The Effect Of Natural Infection With Some Blood Parasites And Their Treatment On The Humoral And Cell Mediated Immunity Of PPR Vaccine In Sheep

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ABSTRACT

Egypt exports the peste des petits ruminants (PPR) vaccine to several Arab, African and Asian countries, where the PPR is rampant. However, the simultaneous blood parasitic diseases, which are considered immunosuppressive agents are widespread among sheep and goats. The purpose of our work is to study the effect of theileriosis and babesiosis and their treatment with Butalex and Imizol respectively, on the immune response of vaccinated sheep with PPR vaccine.

The naturally infected groups of sheep with *T. ovis* and *B. ovis* showed a tendency of lower antibody responses in comparison with the uninfected group, post vaccination, as determined by the serum neutralization test (SNT). In addition, a depression in the cell mediated immunity was apparent between 3-7 days post vaccination (PV).

Complete clinical recovery and improvements in the blood profile were obtained in the treated animals where the packed cell volume (PCV), the haemoglobin content (Hb) as well as the lymphocytic count were accentuated gradually to reach their normal values.

The two aforementioned drugs were satisfactory used in the treatment of the blood parasites simultaneously with vaccination with the PPR vaccine.

INTRODUCTION

Peste des petits ruminants (PPR), is an acute highly contagious viral disease of small ruminants in Africa, Asia and the Middle East. Classically, it is characterized by pyrexia, nasal and ocular discharges, diarrhoea, respiratory distress, mucosal erosive lesions, as well as lymphoid tissue rational syndrome, and death in 80-90% of the acute cases *(1, 2)*.

PPR has a very high rate of morbidity and mortality and effective control of the disease is of high economic importance *(3,4,5,6)*.

In Egypt, the disease was firstly reported by *(7,8,9)* among sheep and goats in Giza governorate, and lastly by *(10)*.

Recently, a homologous vaccine (a strain of PPR virus attenuated and cloned after 22-25 passages in vero cells) has been developed and the minimum vaccine dose is $10^{2.5}TCID_{50}$/ml *(11,12)*. The value of this vaccine lies in the fact that the serological surveys, particularly for rinderpest eradication programmes would be made easier. In Egypt, the presence of PPR virus is now established after its isolation, identification and serological investigation *(7,9,10, 13)* suggested that the Egyptian sheep and goats are threatened by the spread of PPR and recommended that control measures should be undertaken to protect this wealth through vaccination with specific homologous PPR V vaccine.

Both of the veterinary authorities and animal owners direct their attention to provide good protection for their animals through successful vaccination using safe and potent vaccines. However, on the other hand, parasitic diseases such as theileriosis and babesiosis were found to be immunosuppressive agents facing the animal vaccination against many viral vaccines as reported by *(14,15,16)*.

Ovine theileriosis and babesiosis are known to occur in the Mediterranean basin as well as other areas such as Africa, Central Asia, Southern Europe, India and Iran; where the tick vector is present *(17)*. They are considered two important tick-borne diseases that cause the greatest economic losses in sheep and goat-production *(18,19)*. Steps to minimize the effects of the blood parasitic immunosuppression has been taken by using broad spectrum antibiotics and appropriate

anti-protozoal therapics *(20)*. Treatment of theileriosis and babesiosis using the buparvaquone (Butalex) and Imidocarb-dipropinate (Imizol) respectively were recorded by *(21,22,23,24,25)*.

The objectives of this work were to study the effect of the natural infection of sheep with *Theileria* and *Babesia ovis*, and their specific treatment on the humoral and cellular immune response to tissue culture PPR vaccine.

MATERIALS AND METHODS

(1) Experimental animals:

Sixteen sheep (9-24 months old from El-Wadie El-Gedid) were housed in the Veterinary Serum and Vaccine Research Institute and examined for the detection of the different blood parasites (T. ovis or B. ovis). Twelve of the animals were chosen to fulfil our experiment. The animals proved their susceptibility to PPR through the negative results of screening of their sera for the PPR neutralising antibodies.

(2) Viruses:

A. Peste des petits ruminants live attenuated vaccine:

It was kindly supplied by the Rinderpest Department, Serum and Vaccine Research Institute, Abbasia, Cairo. An attenuated strain of PPR virus, Egypt 87, was used to screen sheep for their seronegativity of PPR antibodies as well as for vaccination of tested animals and SNT.

B. Rinderpest vaccine:

The Rinderpest bovine Kabbet-O (R BOK) virus strain, routinely used for vaccine manufacture, was used to screen test of sheep for their seronegativity of rinderpest antibodies, through serum neutralization test *(26)*.

(3) Cell culture:

Vero cell line, established by *(27)* was employed in this study for serum neutralization test. The stock cell line was kindly supplied by Navy American Medical Research Unit-3 (NAMRU-3),Abbasia, Egypt.

(4) Evaluation of the immune response of vaccinated sheep:

It was carried out through the evaluation of cell mediated and humoral immune responses.

Evaluation of cell mediated immune response:

It was carried out according to the following steps:

- Separation of lymphocytes, according to *(28)*.
- Total lymphocytic count, according to *(29)*.
- Standardization of lymphocyte concentration for blastogenesis, where the concentration of mononuclear cells was adjusted to be $5x10^6$/ml of RPMI medium with 10% faetal calf serum.
- Preparation of mitogens (Phytohaemagglutinin, PHA), according to *(30)*.
- Setting up of lymphocyte culture according to *(31)*.

Lymphocyte transformation assay using MTT staining procedure according to *(31)*.

B. Evaluation of humoral immune response:

Serum neutralization test (SNT)

Qualitative and quantitative estimations were performed on the sera of sheep using the microtitre technique as described by *(32)*.

(5) Antiblood-parasite drugs:

(A) Buparvaquone (Butalex):

It was supplied by Pitman – Moore Limited Company, Harefield, England. It was uised for treatment of theileriosis in the tested sheep at the dose of 2.5 mg/kg.body weight injected once intramuscularly (I/M) according to *(33)*.

(B) Imidocarb dipropionate (Imizol):

It was supplied by Pitman – Moore Limited Company, Harefield, England. It was used for treatment of babesiosis in sheep. The drug was injected once I/M at the dose of 3 mg./kg body weight according to *(34)*.

(6) Sample collection:

(A) Serum samples:

Serum samples were collected from sheep before and weekly after vaccination and up to 8 weeks, to monitor the levels of PPR antibody titres.

(B) Blood samples:

Blood samples with, EDTA as anticoagulant, were collected twice weekly post vaccination for measuring the cell mediated immunity. In addition, blood samples were obtained from the animals pre and post vaccination for determination of the packed cell volume (PCV), haemoglobin content (Hb) and leucocytic count using the standard method of *(35)*.

(C) Blood films:

Giemsa stained blood smears were prepared from all sheep and examined pre and post vaccination for the detection of blood parasites and determination of parasitaemia percentages.

(7) Experimental design:

The present work was carried out on 12 sheep obtained from El-Wadi El-Gedid Governorate, Egypt and housed in the Veterinary Serum and Vaccine Research Institute. The animals were divided into 6 groups each consisted of 2 animals:

Group (1): Naturally infected with T. ovis and vaccinated S/C with 1 ml containing 103 TCID50 of PPR V vaccine adapted on Vero cells.

Group (2): Naturally infected with T. ovis and treated with Butalex simultaneously with the vaccination with PPR vaccine.

Group (3) : Naturally infected with B. ovis and vaccinated with PPR vaccine.

Group (4): Naturally infected with B. ovis and treated with Imizol simultaneously with the vaccination with PPR vaccine.

Group (5): Non infected and vaccinated with PPR vaccine.

Group (6): Non infected, non vaccinated control.

RESULTS

The mean results of the cellular immune response of the different groups of animals are illustrated in table (1). It revealed that the mean Delta optical density (ΔOD) values in the infected and untreated groups (1& 3) were lower than the infected and treated groups (2&4) post vaccination. Higher values of ΔOD were obtained from the non-infected and vaccinated group (5). The ΔOD of the non-infected and non-vaccinated control group (6) did not exceed 0.071, during the experimental period.

The results of SNT are presented in table (2). Lower antibody titres were obvious in the infected non-treated groups (1 & 3) from the 1st week till the 8th week post-vaccination. Regarding the infected vaccinated and treated animals (groups 2 & 4), it was noticed that their antibody titres were higher than those of the previous groups (1 & 3). However, the non-infected and vaccinated group (5) revealed an increase in the mean antibody titres from the 7th day and up to the 8th week. The neutralizing antibody titres reached the peak (titre 128) 30 day post vaccination.

Table (3) illustrated the effect of T. ovis and B. ovis on the parasitaemia percentage as well as the haematological values of the different groups. Examination of Giemsa stained blood smears of the naturally infected animals revealed parasitaemia in all the infected groups with different levels. An increase in the lymphocytic count was accompanied with a decrease in the Hb and PCV, compared with the non-infected control group (5) where all the previously mentioned parameters were within the normal limits. The treated groups (2 & 4) revealed a reduction of the parasitaemia. Additionally, the PCV and Hb content showed an elevation.

Table (1): Evaluation of the cell mediated immune response of sheep, vaccinated with PPRV vaccine.

Days post vaccination	Groups of animals					
	(1)*	(2)	(3)	(4)	(5)	(6)
3	0.229**	0.304	0.224	0.294	0.467	0.071
5	0.201	0.212	0.193	0.243	0.350	0.013
7	0.151	0.169	0.125	0.185	0.241	0.011

*Gp. (1): Infected with *T. ovis*. Gp. (2) : Infected with *T. ovis* and treated
Gp. (3) : Infected with *B. ovis*. Gp. (4) : Infected with B. ovis and treated.
Gp. (5) : Non-infected and vaccinated with PPR V vaccine.
Gp. (6) : Non-infected, non-vaccinated control. **Delta optical density values.

Table (2): Means of the serum neutralizing antibody titres following vaccination with PPR vaccine in the different sheep groups.

Groups of animals	Mean of the serum neutralizing antibody titres* / weeks post vaccination								
	0 wpv**	1 wpv	2 wpv	3 wpv	4 wpv	5 wpv	6 wpv	7 wpv	8 wpv
Group (1) Infected with *T. ovis*	0	2	8	8	8	8	8	8	8
Group (2) Infected with *T.ovis* and treated	0	4	16	32	32	32	32	32	32
Group (3) Infected with *B.ovis*	0	2	4	4	4	4	4	4	4
Group (4) Infected with *B.ovis* and treated	0	4	8	16	32	32	32	32	32
Group (5) Non-infected and vaccinated with PPR vaccine	0	8	32	64	128	128	128	128	128
Group (6) Non-infected non-vaccinated control	0	0	0	0	0	0	0	0	0

* Antibody titres are expressed as the reciprocal of the last serum dilution which neutralized and inhibited the CPE of 100-200 $TCID_{50}$/0.1 ml of PPRV.

**wpv = Weeks post vaccination.

Table (3): The means of the parasitaemia percentages and haematological values in the different sheep groups.

Weeks post	Group(1) : Infected with *T.ovis*				Group (2): Infected with *T. ovis* and treated				Group (3): Infected with *B.ovis*				Group (4): Infected with *B.ovis* and treated				Group (5): Non infected and vaccinated				Group (6): Non infected Non vaccinated			
Vaccin-ation	P *	PCV **	Hb ***	L ****	P	PCV	Hb	L	P	PCV	Hb	L	P	PCV	Hb	L	P	PCV	Hb	L	P	PCV	Hb	L
0	2	24	9.8	65	2.3	28	9.6	64	1.5	25	9.4	62	1.2	27	9.5	63	0	33	10.5	56	0	33	11.4	50
1	3.2	27	9.7	68	1.9	30	10.4	60	2.0	28	9.6	64	0.9	31	10.3	60	0	36	11	59	0	34	10.8	51
2	2.5	28	9.4	66	1.0	36	10.5	58	2.8	27	9.1	65	0.5	33	10.8	59	0	32	11.4	57	0	33	11.2	50
3	2.9	27	8.8	69	0.3	37	11.2	57	2.7	27	8.9	64	0.2	37	11.0	57	0	36	10.9	58	0	33	11.0	50
4	3.1	25	9.2	65	0.1	37	11.4	57	3.2	26	9.2	66	0.2	36	11.5	58	0	38	11.5	57	0	34	11.1	51

*Parasitaemia percentage (%)
**Paked cell volume (%)
***Haemoglobin content (g%)
****Lymphocytic count (%)

DISCUSSION

Peste des petits ruminants (PPR) is an acute highly contagious viral disease of domestic and wild small ruminants. It is caused by a morbillivirus and closely related to, but quit distinct from rinderpest virus *(1,36).*

At the end of 1980s, a PPR V strain was successfully attenuated by serial passages in Vero cells. It proved to be very efficient in the protection of sheep and goats against a virulent challenge. The living attenuated PPRV is now widely used in the control of PPR *(12)*.

On the other side, the blood protozoal diseases, such as theileriosis and babesiosis, are considered as immuosuppressive candidates influencing the immune response of the vaccinated animals *(15,37,38)*.

Consequently, it was necessary to study the effect of theileriosis and babesiosis, in naturally infected sheep and their treatment with specific drugs, on the immune response of sheep to the homologous attenuated cell culture of the PPR vaccine.

It was found that the treatment of infected sheep with theileriosis and babesiosis with Butalex and Imizol respectively, resulted in a complete recovery of the infected animals where the piroplasms were gradually reduced within 21 days of treatment. Complete clinical recovery and improvements in the blood profile were obtained in the treated animals two weeks after drug treatment. The haemoglobin concentration and PCV which were decreased initially because of the infection (ranging between 8.8-9.7 g% and 24-28% respectively) increased gradually to reach its highest values (11.5g% and 37% respectively). The general health of the animals was improved. This important observation parallels with those of *(25,39,40)*. Moreover, the lymphocytic counts restored their normal values post-treatment which is similar to previous findings *(41,42)*.

It is suggested that the treatment of the chronically infected sheep with *T. ovis* or *B. ovis* have the dual beneficial effect of reducing the pathogenic effects of theileriosis or babesiosis, thereby permitting restoration of an impaired immune system, thus increasing resistance to other infections.

Regarding the immune response of the experimental animals, the infected animals with *T. ovis or B. ovis* showed a suppressed immune response to PPR vaccine, where the antibody response was low (titre 4) post vaccination in comparison with the uninfected group. (titre 128).

Similar results were obtained by *(15,42)* who reported that *T. annulata* induced a significant abatement on rinderpest vaccine immune-response due to the massive lymphoid cell destruction which diminished the antibody response. They suggested that theileriosis leads to rupture of the lymphocytes. Furthermore, *(37,43)* reported that the vaccinated animals were predisposed to rinderpest virus infection by immunosuppression due to T.annulata infection. In a similar manner, babesia infection suppressed the immune response of sheep to PPR vaccine in which agrees with previous investigators *(19,38)*.

Concerning the cell mediated immunity, the presented findings indicate a probable depression of the cell mediated immune response. This depression was apparent between 3-7 days. Similarly, *(44)* referred that, on mitogenic stimulation, the peripheral blood leucocytes from the infected cattle with *T.annulata*, showed a remarked reduction.

It is important to point out that the inoculated sheep with Butalex or Imizol simultaneously with vaccination with PPR vaccine resulted in highly increased antibody titres of 32 than in the nontreated groups (titre 4). Similar findings were noticed by *(39)*, who suggested that these drugs may enhance the immune system. In addition, the drugs have the ability to optimise the animal immune-response to the inoculated vaccines. Such drugs may stimulate the antigen presenting cells, besides the T and B cells. Moreover,

they probably can generate a large number of memory cells.

From the previous results, it is obvious that all the infected and treated groups revealed a good protective level of antibodies from the 2nd week and up to the 8th week post-vaccination. On the other hand, the titres of the serum-neutralizing antibodies in the infected vaccinated sheep (without treatment) were still lower than those of the vaccinated non infected group. These results are in harmony with those obtained by *(43,45)*, who reported that the protozoal infections might interfere with the vaccination programmes by immunosuppression and shortening the duration of immunity. It is worthy to observe the low levels of antibodies in the non-treated groups. The permissible level of PPR antibody titre is 1 log 10, so the infected nontreated groups of sheep (1 & 3) are considered to have immunofailure *(46)*.

It could be recommended that a considerable attention must be paid to the protozoal immunosuppression. It is of a supreme importance to treat the blood parasite infected animals with the suitable specific antiparasitic drugs simultaneously with vaccination with the PPR vaccine to achieve the promising levels of immunity.

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الملخص العربي

تأثير الإصابة ببعض طفيليات الدم وعلاجها على المناعة السائلة والخلوية للقاح طاعون المجترات الصغيرة فى الأغنام

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معهد بحوث الأمصال واللقاحات البيطرية - العباسية

تصدر مصر لقاح طاعون المجترات الصغيرة إلى بعض الدول العربية والأفريقية والأسيوية حيث ينتشر المرض. فى نفس الوقت تنتشر أمراض طفيليات الدم بين الأغنام حيث تعتبر عوامل مثبطة. ولذلك أجرى هذا العمل لدراسة تأثير الثايليريا والبابيزيا أوفز وعلاجها باستخدام عقار البيوتالكس والإيميزول على الاستجابة المناعية للقاح طاعون المجترات الصغيرة.

أظهرت مجموعات الأغنام المصابة طبيعياً بطفيل الثايليريا والبابيزيا أوفز اتجاه لانخفاض الأجسام المناعية بالمقارنة للمجموعة الغير مصابة بعد التحصين وذلك بإجراء اختبار المصل المتعادل. بالإضافة إلى ذلك لوحظ انخفاض فى المناعة الخلوية بين اليوم الثالث والسابع.

ظهر تحسن واضح فى صورة الدم وذلك فى الحيوانات التى تم علاجها حيث ارتفعت نسبة الاختزال فى خلايا الدم (PCV) وهيموجلوبين الدم. كذلك وصلت نسبة الـ Lymphocytic Count إلى معدلاتها الطبيعية.

يتضح من نتائج البحث أن العلاج بالأدوية السابق ذكرها كانت مرضية ومؤثرة فى علاج طفيليات الدم وذلك عند استخدامها مع التحصين بلقاح طاعون المجترات الصغيرة.