Effect of some antibabesial drugs on the immune response of cattle vaccinated with inactivated bovine ephemeral fever vaccine

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ABSTRACT

This work spot the light on the effect of bovine babesiosis treatment on the immune response of cattle to the inactivated bovine ephemeral fever vaccine using imidocarb dipropionate and diminazene aceturate. Such investigation was carried out through mutual injection of the used drugs with animal vaccination in 8 groups (4calves/group) of native calves of about 6-8 months age included control vaccinated non-treated animals and healthy non-vaccinated and non treated animals. The obtained results revealed that diminazene aceturate depressed the immune response of vaccinated calves showing lower levels of BEF serum neutralizing antibody titer (16) than that induced by using imidocarb dipropionate which enhanced the immune response of vaccinated calves showing higher levels of antibodies (128) than those in the previous treatment and in control non-treated vaccinated calves (64). So, it could be recommended to use imidocarb for treatment of bovine babesiosis either simultaneously or post vaccination with the inactivated BEF vaccine providing enhanced immune response. Morever; imidocarb could be considered as immune stimulant agent to improve the immune response of calves to other vaccines.

Key words: babesiosis, imidocarb, diminazene aceturate, immune response, bovine ephemeral fever vaccine

INTRODUCTION

Babesiosis is the second most widespread blood-borne disease of animals **(**Home **et al, 2000), (Hunfeld et al, 2008)** and **(Gohil et al, 2013)** prominently, is gaining increasing interest as an emerging zoonosis of humans **(Home et al, 2000), (Kjemtrup & Conrad 2000), (Zintl et al ,2003) , (Leiby, 2011)**and **(Gohil et al, 2013).** Babesiosis is a disease caused by intraerythrocytic apicomplexan parasites of the genus Babesia, and transmitted by blood-sucking ticks of the Ixodidae family (hard ticks). Clinically, calves are characterized by fever and intravascular hemolysis manifested by a syndrome of anemia, hemoglobinuria and jaundice. It is also named as tick fever, Texas fever, red water fever **(Bock et al, 2004); (Cooke, 2005), (Gohil et al, 2010)** and **(Gohil et al, 2013).** As a result of the infestation there are retarded growth in calves, death, increased abortion rate and sterility, reduced milk and meat production and escalate the cost of prevention and treatments **(Bock et al, 2004)**.

Recovered animals become resistant carriers and serve as reservoirs of infestation for susceptible animals **(Bock et al, 2004), (Chauvin et al, 2009)** and **(Gohil et al, 2013).** Babesia is transmitted by ticks in which the protozoan passes transovarially, via the egg, from one tick generation to the next **(Gohil et al, 2013).** Vaccine use is not widely available in several countries; consequently treatment of cattle with babesiacides plays a central role in management of this disease (**Marta et al, 2013).** A wide range of antibacterial and antiprotozoal drugs have been used for the treatment of bovine babesiosis **(Adam and Corrier, 1980)** and **(Coetzee et al, 2009).** Imidocarb dipropionate and Diminazene Aceturate are widely used for treatment bovine babesiosis. Imidocarb at the dose rate of 1-2mg/kg body

weight subcutaneously is effective in prevention and treatment of bovine babesiosis and used as a chemoprophylactic at a high dose, 3mg/ kg subcutaneously **(Atif et al, 2012**). It provides protection from Babesia for at least 2 months (**Taylor and McHardy, 1979).** Moreover, the intraperitoneal administration of Imidocarb significantly increased serum IL-10 levels and lowered TNF-alpha levels in mice (**Katayama et al, 2003)** suggesting infective that a novel anti-inflammatory effect of Imidocarb could be utilized to treat inflammatory conditions.

Diminazene aceturate is an antiparasitic drug for treatment and control of protozoa infection in cattle, sheep, horses and dogs. It also protects cattle against Babesiosis and Trypanosomiasis for 2-4 weeks. It is widely used and given in a 7 % aqueous solution by deep intramuscular injection at a dose 3.5 mg/kg. It is well tolerated and will protect cattle from this disease for 2-4 weeks **(Faez et al, 2013).**

Bovine ephemeral fever (BEF) is an economically important disease in cattle and buffalo which occurs mostly in tropical and subtropical climates in Africa, Asia, the Middle East and Australia (**Walker, 2005) .**The disease is characterize by biphasic fever, anorexia, lameness and recumbence (**Burgess and Spradbrow, 1977) .** The disease is caused by a vector-borne single-stranded RNA virus bovine ephemeral fever virus (BEFV) and inflicts significant economic losses, mainly due to reduction in milk production **(Walker, 2005)** .

Both live attenuated and inactivated BEf vaccines are available for field use. The use of inactivated vaccine is considered a safer approach. These vaccine was inactivated by binary ethyleneimin and adjuvanted with, aluminum hydroxide and given as 2ml subcutaneously **(Della-Porta and Snowdon, 1979)**. The ability of commercial inactivated gel adjuvant vaccine

to produce long lasting protective immunity is doubtful, and it may need to be boostered (**Daoud et al, 2001).**

Therefore this study was aimed to investigate the; effect of Imidocarb and Diminazene Aceturate on immune response of cattle vaccinated with Bovine Ephemeral Fever vaccine.

MATERIAL AND METHODS

1.Drugs:

1.1- Imidocarb dipropionate: was obtained from Pitman-Moore Limited, Harefield, England. Firstly Amino ethyl thiourinium bromide (AET) Imidocarb is available in the form of a sterile clear aqueous solution treated sheep erythrocytes was prepared by mixing injectable solution 12%W/V. It was given at a single one volume of packed sheep RBCs to 4volumes of dose of 3mg/ kg body weight by subcutaneous route.

1.2- Diminazene aceturate: was obtained from Pitman-Moore Limited , widely used and given in a 7 % aqueous solution by deep intramuscular injection; 1.05 gm vial is dissolved in 12.5 ml water and a dose of 3.5 mg/kg body weight was used.

2. Bovine ephemeral fever vaccine: Inactivated cell culture BEF vaccine was supplied by department of pet animal and vaccine research (DPAVR); Serum and Vaccine Research Institute, Abbassia, Egypt and used for vaccination of cattle (**Hsieh et al, 2005)** and **(Della-Porta & Snowdon, 1979)** .Two doses of 2ml was given subcutaneously/ animal with 2 weeks interval.

3. Bovine Ephemeral Fever (BEF) virus: Locally isolated Bovine Ephemeral Fever Virus (BEF/AVS/2000) was propagated in BHK-21 monolayer cell culture and used for and SNT, with a titer of 10^6 $TCID_{50}$/ ml **(Azab et al, 2002**). This virus was supplied by the Department of Pet

Animal Vaccine &Research (DPAVR); Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia Cairo.

4. Animals and husbandry: 32 male native bread calves weighing 150-200kg were used. Calves were fed good quality concentrates and hay, with free access to water.

5.-Experimental design: All calves were proved to be seronegative to BEF antibodies. and divided into 8 groups (4calves/group) allocated as follow: Group-1: was vaccinated with the 2 doses inactivated BEF vaccine with 2 weeks interval using 2 ml inoculated subcutaneously. Group-2&3: were given 3 mg / kg body weight imidocarb dipropionate in a single S/C dose and 3.5 mg/kg / body weight diminazene aceturate in a single deep I/M dose at the time of vaccination with BEF vaccine, respectively. Group-4: was injected with imidocarb dipropionate, one week post vaccination with BEF vaccine. Group-5: was injected with diminazene aceturate one week post vaccination. Group-6&7: were injected with imidocarb and diminazene aceturate then vaccinated with BEF vaccine two weeks post injection, respectively. Group-8: was kept as non- vaccinated and non-injected as control

All groups were boostering after two weeks from the first vaccination with the inactivated BEF vaccine except the control one (non vaccinated group).

6. Serum samples: Serum samples were collected from vaccinated and non-vaccinated calves weekly for 4 weeks post vaccination and then every 4 weeks till the end of the experiment (٢٦ weeks). Sera were stored at – 20°C and inactivated at 56°C for 30 minutes before being used for evaluation of the humoral immune response using SNT.

7. Serum neutralization test (SNT): BEF serum neutralizing antibodies were detected by a microtiter assay modified by **Pega et al (2013).**Antibody

tires were expressed as the reciprocal of the final dilution of serum which neutralized and inhibited the CPE of 100 TCIV of BEF according to **Singh et al,(1967).**

8.Baby Hamster Kidney cells (BHK 21 clone 13): These cells were supplied by the Animal Virus Institute, Pirbright, UK and propagated at Pet Animal Vaccine Department, Abbasia, Cairo, These cells were used for virus titration and serum neutralization test.

9. Statistical analysis: The statistical analysis was performed using the ANOVA test- single factore (Sneelor and Cochren). Results are presented as arithmetic mean ± standard errors (SE) **(**Sneelor **and Cochren, 1986**).

RESULTS AND DISCUSSION

Table (1): BEF serum neutralizing antibody titers in vaccinated and inoculated cattle with Imidocarb and Diminazene aceturate

Animal Groups	Mean BEF serum neutralizing antibody titer #										
	1WPV ##	2WPV		1WPB ###	2WPB	3WPB	4WPB	1MPB ####	2MPB	3MPB	4MPB	°MPB
1	2	8		16	32	32	32	64	64	64	64	64
2*	4	16	2nd Dose	32	64	64	64	128	128	128	64	128
3	2	4		8	16	16	32	8	16	8	16	16
4	4	16		32	32	64	64	128	128	64	128	128
5	2	4		8	16	16	16	8	16	16	16	16
6	2	8		16	32	32	64	64	64	64	64	64
7	2	4		8	8	16	8	16	8	8	8	8
8	0	0		0	0	0	0	0	0	0	0	0

Group -1= vaccinated with BEF vaccine only
Group-2= vaccinated simultaneously with BEF vaccine and imidocarb(imizol)
Group-3= vaccinated simultaneously with BEF vaccine and diminazene Aceturate (berenil)
Group-4=vaccinated with BEF vaccine then injected with imizol by 1weeks
Group-5= vaccinated with BEF vaccine then injected with berenil by1weeks
Group-6= injected with imizol then vaccinated with BEF vaccine after 2weeks
Group-7= injected with berenil then vaccinated with BEF vaccine after 2weeks
Group-8 = non-vaccinated and non-injected and kept as control.

Mean BEF serum neutralizing antibody titer = the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100 $TCID_{50}$ of virus. BEF
WPV= week post vaccination
WPB= week post boostering
MPB= month post boostering
* significant at $p \geq 0.05$
N.B: the protective antibody titer of inactivated BEF vaccine is 32

The mean specific BEF neutralizing antibody titers were detectable by the 1st week post vaccination in all animal groups by SNT. The antibody titers that recorded in groups 2&4 injected with imidocarb were high (the titer was 64) by 2nd week post boostering and reached to the peak by 1 month post boostering (128). The specific BEF neutralizing antibodies lasted for ٥th month post boostering with good levels in vaccinated calves (gp 2,4&6).The groups that treated with diminazene aceturate (gp 3,5&7)showed low levels of antibody titers (8-16) as shown in table (1).

From an economic point of view, cattle are the most severely affected animals by babesial infections. The greatest losses occur in fully susceptible cattle introduced into enzootic areas so; bovine babesiosis is an important obstacle to live stock improvement programs in endemic areas. Currently, Diminazene Aceturate and imidocarb dipropionate are the most widely used for treatment of bovine babesiosis. Imidocarb dipropionate salt) is effective at a dose 3mg/ kg subcutaneously or intramuscularly . It provides protection from Babesia for at least 2 months **(Taylor and McHardy, 1979).** Diminazene Aceturate is widely used and given in a 7 % aqueous solution by deep intramuscular injection at a dose 3.5 mg/kg. It is well tolerated and will protect cattle from this disease for 2-4 weeks.

The present results show that imidocarb dipropionate, which has been used as anti-protozoan drug for the prevention and treatment of babesiosis in cattle, in a dose of 3mg/kg B.W. when injected subcutaneously in calves

vaccinated simultaneously and/or one week after vaccination with inactivated BEF vaccine caused a significant increase in antibodies titre of the vaccinated groups (gp. 2&4), with a considerable increase in the group injected two weeks post vaccination(gp. 6). But the groups injected with diminazene aceturate showed a lower levels of antibody titres than that the groups injected with imidocarb (gp3,5&7).These results suggested that the imidocarb dipropionate may stimulate the immune response of vaccinated animals. Similar results were described by **Bengelsdorff, (1989)** who stated that vaccinated calves were protected when SNT> 1.2 log.The imidocarb increased the animal immune response to the BEF vaccine through stimulation of humoral and cellular immunity **(Katayama et al, 2003).** It is also suggested that the immunostimulating effects of the these drug attribute to the release of a mixture of cytokines as it markedly enhanced LPS-inducing IL-10 production which promote antibody formation, increased number of both T and B lymphocytes and enhanced interferon production **(Katayama et al, 2003) .** The significant increase in the antibody titres against BEF vaccine in normal calves injected with imidocarb dipropionate explained the ability of the drug to optimize effective uses of the vaccine **(Ada, 1990)** .The imidocarb injected before (gp. 6)or at the time of vaccination(gp.2) may have a role in the stimulation of antigen presenting cells, T and B cells, so as to generate a large number of memory cells. Administration of imidocarb after vaccination may have similar effects but it decreased with the time as the antigen by the time is fully presented and already recognized by memory cells. The statistical analysis for the result shown in table (1) using ANOVA test- single factor according to **Sneelor and Cochren ,(1986).** It was revealed that there was a significant difference

(at $P \geq 0.05$) between group 2 (vaccinated simultaneously with BEF vaccine and imidocarb) and the other groups.

Conclusion

Administration of imidocarb dipropionate (3mg/kg b.wt) either at the time of vaccination or post vaccination with BEF vaccine stimulates the immune response of vaccinated calves with increased level of antibody titre and was prolonged the duration of protection. Furthermore, improvement in the immune response to BEF vaccine occurred during Imidocarb therapy in vaccinated calves. On contrast it is not recommend to vaccinate calves with inactivated BEF vaccine during or after treatment of bovine babesiosis with diminazene aceturate as anti-protozoan drug. So vaccination of calves with BEF vaccine should be used during treatment against babesiosis with imidocarb as anti-protozoan drug.

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الملخص العربى

تأثير بعض مضادات البابيزا على الاستجابة المناعية للابقار المحصنة بلقاح حمي الثلاث ايام المثبط

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تم خلال هذا العمل بدراسة تاثير مادة الاميزول و البيرينيل المستخدمتان في علاج مرض البابيزا في الابقارعلي الاستجابة المناعية للماشية ضد لقاح حمي الثلاث ايام المثبط ، حيث تم استخدام ثمانية مجموعات من العجول المحلية (اربعة حيوانات لكل مجموعة) عن عمر من ٨-٦ اشهر في تقيم هذا العمل وتم استبيان المستويات المناعية المتكونة فى أمصال الحيوانات المحصنة باستخدام اختبار المصل المتعادل . وتم حقن العجول بالادوية المستخدمة بطريقة متبادلة مع تحصين الحيوانات باللقاح وترك مجموعة كضابط بدون تحصين او حقن.واظهرت النتائج ان استخدام مادة البيرينيل تسبب نقص فى الاستجابة المناعية عند الابقار للقاح حمي الثلاث ايام(١٦)، وأن مادة *الاميزول* ترفع المستوى المناعى *لحمي الثلاث ايام* بشكل ملحوظ حيث اكتسبت الحيوانات المحصنة باللقاح أجساما مناعية نوعية ذات مستويات وقائية أعلى(١٢٨). وعلى ذلك يوصى باستخدام مادة الاميزول اثناء التحصين بلقاح حمي الثلاث ايام المثبط وعدم التحصين باللقاح فى حالة علاج مرض *البابيز* فى *الابقار* بمادة البيرينيل حيث انه يمكن اعتبار مادة الاميزول كمنشط مناعي للعجول المحصنة بلقاحات اخري.