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COMPARATIVE STUDY BETWEEN LOCAL COMBINED INACTIVATED RESPIRATORY VIRUS VACCINE (PNEUMO-3) WITH IMPORTED CATTLE MASTER 4 VACCINE

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Abstract

Comparative studies have been carried out to compare the effectiveness of the local combined inactivated respiratory virus vaccine incorporating IBR, PI-3 and BVD viruses (pneumo-3) and the imported modified live with killed BVD virus (cattle Master 4) vaccines. Five to six months old calves were randomly assigned to 3 groups with 6 animals/ group. Calves in group I were inoculated on days 0 and 14 with 5ml of local combined inactivated respiratory virus vaccine (pneumo-3), and the second group was inoculated on days 0 and 14 with 2ml of modified live virus vaccine with killed BVD vaccine (cattle master 4). The remaining 6 calves were maintained as unvaccinated controls. Immune responses were measured by means of ELISA, serum neutralization assay against the same fractions of the vaccine and haemagglutination inhibition test against PI-3 virus. Both vaccines, either prepared locally or imported gave, full protection against challenge with each disease causing strain with that fraction. All vaccines induced production of antibodies. However, the ratio of neutralizing antibody titre change in IBR, PI-3 and BVD viruses-specific IgG antibody level was lower for calves that received inactivated virus vaccines than for calves that received the modified live virus vaccine. Results suggested that the commercially inactivated vaccine stimulated production of protective antibodies without replication of virus in the tissues of the host leading to decrease the production of neutralizing antibodies in immunized calves.

INTRODUCTION

Both modified live combined inactivated vaccines against IBR, BVD, PI-3 viruses are used extensively as an aid in prevention of bovine respiratory diseases. Modified live virus (MLV) can replicate in host tissues and produce additional viral antigen to stimulate the immune response of the host over a period of several days (Fulton *et al.*, 1995). The MLV may be shed by the host during this time and infect susceptible cattle and may potentiate concurrent infections (Roeder and Harkness, 1986). There is the possibility of MLV becoming latent with later recrudescence when the animal is under stress, thus, posing a danger of shedding and abortion associated with the use of MLV vaccine (McFeely *et al.*, 1968) or congenital abnormalities in the growing foetus (Mansfield *et al.*, 1986).

Inactivated virus vaccines have an advantage in that the vaccine virus does not replicate in the host tissues and live vaccine virus is not shed. However, more inactivated virus antigen must be administered per dose and often in more than one dose to stimulate an adequate immune response before the vaccine is metabolized and eliminated (Schipper *et al.*, 1983). The optimum time for vaccination with either live or inactivated vaccines is very critical since maternal antibodies in young animals interfere with the development of active immunity (Brar *et al.*, 1978). There has been interest in replacing modified live virus vaccines with inactivated virus largely because of safety issues.

This study was conducted to compare persistence of humoral antibodies and the anamnestic response in calves vaccinated with pneumo-3 and Cattle Master-4 vaccines.

MATERIALS AND METHODS

1. Animals

Eighteen mixed breed calves (Freizian X local) of approximately 6-9 months of age were used in this study. These calves were kept in Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.

2. Vaccines

a. Imported modified live IBR, PI-3 and BRSV and killed BVD virus vaccine (Cattle Master 4)

It is used for vaccination of healthy cattle including pregnant cows against bovine virus diarrhoea (BVD), parainfluenza-3 (PI-3), infectious bovine rhinotracheitis (IBR) and bovine respiratory syncytial virus (BRSV). Cattle Master 4 is a freeze dried preparation of chemically altered strains of IBR, PI-3 viruses and modified live BRSV plus a liquid adjuvanted preparation of inactivated cytopathic and non cytopathic BVD virus strains. The liquid component is used to rehydrate the freeze dried components, and to be shaked well and 2ml were administered intramuscularly (2 doses 2-4 weeks apart in accordance with beef quality assurance guide lines and annual re-vaccination with a single dose as recommended).

b. Local combined inactivated vaccine (Pneumo-3)

Contained PI-3 (strain 45) 8 $\log_{10}$ $TCID_{50}$/ml, IBR (Abou Hammad strain) 8 $\log_{10}$

$TCID_{50}$ and BVD-MD (Iman strain) 7 $\log_{10}$ $TCID_{50}$/ml. The antigens were inactivated by binary ethyleneimine and adsorbed by 30% alhydrogel in 50ml bottle. The vaccine was administered in 2 doses 2-4 weeks apart with 5ml intramuscularly. Calves must be vaccinated before age of 6 months, and a single dose every 6 months is recommended. The validity of vaccine was 6 months post-manufacturing. The vaccine was prepared in the Department of Rinderpest Like Diseases and Blue Tongue, in Vet. Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt.

3. Tissue culture

Madin Darby Bovine Kidney (MDBK) cell line culture tested to be free from the non cytopathic (NCP) BVD-MD virus was used in this study or laboratory testing on the vaccines.

4. Experimental Design

The vaccines were evaluated according to the following :

a. Purity

In accordance with the US Code of Federal Regulations (1987) testing 9.CFR 113-26, 113-27, 113-30 and 113-25.

b. Safety

According to 9 CFR (1987) testing 113-41 in calves and 9 CFR 113.38 in Guinea pigs were used in this study.

c. Potency evaluation

To evaluate the potency efficacy of the locally prepared (Pneumo-3) vaccine and imported modified live with inactivated BVD vaccine (Cattle Master 4), controlled experiments were conducted on 18 cattle calves that were divided into 3 groups :

Group 1 : Six animals were vaccinated with pneumo-3 by 2 injections 2-4 weeks apart with 5ml intramuscularly according to Wassel *et al.* (1997).

Group 2 : Six animals were vaccinated with 2ml intramuscularly 2 weeks apart of imported modified live with killed BVD virus (Cattle Master 4) vaccine.

Group 3 : The rest 6 calves were left as normal control.

d. Testing the effectiveness of the 2 vaccines

The effectiveness of the vaccines was tested 4 weeks after the second vaccination by challenging 3 calves of each group with pathogenic strains of BVD, IBR and PI-3 viruses. Calves were infected with 5ml intranasally and 5ml intravenously. Three calves of group 3 were experimentally infected; the 1st with 10ml of NADL strain of BVD-MD $10^{6.5}$/ml, the 2nd with 10ml of Abou Hammad strain of IBR virus 10^7 $TCID_{50}$/ml and the 3rd with 10ml of PI-3 strain 45 10^8 $TCID_{50}$/ml, 5ml instilled intranasally and 5ml injected intravenously according to Wassel *et al.* (1997). The effectiveness of the vaccines was evaluated on the basis of clinical observation, total leucocytic counts, virus isolation and the immune response towards the two vaccines during the following 6 months post vaccination.

5. Sampling

a. Nasal and rectal swabs for trials of virus reisolation : swabs were collected from different experimental groups daily through the first 3 weeks post vaccination (PV) and post challenge (PCh).

b. Blood samples : Non coagulated blood samples for virus reisolation from buffy coat and total leukocytic and differential counts were collected. Coagulated blood samples for preparation of serum for studying seroconversion were conducted.

6. Methods of examination

A. Virological studies

1. Trials of virus reisolation

Trials of virus reisolation were conducted on nasal and rectal swabs, buffy coat. The method used was described by Singh and Baz (1966).

2. Identification of isolated agents

Serum neutralization test was utilized as described by Singh and Baz (1966) for the identification of the reisolated viral agents using monospecific antiserum against each virus.

B. Serological studies

1. Serum Neutralization Test

The test was performed for the measurement of specific BVD, IBR and PI-3 serum neutralizing antibodies in vacccalves according to Fulton *et al.* (1995).

2. Enzyme Linked Immuno Sorbent Assay (ELISA) :

It was used for antibodies to IBR, BVD and PI-3 viruses according to Voller *et al.* (1976).

3. Micro-haemagglutination inhibition test :

It was carried out according to the technique of Cho *et al.* (1985) against PI-3 virus.

RESULTS AND DISCUSSION

Calf pneumoenteritis caused by BVD, IBR and PI-3 viruses affecting animal industry cause high death rate. Synergism between BVD-MD virus and other agents as IBR and PI-3 (Werner Heuschle, 1984) and its predilection to replicate in and damage lymphoreticular tissues is well known (Muscoplat *et al.*, 1973). This result is a significant suppression of the animals non-specific and specific defence mechanism against other organisms to which it may be concurrently exposed. Moreover, the virus could cross the placenta causing foetal infection leading to foetal death, resorption, abortion, cerebellar hypoplasia, necrotic dermatitis, alopecia and pulmonary aplasia or it may result of birth of apparently healthy normal calf.

BVDV modified live vaccine also may be immunosuppressive in calves (Neaton, 1986). Vaccination with attenuated vaccine strains may potentiate concurrent infections (Roeder and Harkness, 1986) BVDV MLV depressed and impaired the number of circulating bovine neutrophils in a manner similar to the virulent virus (Kaeberle and Roth, 1980). For all mentioned before, BVD virus must be used as killed vaccine as in cattle master 4 or pneumo-3.

Vaccination of animals against these diseases is one of the most important control measurement of these diseases. The combined inactivated respiratory virus vaccine (Pneumo-3) is effective and safe to the pregnant animals although it gives short period of immunity and need of 2 doses to protect calves for 6 months. The

modified live vaccine (Cattle Master 4) has long period of immunity, but has some disadvantages such as its theoretical risk on pregnant animal, or it could be transferred to other species which it might be pathogenic for them, or it could be returned to its natural virulence and antigen blockage or interference in multiple antigen products, or shedding virus to non-vaccinated animal. So, it is important to compare and evaluate the effect of both types of vaccines in calves.

At first, all the used calves were kept under observation post vaccination with both vaccines. The results revealed that animals vaccinated with (Cattle Master 4) vaccine (Group 1) had an elevation of body temperature (40°C) from 2nd day to 4th day post-vaccination (as post-vaccinal reactions), but in group 2 which were vaccinated with (Pneumo-3) the body temperature of all animals were with in normal. These results are in agreement with that obtained by Chennekatu *et al.* (1967) who concluded that within 10 to 20 days after vaccination of the animals a disease like syndromes occurred in all numbers of calves and other complication associated with vaccination with modified live virus vaccines.

The vaccinated challenged calves with (Cattle Master 4) showed a slight increase in body temperature from 3rd day to 5th day post-challenge (PCh), slight serous nasal and ocular discharges were observed in 2 calves for few days post challenge but clinical signs were not observed in vaccinated challenged calves by local inactivated vaccine (Pneumo-3) agree with Wassel *et al.* (1997). The non-vaccinated control infected calves showed signs of illness in the form of pneumo-enteric manifestation in general. The clinical respiratory manifestations had developed after 5 days post infection and remained up to 4 days (Kretzschmar, 1980).

The effect of post-vaccination with both types of vaccines on leucocytic count was studied. The results indicated that the leucocytic count of animals in group 1 revealed a slight leucopenia on the 3rd day post-vaccination then followed by leucocytosis from (1-3 week) post-vaccination with modified live (Cattle Master 4). The group which was vaccinated with inactivated (Pneumo-3) (group 2) had significant increase of leucocytic count for 2nd week post-vaccination than that obtained by control group.

The results were parallel to that of James and Merlin (1983) who found that modified live vaccines caused leucopenia. This is mainly attributed to the sequestration of neutrophils at the site of tissue damage and thus the marginal and post mitotic neutrophils pool have been exhausted after 48 hours. The proliferating pool is capable of

supplying enough cells to resupplying peripheral blood causing the leucocytosis. Ebeid *et al.* (1993) reported an increase in leucocytic count post vaccination with inactivated vaccine and the increase in total leucocytic count post vaccination might clear the pictures of antibody formation.

After studying the total leucocytic count post vaccination with both vaccines, the differential leucocytic count profile was interpreted to know the effect of vaccination on different types of leucocytes. The obtained results revealed that vaccinated animals groups had increased for lymphocytic count and decreased of neutrophil count post vaccination than that obtained by control calves.

The increase of lymphocytes in relation to decrease of neutrophils suggested superiority of modified live vaccine as the lymphocytes which are antibodies producer were increased more early post vaccination with this vaccine than did with inactivated vaccine (Kaeberle and Roth, 1980). Botros *et al.* (1995) reported that animals receiving the live attenuated Smithburn vaccine did not show any changes throughout 28 days observation. Ebeid *et al.* (1993) explained that there is no significant difference through 21 days post vaccination with inactivated RVF vaccine.

As shown in Table 1, there were no reisolation of BVD, IBR and PI-3 viruses from the vaccinated calves either vaccinated with (Cattle Master 4) or vaccinated with (Pneumo-3). In the non vaccinated infected calves, the viruses were recovered throughout the observation time (3 weeks). In vaccinated challenged calves, viruses were recovered from 3-7 days post-challenge (PCh) of low percent in (Pneumo-3) and from 1-10 days with high percent in (Cattle Master 4). Table 1 shows that virus recovery started on day 1 to 14 days after infection reaching a peak on day 5-7 with viraemia on days 5 to 14 (Neaton *et al.* 1986; Bolin *et al.*, 1991).

No IBR, BVD and PI-3 viruses could be detected from contact control calves throughout the experimental period, this indicated that there was no shedding viruses as reported by Wassel *et al.* (1997).

The humoral immunity of calves was studied by SNT and ELISA tests. Tables 2 and 3 showed that animals of group 1 which were vaccinated with inactivated vaccine antibodies appeared at 3rd week and reached its protective level between 4-6 weeks, and disappeared at the end of 6th month post-vaccination. In group 2 which was vaccinated with modified live (Cattle Master 4), the anti-IBR and PI-3 antibodies were start-

ed to appear at the 1st week post-vaccination and reached its peak level at 6th-12th week post vaccination and gradually decreased till the end of experiment and the anti-BVD antibodies in this group resemble the curve of antibodies in the inactivated vaccine. The BVD antibodies were developed in calves vaccinated with commercial vaccines (killed and live) later than those induced by IBR and PI-3 but the induced BVDV antibodies were maintained through day 140 by both vaccines (Fulton *et al.*, 1995). Also, PI-3 virus haemagglutinating antibodies run parallel to the results of SNT and ELISA tests (Table 4), and the protective level of antibodies was found till 6 months post-vaccination as reported by Mohanty and Lilli (1964).

These findings were in agreement with the result of Wassel *et al.* (1997) who found that the protective antibody level of calves vaccinated with local combined inactivated respiratory virus vaccine was detected till 6 months post-vaccination against 3 viruses. Sutton (1980) explained that calves vaccinated with live attenuated vaccine had protective level till the end of experiment (one year post-vaccination), and should be used under restricted age prior to breeding, so that there would be no interference between colostrally transferred maternal antibodies and shed no viruses to unvaccinated calves. Schipper *et al.* (1983) and Mansfield *et al.* (1986) applied comparison of attenuated and inactivated bovine virus diarrhoea vaccines and found that the inactivated vaccine was regarded as safer and just as effective as the live vaccine.

Low antibody response in the group vaccinated with inactivated vaccines than that induced by modified live vaccine, but, the inactivated vaccines stimulated production of protective antibodies without replication of virus in the tissues of the host.

No adverse reaction after vaccination was observed and loss from disease was markedly less than that of the control group.

The fact that the control calves remained seronegative for the three viruses allover the experiment indicated that no live virus infection was active on the premises and that the antibody production in vaccinated calves was stimulated by the vaccination.

Results of the study reported in this paper show that use of killed vaccine is safe and effective method to reduce losses and control respiratory virus infections (IBR, BVD and PI-3) in Egypt.

Table 1. Recovery rate of reisolated viruses post-vaccination and post-challenge by virulent BVD, IBR and PI-3 viruses.

Site of isolation	Time/ day	Reisolated viruses post challenge						Recovery rate /type vaccine		Reisolated viruses postinfection			Virus recoveryrate / day		
		IBR		PI-3		BVO									
		Inactivated	Modified	Inactivated	Modified	Local	Imported	Local	Imported	IBR	PI-3	BVD	IBR	PI-3	BVD
Buffy coat	1	0/3	3-Feb	0/3	3-Mar	0/3	3-Jan			0/3	0/3	0/3			
	3	33	3-Mar	3-Mar	3-Mar	3-Feb	3-Feb			3-Mar	3-Feb	3-Mar			
	7	0/3	3-Feb	3-Feb	3-Feb	3-Feb	3-Feb	Nov-54	20/54	3-Mar	3-Mar	3-Mar	18-Dec	18-Sep	18-Jul
	10	0/3	0/3	0/3	0/3	0/3	0/3	20.37%	37.03%	3-Mar	3-Mar	3-Jan	66.66%	50%	38.88%
	14	0/3	0/3	0/3	0/3	0/3	0/3			0/3	3-Jan	0/3			
	21	0/3	0/3	0/3	0/3	0/3	0/3			0/3	0/3	0/3			
Nasal swabs	1	0/3	3-Jan	0/3	3-Feb	0/3	0/3			3-Jan	3-Feb	0/3			
	3	33	3-Mar	3-Mar	3-Mar	3-Mar	3-Mar			3-Mar	3-Mar	3-Feb			
	7	2	3-Mar	3-Feb	3-Mar	3-Feb	3-Mar	13/54	28/54	3-Mar	3-Mar	3-Mar	18-Dec	14/18	18-Nov
	10	0/3	3-Feb	0/3	3-Mar	0/3	3-Feb	24.07%	51.85%	3-Mar	3-Mar	3-Mar	66.66%	77.77%	61.11%
	14	0/3	0/3	0/3	0/3	0/3	0/3			3-Feb	3-Mar	3-Mar			
	21	0/3	0/3	0/3	0/3	0/3	0/3			0/3	0/3	0/3			
Rectal swabs	1	0/3	0/3	0/3	0/3	0/3	0/3			0/3	0/3	0/3			
	3	0/3	3-Jan	0/3	3-Feb	0/3	3-Feb			0/3	3-Mar	0/3			
	7	0/3	3-Feb	3-Feb	3-Feb	0/3	3-Jan	Feb-54	14/54	3-Feb	3-Feb	3-Mar	18-Feb	18-Jul	18-Sep
	10	0/3	3-Jan	0/3	3-Feb	0/3	3-Jan	3.70%	25.92%	0/3	3-Feb	3-Mar	11.11%	38.88%	50%
	14	0/3	0/3	0/3	0/3	0/3	0/3			0/3	0/3	3-Mar			
	21	0/3	0/3	0/3	0/3	0/3	0/3			0/3	0/3	0/3			
Virus recovery per type of virus		5.54	20/54	Dec-54	25/54	Sep-54	17/54	26/162	62/162	26/54	20/54	27/54			
Percentage		9.25%	37.03%	22.22%	46.29%	16.66%	31.48%	16.04%	38.27%	48.14%	37.03%	50%			

* No virus isolation could be detected from vaccinated groups and control non infected groups post infection by the virulent viruses.

Table 2. Mean serum neutralizing antibody of calves vaccinated with pneumo-3 and cattle master 4 vaccines as well as control calves expressed as log_{10}.

Time post vaccination/ weeks	Serum neutralizing antibody titre expressed as log_{10}								
	Local inactivated vaccine (Pneumo-3) group (1)			Imported cattle master 4 (Group 2)			Control animals (Group 3)		
	IBR	PI-3	BVD	IBR	PI-3	BVD	IBR	PI-3	BVD
Zero	0.30	0.20	0.00	0.30	0.00	0.60	0.25	0.30	0.20
1st	0.30	0.20	0.00	0.90	1.00	0.60	0.20	0.30	0.40
2nd	0.60	0.55	0.00	1.50	1.80	0.30	0.30	0.30	0.30
3rd	1.55	1.40	1.15	2.00	2.10	1.00	0.03	0.30	0.00
4th	1.80	1.90	1.70	2.50	2.65	1.65	0.03	0.02	0.00
6th	2.00	2.10	1.90	2.80	2.90	1.80	0.03	0.04	0.00
8th	1.90	2.00	1.80	3.00	3.20	1.70	0.20	020	0.00
10th	1.80	2.00	1.80	3.10	3.25	1.70	0.00	0.00	0.00
12th	1.70	1.95	1.75	2.95	3.00	1.55	0.00	0.00	0.00
14th	1.50	1.80	1.60	2.80	2.85	1.30	0.00	0.00	0.00
16th	1.35	1.50	1.40	2.40	2.55	1.20	0.00	0.00	0.00
20th	1.20	1.30	1.25	2.00	2.25	1.00	0.00	0.00	0.00
24th	1.00	1.00	1.00	1.90	2.10	0.95	0.00	0.00	0.00
28th	0.85	0.95	0.90	1.75	1.85	0.80	0.00	0.00	0.00

Table 3. Mean ELISA unit of calves vaccinated with modified live (cattle master 4) and combined inactivated respiratory virus vaccine (pneumo-3) as well as control calves.

Source of vaccine	Constituent		Nature of vaccine	Weeks Post Vaccination									
				0	2	4	6	8	12	16	20	24	28
Imported vaccine	BVD	Vaccinated calves	Killed virus vaccine	0.90	0.60	2.00	2.40	2.70	2.30	2.20	1.85	1.60	1.00
Local vaccine			Killed virus vaccine	0.60	0.95	2.30	2.65	2.90	2.50	2.35	2.00	1.80	1.20
Imported vaccine	IBR		Modified live vaccine	0.80	2.10	3.20	3.65	3.55	3.40	3.00	2.60	2.30	1.80
Local vaccine			Killed virus vaccine	0.65	1.00	2.80	2.75	2.50	2.30	2.10	1.80	1.50	1.30
Imported vaccine	PI-3		Modified live vaccine	0.55	1.60	3.40	3.70	3.80	3.50	3.25	2.80	2.50	1.90
Local vaccine			Killed virus vaccine	0.85	0.90	2.70	2.80	2.75	2.40	2.55	1.90	1.65	1.45
Control non infected		Contact control	PI-3	0.30	0.25	0.50	0.54	0.42	0.35	0.40	0.22	0.30	0.25
			IBR	0.40	0.30	0.00	0.65	0.55	0.60	0.40	0.50	0.30	0.00
			BVD	0.20	0.40	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 4. Results of haemagglutination inhibition test against PI-3 virus in calves vaccinated with local combined inactivated vaccine (pneumo-3) and modified live and killed vaccine (Imported vaccine).

Time post vaccination	Log_{10} haemagglutinating inhibiting titres post vaccination		
	Local vaccine (Pneumo-3)	Imported vaccine (Cattle Master-4)	Contact control group
Zero day (1st dose)	0.3	0.45	0.35
1 WPV	0.35	1.5	0.45
2 WPV (2nd dose)	1.25	1.95	0.25
3 WPV	1.85	2.35	0.35
4 WPV	2.15	2.5	0.45
6 WPV (Challenge)	2.25	3.2	0.45
8 WPV	2.45	3.8	0.2
10 WPV	2.8	3.4	0.25
12 WPV	2.5	3.25	0.3
14 WPV	2.2	2.9	0
16 WPV	2.15	2.5	0
20 WPV	1.8	2	0
24 WPV	1.5	1.85	0
28 WPV	1	1.5	0

WPV : Weeks Post-Vaccination.

Reciprocal of the highest serum dilution completely inhibiting agglutination of 0.5% Guinea pig erythrocytes by 4 haemagglutinating units of PI-3 virus.

The minimum accepted protective HI titre for PI-3 virus is 1.20 log10 or 1:80 (Mohanty and Lillie, 1964).

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دراسة مناعية بين اللقاح الجموعي التنفسي الفيروسي الميت المحلي (نيمو -٣) واللقاح المستورد الحي المستضعف (مستر-٤)

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تمت مقارنة لدراسة كفاءة اللقاح التنفسي الجماعي الميت المحلي (نيمو-٣) والذي يحتوى على كل من فيروس الميكوزا والتهاب القصبة الهوائية والبارا أنفلونزا - ٣ والسينسيتي التنفسي وعترة مثبطة لفيروس الميكوزا.

وقد أختبر تأثير كل من اللقاحين على الحيوانات باستخدام ٣ مجموعات من العجول. تم حقن المجموعة الأولى بجرعة مزدوجة ٥سم من اللقاح الجموعي الميت (نيمو -٣) بينهم ١٤ يوم وكذلك تم حقن المجموعة الثانية بجرعة مزدوجة ٢ سم من اللقاح الحي المستضعف والمجموعة الثالثة تركت كضابط للتجربة وأوضحت الاختبارات المختلفة وجود تباين في القوى التكوينية للأجسام المناعية المعادلة المضادة لكل من اختبار السيرم المتعادل واختبار الإليزا بالنسبة للفيروسات المتشابهة لكل من اللقاحين وأجسام التلازن الدموي المناعية المضادة لفيروس البارا أنفلونزا-٣.

أظهر عد كرات الدم البيضاء انخفاضاً حاداً عند استعمال اللقاح الحي المستضعف عن اللقاح الجموعي الميت (نيمو-٣)

وعند إجراء اختبار التحدي لكل من اللقاحين بالعترات الضارية بعد شهر من الجرعة الثانية لم تظهر اعراض مرضية على الحيوانات المحصنة بينما أظهرت اعراضاً على الحيوانات الأخرى التي لم تحصن كذلك تم عزل الفيروسات والتأكد من وجودها باستخدام Specific antisera أجسام مناعية مضادة لكل فيروس.

كما أوضحت النتائج انخفاض القوى المناعية التكوينية للقاح الميت عن اللقاح الحي المستضعف وذلك لأن اللقاح الميت لا يعتمد على تكاثر الفيروس داخل جسم الحيوان مما يؤدي إلى انخفاض الأجسام المناعية المتكونة، على الرغم من ذلك ينصح باستخدام اللقاح الجموعي الميت لمقاومة الأمراض التنفسية المعوية الفيروسية في العجول حيث أنه آمن ولا يساعد على انتشار الأمراض.