of *B.bovis* using SDS-PAGE and specific positive sera.

From tables (2) and (3) our results revealed that the immunosuppressive effect of the *B.bovis* isolates might be due to one or more of the 19 homologous antigenic structures which shared between the different isolates and their molecular weight were 160-110-85-77-70-67-60-49-45-39-35-33-23-18 and 12 KDa.These results were agreed with Wright et.al.(1988) who considered the multitude of path physiological effects of acute babesiosis might be due to the 20 KDa. Protease and other proteolytic enzymes which interacted with components of the host defense systems of *B.bovis* infected calves.

The details of antigenic profile were more distinguished in Western blot (Fig. 3) than SDS-PAGE which silver nitrate stained (Fig. 2) . It might be due to highly sensitivity of Western blot developer (4-chloro-naphthol and H_2O_2 in the presence of peroxidase of secondary conjugated IgG). Also Sohagan (1992) characterized Babesia species antigens using immunogens rather than SDS-PAGE.

In conclusion, imuno-suppression of cattle suffering from *B.bovis* infection could be of considerable veterinary importance. However, this immuno-depperssion may interfere with vaccination campaigns and predispose animals to other infective agents (parasitic bacterial or viral). SDS-PAGE followed by Western blot is sensitive and specific for detection the profile of B. bovis spe-

Table (1): *B.bovis* Parasitaemia and Packed cell volume (PCV) of calves during the period of the experiment.

Period post vaccination	Parameters	Mean parasitaemia and PCV of calves		
		Group 1	Group 2	Group 3
At vaccination	parasi taemia PCV	0 38 ± 2	2% ± 0.5 30 ± 2	0 39 ± 1
4 weeks post vaccination	parasi taemia PCV	0 38 ± 1	0.7% ± 0.2 32 ± 3	0 39± 2
8 weeks post vaccination	parasi taemia PCV	0 39± 1	0.4 % ± 0.1 33 ± 1	0 38± 1
12 weeks post vaccination	parasi taemia PCV	0 39 ± 1	0.1% ± 0.05 33 ± 1	0 38 ± 2
16 weeks post vaccination	parasi taemia PCV	0 38 ± 1	>01% 34 ± 2	0 38 ± 2

Group1 : non infected calves Group 2 :calves infected with Babesiosi Group 3 control

Table (2): Mean Serum neutralization titers of calves vaccinated with FMD vaccine in different groups.

Period post vaccination	Mean serum neutralization of calves		
	Group 1	Group 2	Group 3
At vaccination	0.3*	0.3	0.4
4 weeks post vaccination	1.9	1.4	0.4
8 weeks post vaccination	1.8	1.2	0.3
12 weeks post vaccination	1.3	1.1	0.4
16 weeks post vaccination	1.2	1.0	0.4

*antibody titers expressed as $\log_{10}$ of the reciprocal of the 50% serum end –point dilution.

Table (3): Antigens profile of *B.bovis* isolates characterized by SDS-PAGE and Western blot.

Molecular weights of antigens in KD_a	Isolate no.1	Isolate no.2	Isolate no.3
165	-	+	+
160	+	+	+
155	-	-	+
153	+	+	-
152	+	+	+
144	+	+	-
140	-	+	-
132	+	+	+
120	-	+	+
115	-	-	+
112	+	+	-
110	+	+	+
85	+	+	+
77	+	+	+
70	+	+	+
67	+	+	+
62	-	+	+
60	+	+	+
49	+	+	+
45	+	+	+
40	+	+	+
39	+	+	+
37	+	+	-
35	+	+	+
33	+	+	+
30	+	+	+
23	+	+	+
20	+	+	-
18	+	+	+
12	+	+	+

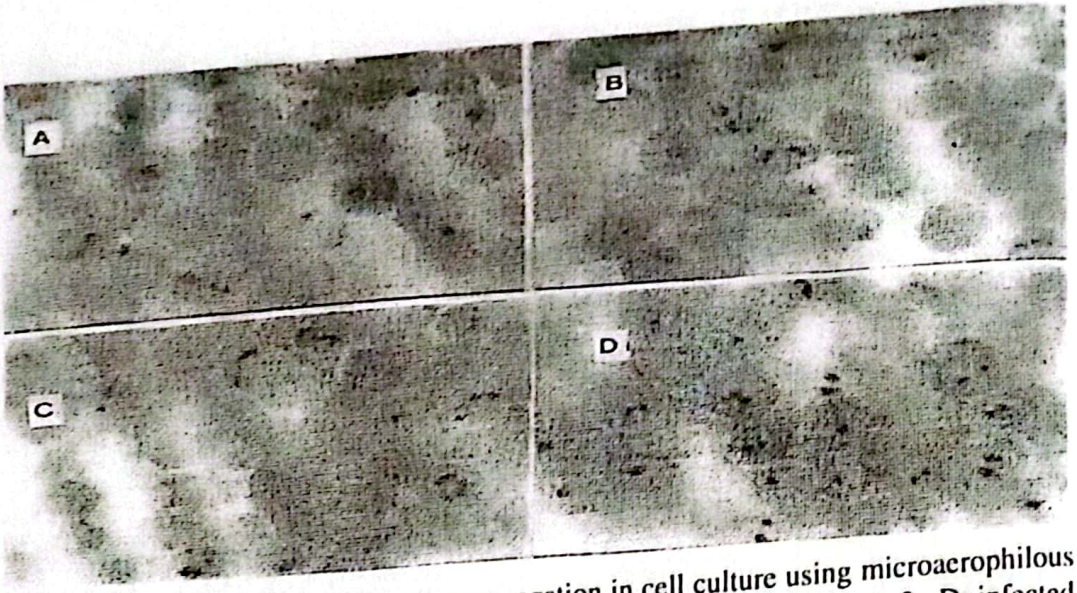

Fig. (1): *B. bovis* infected RBCs after propagation in cell culture using microaerophilous stationary phase. A: Isolate no. 1, B: Isolate no. 2, C: Isolate no. 3, D: infected RBCs after percoll density distribution

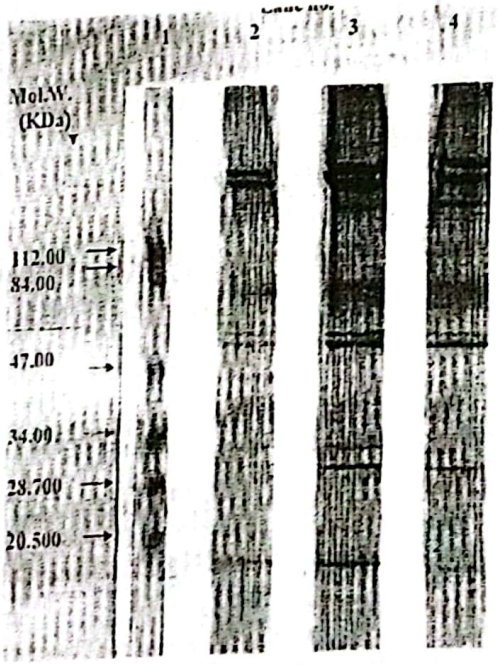

Fig (2): Antigenic profile of the three isolates of *B. bovis* demonstrated by gel electrophoresis and silver nitrate staining. Lane no. 1 : Known standard molecular weights, Lane no. 2 :isolate 1, Lane no. 3 :isolate 2, Lane no. 4 :isolate 3.

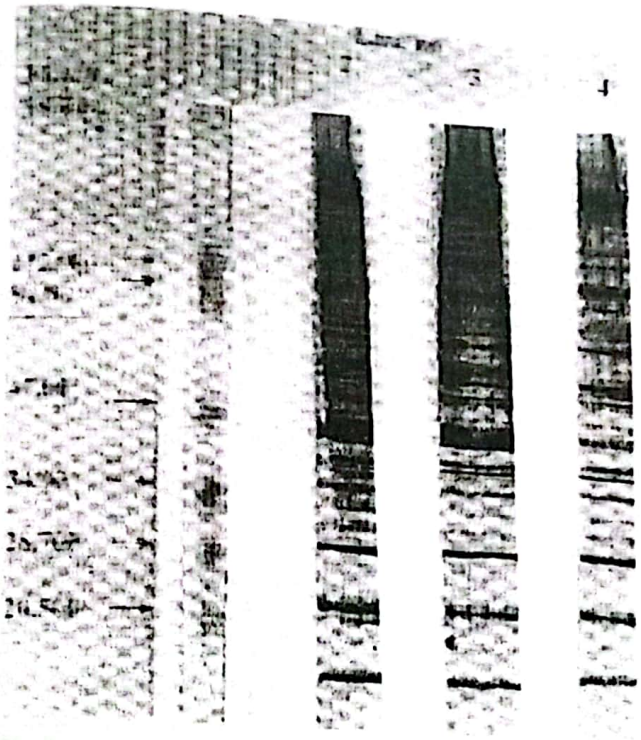

Fig. (3): Reactivity of the three isolates with *B. bovis* hyper immune serum as detected by immuno (Western) blot. . Lane no. 1 : Known standard molecular weights, Lane no. 2 :isolate 1, Lane no. 3 :isolate 2, Lane no. 4 :isolate 3.

cific antigens sharing between the isolates (common) and isolate variant immunogens.

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