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TRIAL FOR PREPARATION AND EVALUATION OF A COMBINED VACCINE FOR IBRV, BVDV, PI-3V, P. MULTOCIDA AND P. HAEMOLYTICA IN EGYPT.

(With 3 Tables & 3 Figures)

By

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محاولة لتحضير وتقييم لقاح مركب من فيروسات التهاب القصبه البقرى المعدى والميكوزا والبارأنفلونزا -٣ والباستيريلا ملتوسيدا والهيموليتكا فى مصر

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تم تحضير لقاح مركب من فيروسات التهاب القصبه البقرى المعدى، والميكوزا والبارأنفلونزا -٣ والباستيريلا الملتوسيدا والهيموليتكا والمثبط بالفورمالين ٢ر٪ وأستخدام الألومنيوم هيدوكسيد جيل كمادة مدمصة وتم تقييم هذا اللقاح بعمل أختبار النقاوه وأختبار السلامه بالحقن ١٠ جرعات فى العضل فى العجول والخنزير ٥ر٠ سم٢ فى البطن الغينى واختبار كفاءة اللقاح فى العجول بالحقن ٥سم٢ فى العضل مرتان بينهما اسبوعان وتم باجراء أختبار التعادل فى السيرم وأختبار الاليزا وأختبار التحدى على مصل العجول المحصنه فى الفئران بالنسبه للباستيريلا الملتوسيدا والهيموليتكا وأعطى اللقاح نتائج جيده من حيث أختبار السلامه والنقاوه وكان مستوى الأجسام المناعيه قد وصل إلى أعلى معدل فى الفئران من ٨-١٢ أسبوع بعد الحقن وأستمر فى المعدل المطلوب لمدة ٦ شهور وذلك بإجراء أختبار التعادل فى السيرم والاليزا.

SUMMARY

An inactivated combined vaccine of Infectious Bovine Rhinotracheitis (IBR), Bovine Viral Diarhoea (BVD), Parainfluenza-3 (PI-3) viruses and P. multocida and P.haemolytica using 0.2% formalin and alum gel as adjuvant. Fourteen susceptible calves were vaccinated twice 2 weeks apart using 5 ml I/M. The vaccine was tested for safety in calves and Guinea pigs and for the

sterility. Sera of immunized calves showed high antibody levels using serum neutralization test (SNT) which reached 4 for IBR, 8 for BVD and 8 for PI-3 after 5 week post vaccination. The ELISA was used to measure the level of antibodies which came in harmony with those of SNT. The average ELISA titre reached 4446 in IBR, 5984 in BVD and 4508 in PI-3 viruses, 3808 in P. mutocida and 3890 in P.haemolytica, a monovalent vaccine to each fraction in combined vaccine was used as a control. Passive mouse protection test was also used to evaluate the potency of pasteurella fractions in combined vaccine, where protection ranged from 60-100 for a period of 6 months post vaccination.

Key words: *Vaccine preparation-IBR, BVD, PI3, Pasteurella-Egypt.*

INTRODUCTION

Shipping fever is a widely used term to describe respiratory diseases that occurs in cattle following their assemblage and shipment from one locality to another (Hamdy et al., 1958 and Heddleston et al., 1962).

Patolay and Christensen (1959) indicated that a multiplicity of agent may be involved since numerous microbial agents have been isolated from cattle including viruses and bacteria.

Wood et al. (1973), Reggiardo (1979) and Potgieter et al. (1984) reported that IBR, BVD, PI-3 viruses, P. multocida and P. haemolytica are important pathogens frequently present in diseased respiratory tract tissues and indicating that synergism may occur between them.

In Egypt, bovine respiratory disease has been reported by Singh and Baz (1966), Fayed (1973), Baz (1975), Hafez et al. (1976) and Badr El-Din (1988), where the disease caused high economic losses and deaths in farm animals.

Vaccination against the common bovine respiratory pathogen has been used as a mean of protecting calves from IBR, BVD and PI-3. The disease is most serious when associated with bacterial infection that act synergistically to cause severe pneumonia (Jericho and Langford, 1978 Jericho et al., 1982).

The purpose of the present work is to prepare a combined vaccine for the first time in Egypt that could effectively fight IBR, BVD, PI-3 viruses, P. multocida and P. haemolytica, through studying immune response at different intervals in vaccinated calves.

MATERIAL and METHODS

Viruses:

a. Infectious Bovine Rhinotracheitis (IBR) virus Abou Hammad strain (Hafez et al., 1976).

b. Bovine Viral Diarrhoea (BVD) mucosal disease virus Iman strain (Baz, 1975).

c. Parainfluenza-3 (PI-3) virus strain 45 (Singh and Baz, 1966).

These viruses (IBR, BVD and PI-3) Egyptian vaccinal strains were kindly supplied from Rinderpest Like Diseases Department, Vet. Serum and Vaccine Research Institute, Abbasia, Cairo.

Bacterium:

P.haemolytica local strain type -A1 (Aboul Soaud et al., 1992).

p.multocida local vaccinal strain (Geneidy and El-Affandy, 1963).

These local strains were received from Aerobic Bacterial Vaccine Department, Vet. Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt.

Vaccines:

a. IBR alum gel inactivated vaccine prepared according to Liao et al. (1992) and El-Sabbagh (1993).

b. BVD alum gel inactivated vaccine prepared according to Liao et al. (1992) and Ghaly (1993).

c. PI-3 alum gel inactivated vaccine prepared according to Liao et al. (1992) and Samira (1992).

d. *e.p.multocida* and *P. haemolytica* inactivated alum gel separately vaccine prepared according to Abdoul Soaud (1990).

f. Combined inactivated vaccine composed of IBR, BVD, PI-3, P. multocida and P. haemolytica. They were prepared inactivated separately with 0.2% formalin, for 18 hours at 4°C, mixed equally together and then addition of 10% of alum gel, the concern of antigen before inactivation were as follow IBR virus 10^7 $TCID_{50}$ / ml, BVD virus 10^5 $TCID_{50}$ / ml, PI-3 virus $10^{6.5}$ $TCID_{50}$ / ml P. multocida and P. haemolytica that according to Matsuoka et al. (1972), Aboul Soaud (1990) and Liao et al. (1992).

Cell cultures:

Monolayer MDBK cell cultures were grown and maintained as described by Samira (1992).

Animals:

Fourteen susceptible calves, 6 months old, were used.

Vaccine Evaluation:

The monovalent as well as the multivalent prepared vaccine were evaluated according to the following:

a. Purity in accordance with the United State Code of Federal Regulation (1987) testing 9 CFR 113.26 and Samira (1992).

b. Safety: 1. Guinea pig safety test according to the United State Code of Federal Regulation (1987) testing 9 CFR 113.38 and Samira (1992).

2. Calf safety test according to the United State Code of Federal Regulation (1987) testing 9 CFR 113, 41 and Samira (1992).

c. Potency or seroconversion.

I. Aginst IBR, BVD and PI-3 (viruses fraction):

i. Serum neutralization test using tissue culture according to Matsuoka et al. (1972) and Jericho et al. (1982) and the titre was calculated according to Reed and Muench (1938).

ii. Enzyme Linked Immunosorbent Assay (ELISA) for antibodies to IBR, BVD and PI-3 viruses was carried out as described by Suri Bubul et al. (1984, Durham and Sillars (1986) and Peter and Lori (1990).

II. Against P. multocida and P. haemolytica (bacterial fraction):

i. Passive mouse protection test: the technique used was that of Bain (1963) and Alwis and Carter (1980).

ii. Enzyme Linked Immunosorbent Assay (ELISA) for detecting antibodies to P. multocida and P. haemolytica in calves. The technique used was that of Marshall et al. (1981), Solano et al. (1984) and Aboul Soaud (1990). The ELISA titre was calculated according to Synder et al. (1984).

RESULTS

The preliminary studies of alum gel inactivated combined IBR, BVD, PI-3, P. multocida and P. haemolytica and each monovalent one gave good satisfactory results of sterility and safety of 10 X dose in calves (with no rise of body temperature and as it remain within the normal 38.2 - 38.3°C for 10 days and no respiratory syndromes). Also, the result of safety test in Guinea pig was good, it remained healthy for a period of 10 days post vaccination.

The result of serum neutralization test (SNT) in calves were shown in table (1) and Fig. (1) for combined vaccines, the immune response of calves it reached 4 for IBR, 8 for BVD and 8 for PI-3 on the 5th week post vaccination and it remained till 24 weeks post vaccination and it reached its maximum from the 2 weeks till 12 weeks (32 for IBR, 64 for BVD, 64 for PI-3) post vaccination.

The level of antibodies as measured by ELISA, the titre ranged as shown in Table (2) and Fig. (2 and 3) for combined vaccines 4446 for IBR, 5984 for BVD and 4508 for PI-3, 3808 for P.multocida and 3890 for P.haemolytica.

The average ELISA titre in combined vaccine reached its maximum from 8-12 weeks post vaccination as shown in Table (2) and Fig. (2 and 3). The result of passive mouse protection test in serum of vaccinated calves as indicated in Table (3) for pasteurella in combined vaccine gave good protective results from 2 weeks post vaccination till 24 weeks post vaccination. Non of serum samples before vaccination showed the presence of antibodies against pasteurella fractions.

The obtained results of monovalent vaccines came within the levels of those of combined ones as shown in Tables (1, 2 and 3) and Fig. (1, 2 and 3).

DISCUSSION

The results indicated that inactivated IBR, BVD, PI-3 viruses are effecacious with inactivated P. multocida and P. haemolytica, also, the consistency of these results indicates that the combined vaccine can be made uniformly from lots of inactivated IBR, BVD, PI-3, P.multocida and P.haemolytica vaccines.

The result of SN of tested vaccines gave a protective neutralizing titres after 5 week post vaccination (which 4 for IBR, 8 for BVD and 8 for PI-3) which considered of good immunity and agreed with neutralizing titre obtained by Phillips (1968), Straub and Mawhinney (1988), Merwin (1989), Samira (1992) and Ghaly (1993) (since the achieved neutralizing titre 4 for IBR, 8 for BVD and 8 for PI-3).

Also, from measuring the serologic response (2 IBR, 4 BVD, 4 PI-3 after 2 weeks post vaccination) after each vaccination indicated 2 doses of an inactivated vaccine given I/M from 14 - 21 days apart were required before a good consistent SN response was detected. These results agreed with those of Matsuoka et al. (1972) and El-Sabbagh (1993) where they used 2 doses of vaccination after 2 weeks after the first dose.

Because the result of serum neutalization was satisfactory, The virus challenge test was not done which came in agreement with the US Code of Federal Regul. (1987) 9 CFR 113.215 of animal and animal products (when the results of a valid serum neutralization test are satisfactory, the vaccinated and control animals may not challenged with virulent viruses). Besides to avoid spreading of infection when using virulent virus strains during challenge.

The levels of antibody titre as measured by ELISA was satisfactory for comined vaccine for virus fractions where it reached 4446 for IBR, 5984 for BVD and 4508 or PI-3 these good ELISA titres where agreed with those obtained by Payment et al. (1979) and Suri Bubul et al. (1984) where the ELISA titre reached 1:8 to1:8192, for virus fractions.

The result of antibodies of ELISA titres for pasteurella fractions in combined vaccine were satisfactory as it reached 4808 for P.multocida and 3890 for P.haemolytica in combined vaccine.

These findings agreed with high levels of antibodies measured by ELISA reported by Marshall et al. (1981), Solano et al. (1984) and Aboul Soaud (1990). The result of passive immunization of mice using the immune sera obtained from the vaccinated calves and then challenge by lethal dose of virulent pasteurella indicated that the combined and monovalent vaccines gave good protective results from 2 weeks post vaccination till 24 weeks post vaccination. These results agreed with those obtained by Bain (1963), Alwis and Carter (1980), and Aboul Soaud (1990).

So, as result studying of the immune response of calves at different interval we could use this combined IBR, BVD, PI-3 viruses and P.multocida and P.haemolytica vaccine to fight bacteria and viruses that act synergistically to cause severe pneumonia in Egyptian farm animals.

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Table (1) : Immune response of calves vaccinated with combined inactivated alum gel vaccine of (IBR, BVD, PI-3 viruses and P. multocida and P. haemolytica compared with the monovalent virus ones, as measured by SNT.

Type of vaccine	Serum antibodies titres in post vaccination time *									
	Weeks Post Vaccination									
	0		2		5	8	12	16	20	24
Combined IBR-V vaccine	0	First vaccination	2	Second vaccination	4	32	32	16	8	4
Monovalent IBR-V vaccine	0		2		4	32	64	32	16	8
Combined BVD-V vaccine	0		4		8	32	64	32	16	8
Monovalent BVD-V vaccine	0		4		8	64	64	64	32	8
Combined PI-3 V vaccine	0		4		8	64	64	32	16	8
Monovalent PI-3V vaccine	0		4		32	64	128	64	32	8
Non vaccinated control calves	0		0		0	0	0	0	0	0

* Titre expressed as reciprocal of serum dilution.

Table (2) : Level of antibodies in calf sera following vaccination with inactivated alum gel vaccine (IBR, BVD, PI-3 viruses and P. multocida and P. haemolytica compared with monovalent ones, as measured by ELISA.

Type of vaccine	Titre range	Average ELISA titre *								
		Weeks Post Vaccination								
		0		2	5		8	12	16	20	24
Virus fraction Combined IBRV vaccine	1000-4500	0	First vaccination	1064	1274	Second vaccination	2006	4448	3558	2236	2004
Monovalent IBRV vaccine	1000-5500	0		1094	1386		2218	5142	4866	3078	2814
Combined BVDV vaccine	2000-6000	0		1562	1886		2602	5984	3438	3006	2282
Monovalent BVDV vaccine	2000-6500	0		1826	2044		3956	6180	4276	3194	2946
Combined PI-3V vaccine	1500-5000	0		1424	2256		3616	4510	3558	2466	2256
Monovalent PI-3 V vaccine	2500-8000	0		2282	3726		4560	7658	4560	3846	2344
Bacterial fractions Combined P. multocida vaccine	2000-5000	0		1600	2744		3324	4810	3336	2864	2120
Monovalent P. multocida vaccine	2000-6000	0		1694	2514		3992	5776	4744	2858	2106
Combined P. haemolytica vaccine	1500-4000	0		1408	2820		3164	3892	2974	2410	2034
Monovalent P. haemolytica vaccine	2000-6000	0		1856	3114		3884	5904	4066	3128	2438
Non vaccinated control calves	0000	0		0000	0000		0000	0000	0000	0000	0000

* Titre express as reciprocal of log serum dilution.

Table (3) : Mouse protection test in serum of calves vaccinated with combined vaccine of P. multocida, P. haemolytica and IBR, BVD PI-3 viruses compared with each monovalent of Pasteurella vaccines.

Type of vaccine	No. of mice	% of passive mouse protection in time post vaccination																									
		Weeks Post Vaccination																									
		0				2				5			8			12			16			20			24		
		D	S	%		D	S	%		D	S	%	D	S	%	D	S	%	D	S	%	D	S	%	D	S	%
Combined P. multocida vaccine	5	5	0	0	First vaccination	2	3	60	Second vaccination	1	4	80	0	5	100	0	5	100	1	4	80	1	4	80	2	3	60
Monovalent P. multocida vaccine	5	5	0	0		2	3	60		1	4	80	0	5	100	0	5	100	1	4	80	1	4	80	2	3	60
Combined P. haemolytica vaccine	5	5	0	0		2	3	60		1	4	80	0	5	100	0	5	100	1	4	80	1	4	80	2	3	60
Monovalent P. haemolytica vaccine	5	5	0	0		2	3	60		1	4	80	0	5	100	0	5	100	1	4	80	1	4	80	2	3	60
Non vaccinated control calves	5	5	0	0		5	0	00		5	0	00	5	0	0	5	0	0	5	0	00	5	0	0	5	0	00

D * Dead within 24 - 72 hours.

S * Survived within 24 - 72 hours.

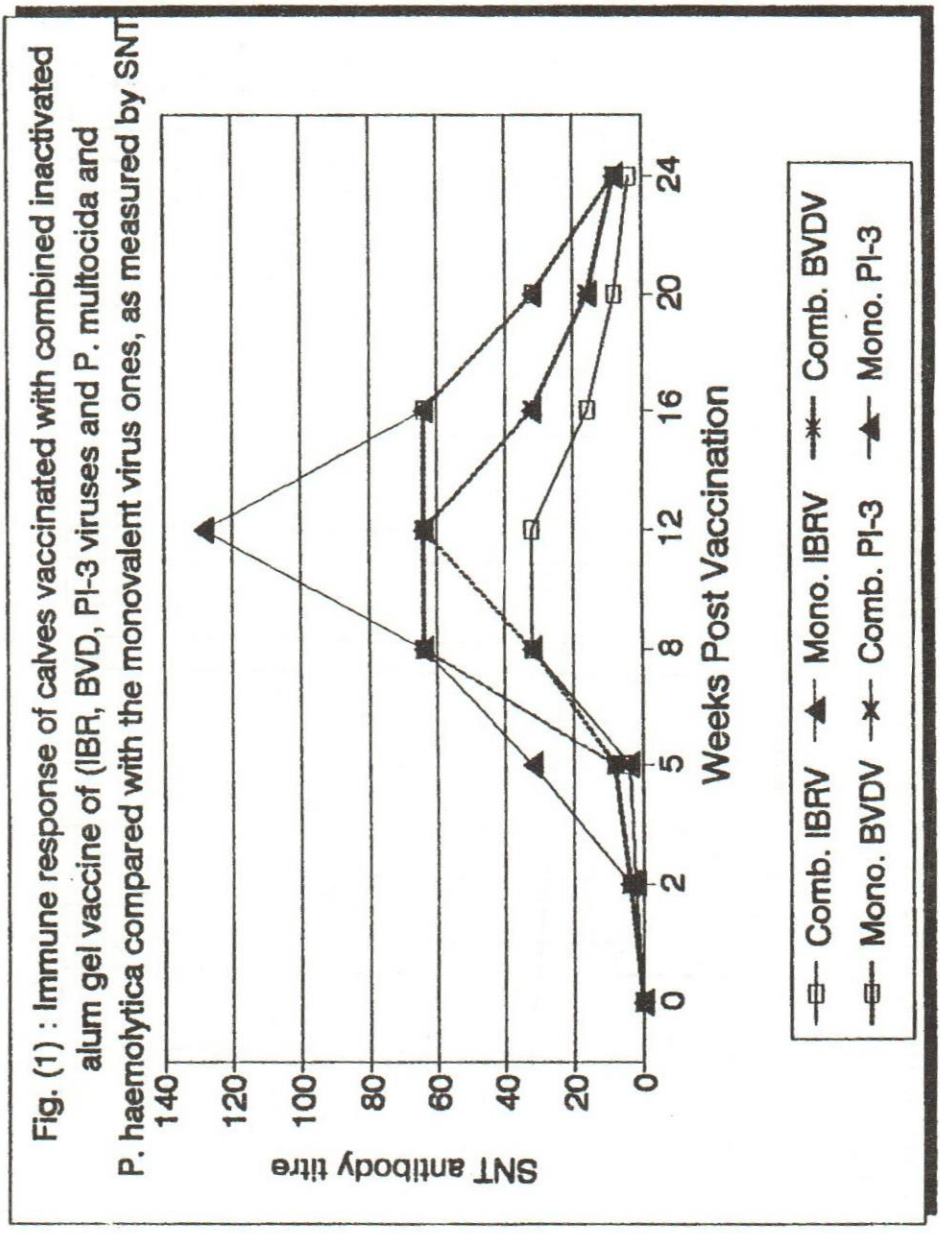

Fig. (1) : Immune response of calves vaccinated with combined inactivated alum gel vaccine of (IBR, BVD, PI-3 viruses and P. multocida and P. haemolytica compared with the monovalent virus ones, as measured by SNT

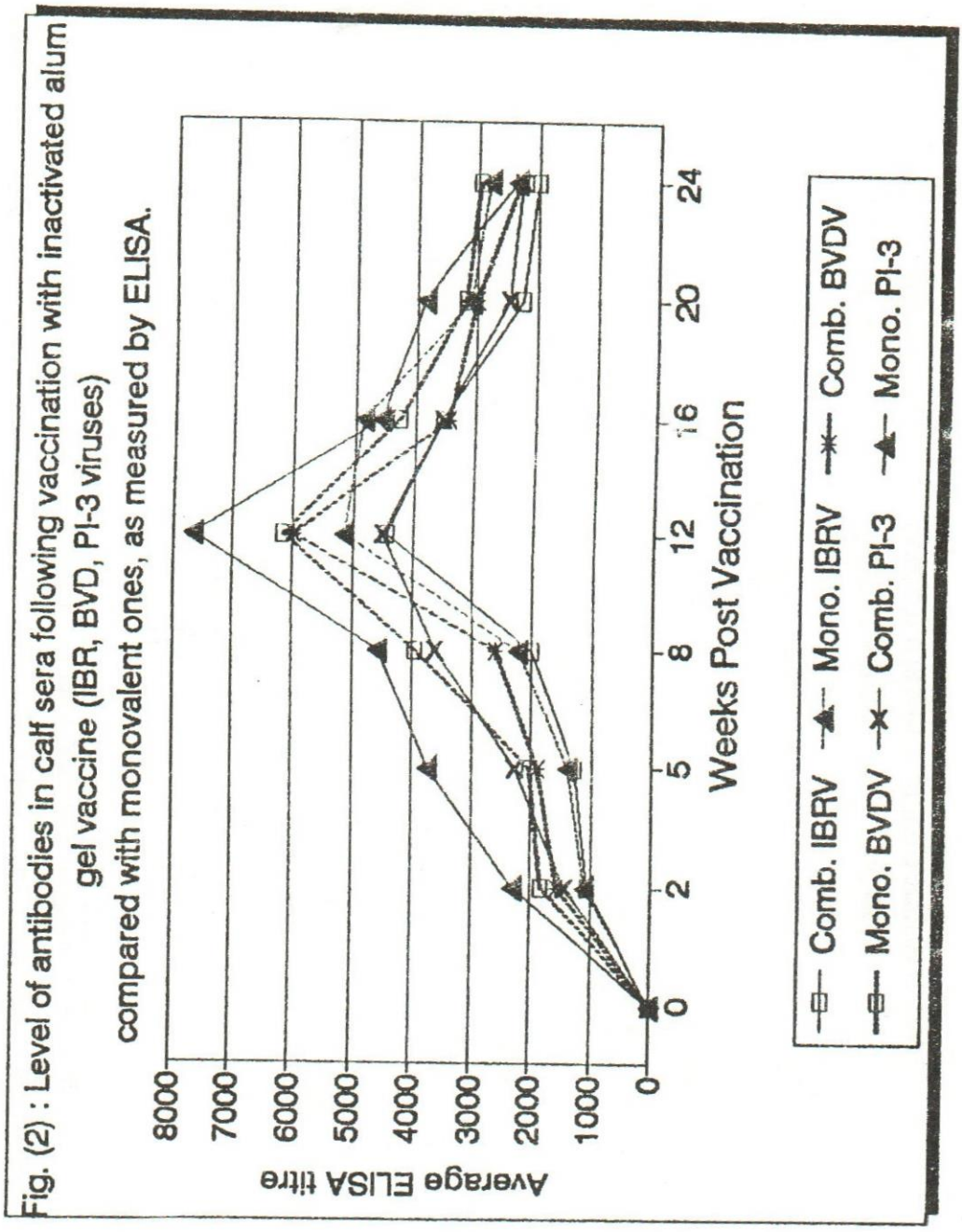

Fig. (2) : Level of antibodies in calf sera following vaccination with inactivated alum gel vaccine (IBR, BVD, PI-3 viruses) compared with monovalent ones, as measured by ELISA.

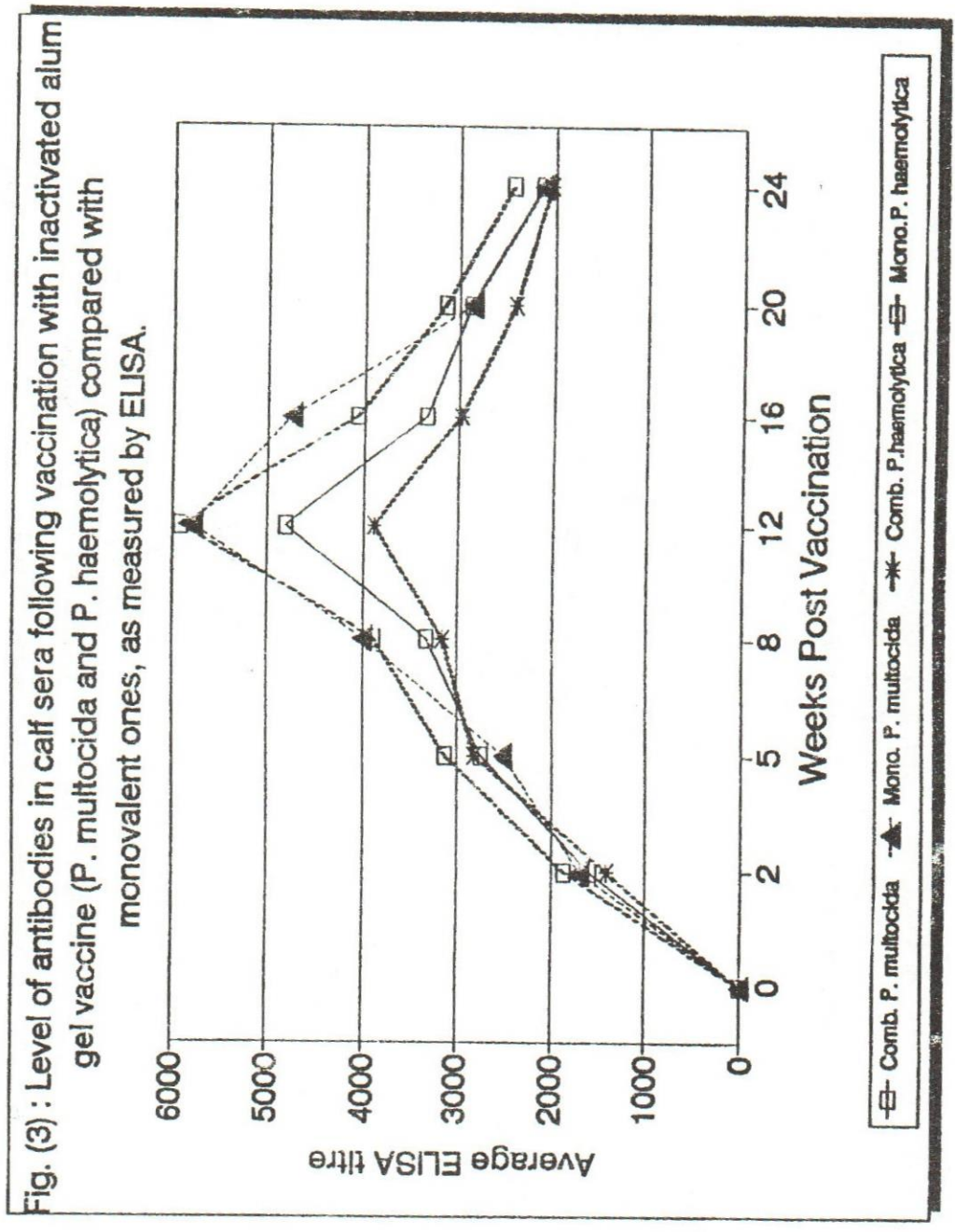

Fig. (3) : Level of antibodies in calf sera following vaccination with inactivated alum gel vaccine (P. multocida and P. haemolytica) compared with monovalent ones, as measured by ELISA.