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PREPARATION OF A SPECIFIC PESTE DES PETITS RUMINANTS (PPR) VIRUS VACCINE

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SUMMARY

A specifc Peste des Petits Ruminants (PPR) virus vaccine was prepared by viral passage of the local strain of PPR virus (Egypt - 87), 20 times on Vero cells . The preparared vaccine was screened for fungi, mycoplasma, aerobic and anaerobic bacteria. Identity, safety, potency and excretion of such vaccine virus were tested. Balady sheep and Balady goats were inoculated subcutaneously each with the detected protective dose(10^3 $TCID_{50}$) of the vaccine virus. The level of PPR neutralizing antibodies was followed up in the sera of vaccinated animals from the first week till the 52nd week (one year) post vaccination . The antibody titres reached their peak (128) in sheep and (256) in goats by the 3 rd week post vaccination and remained unchanged up to one year later. The vaccinated animals did not show any thermal elevation in rectal temperature or abnormal clinical signs.

It was concluded that the prepared live attenuated cell culture PPR vaccine is a safe and potent specific vaccine and could be used safely to protect sheep and goat industries against PPR disease.

INTRODUCTION

Peste des Petits Ruminants (PPR) is a contagious viral disease of small domestic ruminats characterized by pyrexia, catararrhal nasal and ocular dischage, necrotic stomatitis and an intestinal mucosal and lymphoid tissue reaction syndrome. The disease may also be known as pseudorinderpest of small domestic ruminants, stomatities of sheep and goats, pneumoenteritis complex and kata (Appiah, 1982).

Early observations were recorded in

Nigeria where cases of the disease were reported in Nigerian goats (**Henderson, 1930 and 1933**) . The fisrt labelling of the disease as peste des petits Ruminats was in Ivory Coast (**Gargadennec and Lalanne, 1942**).

Among Egypt, the disease was reported for the first time by **Ikram et al. . (1988)** and lastly by **Mouaz et al. (1995)**.

It was believed that PPR is a strain of rinderpest virus adapted and pathogenic for goats and sheep (**Mornet et al., 1956**) but recently, it has been confirmed that PPR is related to, but quietly distinct from rinderpest virus (RP) (**Hamdy et al., 1976 and Gibbs et al., 1979**). They classified it as the fourth member of genus Morbillivirus.

Depending on the relationship between RP, some workers used RP vaccine to protect sheep and goats against PPR disease as **Hamdy (1976) Gibbs et al. (1979), Bourdin et al.(1970), Taylor(1979), Bonniwell (1980), Taylor (1983), Wosu et al. (1990)** . These workers obtained good protective results, but others found that vaccination of sheep and goats with RP vaccine, resulted in low level of formed antibodies which are non specific (heterologous) to PPR, and may cause failure of vaccination if a specific PPR vaccine used (**Plowright and Taylor 1967; Dutrnell and Eid, 1973 ; Mariner et al., 1993; Taha, 1995 and Hussein, 1998**).

Diallo et al., (1989) described the attenuation of PPR (Nig- 75/1) by serial passage in vero cells and obtained a potential homologous vaccine against PPR. They found that the minmum vaccine dose is $10^{2.5}$ $TCID_{50}$ Also, **Adu et al., (1990)** noticed a progressive loss of the virulence of PPR virus by its passages in Vero cells, so they suggested that it will not be long before a homologous PPR vaccine obtained. In addition, **Taha, (1995) and Hussein (1998)** recorded high levels of specifc PPR neutralizing antibodies in sheep and goats inoculated with the vero cells attenuated PPR virus.

It is thought that the present work would be of great importance in the efforts done to protect the national sheep and goat wealth against such disase where the main object of this work is to produce a specifc safe and potent PPR vaccine.

MATERIAL AND METHADS

1- Viruses:

1.1. Rinderpest virus (RPV) :

A living attenuated Vero cell adapted rinderpest virus " Kabeta-O" strain **(Osman et al., 1990)** was used for screening of the sera of experimental aminals using SNT.

1.2. Peste des petits Ruminants virus (PPRV) :

A Vero- cell adapted PPRV (EGYPT -87) at its 7th passage was supplied in

a lyophilized form by Ames Iowa Laboratories, USA. This viral passage was considered to be the virulent strain and the seed material.

3. Cell cultures :

African Green Monkey Kidney cell line culture stablished by Yasumura and Kawatika (1983) (passaged and preserved at the Department of Rinderpest Research,Vet. Suerum and Vaccine Research Institute) were used for the vaccine preparation and SNT.

4. Animals :

Thirty Balady sheep and 30 Balady goats (8-12 months old) were included in the present work. These animals were examined clinically and serologically where they were found to be healthy free from internal and external parasites and fully susceptible to RP and PPR . The animals were kept under hygenic measures and clinical observations for 15 days before exposure to experimantal work.

Each animal species was divided into 5 groups as follows:

1- The first group consisted of 9 animals for the detection of viral pathogenicity of the different viral passage (the 7 th, 15th and 20th) where each virus passage was inoculated in each of 3 animals (sheep or goats).

2- The second group included another 9 animals to dectect the protective dose of the vaccine. Different dilutions of the prepared vaccine (10^2, 10^3 and 10^5) were tested in each 3 animals / dilution . These animals were clinically observed for 21 days post inoculation and then challenged with the 7 th virus passage.

3- Group three was consisted of 3 animals of each species receiving 100 protctive dose of the prepared vaccine to ensure the vaccine safety.

4- Group four included 6 animals of each species and used for the seroconverison test after animal vaccination with the protective dose.

5- Group five, was the test control consisting of 3 animals kept in contact with the vaccinated animal.

5. Virus Titraton :

The infectivity titre of each virus passage was estimated using the microtitre technique according to Rossiter and Jessett (1982).

6. Serum Neutralizaton Test (SNT) :

Both qualitative (screening) and quantitative SNT were carried out on the serum samples obtained form all animals before starting of the experimental work

then on definite intervals post vaccination. The test was carried out according to Rossiter and Jessett (1982) and the antibody titre was calculated as the reciprocal of the end point of serum dilution which the CPE of 100-200 $TCID_{50}$ of the virus (Singh et al., 1967).

7. PPR Vaccine Preparation :

When it was confirmed that the virus of PPR lost its pathogenicity completely for sheep and goat (at the 20th passage) a batch of the vaccine was prepared by harvesting the infected fluid of infected Vero cells in roller bottles showing fully CPE . This fluid was centrifuged at 2000 rpm for 10 minutes and the precipitate was discarded . The obtanied fulid was then mixed with a suitable stabilizer (20% sucrose and 10% lactalbumin hydrolysate) at the proportion of 1 stabilizer :3 infected fluid . This mixture was dispensed in 2 ml quantities in each of neutral vials; of 5 ml capacity and lyophilized using the freeze drier. the virus titre was estimated before and after lyophilization .

8. Quality control of the prepared vaccine:

The control of the prepared PPR vaccine included tests for sterility (Anon, 1994) to ensure the freedom of the vaccine from bacterial, fungal and mycoplasma contaminants.

Potency test was carried by inoculaton of different titres (concentrations) of the virus vaccine (10^2, 10^3 and 10^5) in fully susceptible sheep and goats inoculated subcutaneously . Rectal temperature and clinical observations of these animals were recorded for 21 days post vaccination and 15 days post challenge with 10^5 $TCID_{50}$ of the virulent virus for each animal.

safety test was carried out by injecton of 100 protective dose (10^3 $TCID_{50}$) of the vaccine in each of 3 sheep and 3 goats.

9. Seroconversion of PPR antibodies in Vaccinated animals :

Six sheep and six goats were vaccinated with the protective dose (S/C) and the level of PPR neutralizing antibodies was followed up in their sera up to one year post vaccnation.

RESULTS

Table (1) : Pathogenicity of PPR viral passages in sheep and goats

Virus passage number	Clinical manifestations in	
	Sheep	Goats
7th	- Rise in rectal temperature (41°C) - Nasal discharages	- Rise in rectal temperature (41.5°C) - Nasal and acular discharges. - Diarrhoea.
15th	- Slight rise in rectal temperature (40-40.5°C). - Less nasal discharge.	- Rectal temperature (40.5°C) - Nasal discharge.
20th	No clinical manifestations	

Table (2): Results of sterility tests applied on the prepared PPR vaccine

The used media	The tested organisms		
	Bacteria	Fungi	Mycoplasma
1- Thioglycolate	-ve	--	--
2- Soya bean casein digest	-ve	--	--
3- Sabouraud's	--	-ve	--
4- Solid and fluid mycoplasma media	--	--	-ve

Table (3) : Infectivity titre of PPR vaccine.

Time	Infectivity titre expressed as log_{10} $TCID_{50}$/ml
Before lyophilization	7.0
After lyophilization	7.0

Table (4) : Potency of PPR vaccine.

The used dose for each animal	Sheep		Goats	
	Mean antibody titre	Protection % after challenge	Mean antibody titre	Protection % after challenge
10^2 $TCID_{50}$	4	60%	8	60%
10^3 $TCID_{50}$	64	100%	128	100%
10^5 $TCID_{50}$	64	100%	128	100%

Table (5): Safety of PPR vaccine in sheep and goats vaccinated with 10^5 $TCID_{50}$.

Items	observations
- Clinical manifestations	Not recorded
- Virus recovery from nasal, ocular and faecal swabs	-ve
Transmission to contact animals	Not recorded

Table (6): Seroconversion of PPR neutralizing antibodies in the sera of vaccinated sheep and goats.

Periods post vaccination	Mean PPR neutralizing antibody titres*	
	Sheep	Goats
0	0	0
1	2	8
2	16	32
3	32	128
4	64	256
8	64	256
12	64	256
16	64	256
20	64	256
24	64	256
36	64	256
48	64	256
52	64	256

* Week post vaccination
** Antibody titre: The reciprocal of serum dilution neutralied 100-200 $TCID_{50}$

DISCUSSION

Pests des Petits Ruminants (PPR) is one of the most devastating viral diseases of small ruminants affecting sheep and goat industry causing great economic losses. So, it is of great importance to control the disease saving the animal wealth specially in a developing country as Egypt where there is an increasing demand for animal protein.

The use of a non specific vaccine as rinderpest vaccine for protection of sheep and goats may result in some problems as mentioned by **Plowright and Taylor (1967); Dutrnell and Eid (1973) and Mariner et al., (1993)**. Such problems include low titres of antibodies, failure to vaccination with heterologous or even homologous vaccine.So, the present study aimed to produce a specific safe and potent vaccine against PPR.

Among the pathogenicity of PPR viral passages, it was found that the 7th and 15th passage were still virulent to some extent where inoculated animals showed clinical manifestation (Table 1) presented by rise of rectal temprature, nasal and ocular discharges (in sheep)in addition to diarrhoea (in goats). These signs were more sever in goats than in sheep the thing which could be attributed to the more susceptibility of goat to the disease. These findings come in agreement with those of **Appiah (1982) and Mouaz et al., (1995).**

Also, Adu et at., (1990) noticed progressive loss of the pathogenicity of PPR virus by its passage of PPR virus was completely attenuated and non pathogenic for goat and sheep.

Followed up the protocol of control tests for sterility specified in the Office international des Epizooties (OIE). Code for Rinderpest Vaccine Production (Anon, 1994), the prepared PPR vaccine was found to be free from aerobic and anaerobic bacteria, fungi and mycoplasma (Table 2). Among the infectivity titre of PPR vaccine (Table 3), it was found that the virus titre pre - and post lyophilization was 10^7 $TCID_{50}$ afforded a sufficient amount of the virus to safe guard a surplus for transport and storing conditions as recommended internationally for rinderpest vaccine, the closely related virus (**Anon, 1994**).

Table (4) showed that the vaccinal dose of choice is 10^3 $TCID_{50}$ where it resulted in 100 % protection as 10^5 $TCID_{50}$ while 10^2 $TCID_{50}$ was less protective (60 %). These results approach or nearly agree with those of **Diallow et al.,(1989)** who found that the immune dose of a Vero cell adapted PPR vaccine is $10^{2.5}$ $TCID_{50}$.This slight difference, which could be considered non significant; could be attributed to the used viral strain and the breed of vaccinated animals.

Concerning potency and safety of the prepared PPR vaccine (Table 4 and 5), it was found that the vaccinated goat and sheep showed successfully appreciable results even with small doses resulted in serum neutralizing antibody titres 4 in sheep and 8 in goats while the protective dose and higher dose resulted in a good level of antibodies and a protection % of 100. These titres; even the low often are considered protective in agreement with Plowright and Taylor (1967) and Anon (1994). In addition, all animals did not show any post vaccinal reactions and contact animal remained healthy and there is no virus shedding of vaccinated animals (Table 5).

Followed up the level of formed antibodies in vaccinated animal with the protective dose, it was found that such anitbodies began to appear from the 2 nd week post vaccination reaching their peak in animal sera by the 3 rd- 4 th week post vaccination and remained unchanged up to 52 week (about one year). These findings were found to be confirmed by Taha (1995) and Hussein (1998).

It could be concluded that the prepared live attenuated cell culture PPR vaccine is a specific safe and potent vaccine to protect sheep and goats against PPR disease .

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