قسم: الامراض الباطنه والمعدية.
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القوة المناعية لثلاثة لقاحات من التفحم العضلي

فهمي عوض، اقبال فراج،× عبدالسلام زكي،× محمدعبيد×

تمت دراسة القوة المناعية لثلاثة لقاحات من التفحم العضلي باستعمال ثلاثة محصنات: لقاح مرسب بسلفات الشبه، لقاح مرسب بهيدروكسيد الشبه مع مادة السابونين، ولقاح زيتي. وتم اختبارها في الأرانب الهندية بواسطة اختبار التحدى وتبين من هذه الدراسه أن اللقاح الزيتي أعطى أفضل مناعة بعد عشرة أيام وبعد ٢٠ يوما تساوت اللقاحات الثلاثة.

وجد أن درجة التجمع في أمصال الأرانب الهندية تعادل درجة المناعة المختبره بطريقة التحدى.

تمت دراسة القوة المناعية للثلاثة لقاحات في الأغنام والمواشي وذلك باستخدام اختبار التجمع . ولقد لوحظ أن اللقاح الزيتي أعطى أفضل النتائج . الاغنام المحصنه بجرعة واحده من اللقاح الزيتي ظلت مكتسبة مناعة عالية حتى نهاية السنة . أما في المواشي والجاموس فوجد أن الحيوانات المحصنة باللقاح الزيتي كان مستوى الأجسام المناعية كاف عند ٩ شهور من التحصين.

تمت دراسة لمقارنة الصله بين وجود أجسام مناعية للتجمع في أمصال الأغنام المحصنه ومقدار احتمال هذه الحيوانات للعدوى بميكروب الكلوستريديم شوفياى ووجد أن هذه الحيوانات تتحمل العدوى حسين يكون كمية المصل اللازمة لتجمع الانتجين ٥ر ميكرولتر .

× : معهد بحوث الأمصال واللقاحات البيطرية ـ بالعباسية.

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THE IMMUNIZING POWER OF THREE BLACKLEG VACCINES

(With 4 Tables and 3 Figures)

By
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SUMMARY

1- The immunizing power of three blackleg vaccines (alum precipitated vaccine, aluminium gel with saponin adsorbed vaccine and oil adjuvant vaccine) was tested in guinea-pigs. The oil adjuvant vaccine had the highest power of protection 10 days after immunization with one dose of the vaccine. Twenty one days after immunization, the three vaccines were equal in their power of protection.

2- Studies on the immune level of vaccinated guinea-pigs showed that the agglutination titre obtained from testing individual guinea-pig sera was parallel with protective immunity against challenge with Cl.chauvoei spore suspension.

3- Studies on the immune response of the three blackleg vaccine was investigated in sheep and buffaloes using agglutination test showed that the oil adjuvant vaccine gave the best and most rapid response. In sheep also vaccinated with a single dose of oil adjuvant vaccine retained a high agglutination until the end of one year, but in cattle and buffaloes the titre was satisfactory up to 9 month for the same vaccine.

4- An agglutination titre of 0.5 uL of sheep serum or less required to agglutinate Cl.chauvoei standardized antigen indicated full protection against challenge.

INTRODUCTION

In Egypt, blackleg vaccine is considered the most important clostridial vaccine. Each year 1/2 million doses of this vaccine are injected. The vccine used now is an alum precipitated vacine and is given in 2 doses followed every 6 months with a booster doses.

Results of the use of oil emulsion clostridial vaccines in guinea-pigs have been reported by STERNE et al. (1962), JANSEN (1962), THOMSON and BATTY (1967), IOUSTAU (1968) and THOMSON et al. (1969). FARRAG et al. (1977) compared aluminium potassium sulphate and aluminium gel with saponin as adjuvant for polyvalent vaccine and found that the difference between both vaccines was neglicable. FARRAG et al. (1984) compared aluminium pota-

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ssium sulphate, bentonite, kaoline and Resella oil and lanoline with polyvalent clostridial vaccine in guinea-pig and sheep. They found that the oil adjuvant gave the best results.

Blackleg vaccines have been tested by challenge in guinea-pigs as a routine measure, the use of sheep and cattle in tests involving challenge is unpractical and expensive. The use of serum aglutination technique has been reported by HANDERSON (1932), THORLAND (1953) and CLAUS and MACHEAK (1972).

The present work was designed to compare the immunizing power of 3 blackleg vaccines in guinea-pigs, sheep and cattle using plate agglutination test as a laboratory approach to measure circulating antibodies and comparing it with the ability of vaccinated animals to resist challenge against viable organisms.

MATERIAL and METHODS

Strains :

Laboratory stock strains of Cl.chauvoei.

Preparation of Cl.chauvoei culture for vaccine production

A local isolated strain from a blackleg case of cattle was used. The culture was prepared according to GADALLA et al. (1974). After the final product was safe, it was devided into three portions, to the 1st part aluminum potassium sulphate was added to make a final concentration of one percent (vaccine 1). To the 2nd part saponin was added in a concentration of 1 gram for 1500 ml and then aluminium gel was added to make a final concentration of 25 percent (vaccine 2). The 3rd part of the culture was emulsified with oil (Lanoline and Resella 17) according to the method described by GENEIDY et al. (1967) (vaccine 3).

Spore suspension :

It was prepared according to COOPER et al. (1960) and the MLD determined according to GADALLA and FARRAG (1967). It was 0.1 ml of 1/3200 dilution of Cl.chauvoei spore suspension.

Preparation of aglutinating antigen :

It was prepared and standerdized according to CLAUS and MACHEAK (1972).

PlaTE AGGLUTINATION TECHNIQUE :

The method and determination of titre were conducted as described by CLAUS and MEACHEAK (1972).

Immunizing effect of the three vaccines on guinea-pigs :

One hundered and ninety two guinea-pigs were devided into 4 groups of 48 each. The three groups were inoculated each with one of the three blackleg vaccines. Animals in the 1st and 2nd group were vaccinated subcutaneously with 2 ml of alum precipitated and aluminium gel with saponin vaccine respectively. The third group was vaccinated intramuscularly with one ml oil adjuvant vaccine. The fourth group was left as unvaccinated control.

Twelve guinea-pigs from each group and 12 of the controls were challenged with Cl.chauvoei spore suspension 10, 20, 30 and 40 days after vaccination.

Immunizing power of the three vaccines in sheep :

Thirty six 2 year old sheep were devided into equal 6 groups of 6 animals each. Animals in the first group were each inoculated intramuscularly with 2 ml oil adjuvant vaccine.

Animals in the second group were each inoculated subcutanously with 2 ml of alum precipitated vaccine. Animals in the third group were each inoculated subcutaneously with 2 ml of alum precipitated vaccine and were revaccinated with one ml 21 days after the first dose. Animals in the fourth and fifth group were vaccinated in the same manner but with aluminium gel vaccine. Those of the 6th group were kept as unvaccinated controls.

Blood samples were collected from the vaccinated sheep 10, 21, 35 days after vaccination and then monthly for one year.

Immunizing power of the three vaccines in bovines :

Seven buffaloes and 6 cows were divided into 3 groups of four aimals each and one buffalo was kept as unvaccinted control.

Animals in the first group each was inoculated intramuscularly with 3 ml oil adjuvant vaccne.

Animals in the second group each was inoculated subcutaneously with 3 ml of alum precipitated vaccine and revaccinated 21 days later with 2 ml of the same vaccine.

Animals in the third group were vaccinated in the same manner by aluminium gel and saponin.

Studies on the relationship between agglutination test and protective immunity in guinea-pigs and sheep :

A group of 45 guinea-pigs were inoculated with one ml of blackleg oil adjuvant vaccine, 15 days later heart blood was collected and examined for agglutination titre. On the 17th day of vaccination, the surviving 36 guinea-pigs with 5 unvaccinated control were challenged with different amounts of MLD of Cl.chauvoei spore suspension according to their agglutination titre.

In the same time the experiment was repreated in sheep one year after vaccination. Fifteen of the 30 vaccinated sheep with different agglutination levels were challenged with 2ml Cl.chauvoei overnight culture mixed with 0.5 ml 5% Ca Cl_2, 5 unvaccinated sheep were also challenged as control.

RESULTS

The immunizing power of the three vaccines :

1- **In guinea-pigs :**

The results are illustrated in Table 1 and 2.

Table (1)

The immune response to the three vaccines in guinea-pigs

No. of group	Interval after vaccination	No. of MLDS inoculated	Percent of protection in 12 g.p.			Percent of protection in controls
			Vaccine I	Vaccine II	Vaccine III	
I	10	16	33.3	50	91.6	0
II	20	32	91.6	91.6	91.6	8.3
III	30	64	91.6	91.6	91.6	0
IV	40	96	83.3	91.6	91.6	0

Eash of the unvaccinated controls received one MLD of Cl.chauvoei spore suspension.
g.p. = guinea-pig.

This table indicates that the oil adjuvant vaccine had the highest power of protection 10 days after vaccintion. Twenty days after vaccination the three vaccines were equal in their power of protection, but 40 days after vaccination, the alum precipitated vaccine gave less protection than the other two vaccines.

Table (2): Relationship between Cl.chauvoei agglutinin response and protective immunity developed in immunized guinea-pigs

guinea-Pig. No.	agglutination titre(uL)	challenge dose	result	guinea-pig No.	agglutination titre(uL)	challenge dose	result
1-3	0.04	45 MLD	S	27-29	0.5	32 MLD	S
4-7	0.02	45 MLD	S	30-31	1	20 MLD	S
8-11	0.05	45 MLD	S	32	2	20 MLD	S
12-18	0.1	32 MLD	S	33	5	20 MLD	D_3
19-21	0.2	32 MLD	S	34-36	20	20 MLD	D_2
22-26	0.4	32 MLD	S	Control 1-5	0	1 MLD	D

S = Survived
D = Dead within 18 hours.
D_2 = Dead 48 hours after challenge.
D_3 = Dead within 3 days.
0 = Un aglutinins.

Table (2) indicates that the agglutination titre obtained from testing guinea-pigs was parallel to the protective immunity against challenge with Cl.chauvoei sporé suspension. An agglutination titre of 2 uL of serum or less required to agglutinate the antigen indicated full protection against challenge.

2- In sheep :

The results are illustrated in Table (3 & 4) and Fig. (1). Table (3 and Fig. 1) indicate that the agglutination titre 10 days after vaccination was higher in the group of sheep vaccinated with oil adjuvant vaccine. Twenty one days after vaccination, there was an increase in the agglutination titre of the group of sheep vaccinated with both the alum precipitated and the aluminium gel with saponin adsorbed vaccine. After 35 days, the titre in the animals vaccinated with one dose of either alum precipitated or aluminium gel adsorbed vaccine began to decline until it reached to low level at 5 months. The groups of sheep vaccinated with two doses of either the alum precipitted vaccine or the aluminium gel adsorbed vaccine showed a rise in agglutination titre after 25 days similar to that obtained from sheep vaccinated with oil adjuvant vaccine, after which titre of sheep vaccinated with either of the aluminium salts vaccines began to decrease gradually reaching a low level by the end of the year. Animals vaccinated with one dose of oil adjuvant vaccine retained a high agglutination titre at the end of the year.

Table (4) indicates that the agglutination titre obtained in sheep correlates with protective immunity against challenge with 2 ml. Cl.chauvoei culture. An agglutination titre of 0.5 uL of serum or less required to agglutinate Cl.chauvoei antigen indicated full protection against challenge.

3- In bovines :

Results obtained are shown in Table (5) and Fig. (2).

Table (5) and Fig. (2) indicate that the agglutination titre 10 days after vaccination was higher in animals vaccinated with oil adjuvant vaccine and aluminium gel vaccines than in animals vaccinated with alum precipitated vaccine. After 21 days of vaccination there was a slight increase in agglutination titre in groups vaccinated with alum and aluminium gel precipitated vaccines and a demonstrable increase in the group vaccinated with oil adjuvant vaccine, 35 days after vaccination there was a demonstrable increase in the three groups particularly in the group vaccinated with alum precipitated vaccine. From 2 months onward there was a gradual decline in the aglutination titre in all groups, which was more rapid in the animals vaccinated with the alum and aluminium gel precipitated vaccines, than those vaccinated with the oil adjuvant vaccine which remained at a good level until 9 month post-vaccination.

Table (3)

Immune response of sheep vaccinated with 3 types of blackleg vaccines during a period of one year

Types of Vaccines	No. of doses	Average agglutination titre (&) (µL) in different intervals after vaccination													
		prevaccination	10 dy	21 dy	35 dy	2 m	3 m	4 m	5 m	6 m	7 m	8 m	9 m	10 m	11 m	12
oil adjuvant	1	**	0.015	0.015	0.015	0.02	0.022	0.035	0.05	0.064	0.065	0.10	0.147	0.273	0.274	0.3
alum precipitated	2	**	0.1	0.092	0.01	0.011	0.02	0.027	0.03	0.14	0.235	0.338	0.44	1.16	2.7	4.33
	1	**	0.1	0.117	0.22	0.307	0.75	1.36	4.3	8.2	**	**	**	**	**	**
aluminium gel adsorbed	2	**	0.1	0.05	0.013	0.014	0.014	0.03	0.04	0.121	0.25	0.64	1.0	1.4	3.0	4.0
	1	**	0.2	0.113	0.12	0.14	0.24	0.27	1.06	3.5	7.73	**	**	**	**	**

& = Aglutination titre is defined as number of microliters of serum required to provide definite aglutination of standardised antigen.

dy = days. m = months. ** = no detectable agglutinins.

Table (4)

Correlation between Cl.chauvoei agglutinin level and protective immunity against challenge in sheep

Sheep No.	Agglutination titre (uL)	Result	Sheep No.	Agglutination titre (uL)	Result
5646	0.5	S	5621	5	S(L)
6366	0.1	S	2184	5	S(L)
1802	0.5	S	7540	5	S(L)
5642	0.4	S	7706	5	S(L)
1458	0.5	S	2284	4	S(L)
7678	0.5	S	7708	1	S=
6902	20	D	0172	40	D
66	2	S=	176"C"	0	D_2
106"C"	0	D	177"C"	0	D
107"C"	0	D	104"C"	0	D

"C" = Unvaccinated control
S = Survived
0 = No detectable aglutinins
D = Dead within 18 hours.
D = Dead within 48 hours.
S= = Slight lamness.
S(L) = The animals developed swelling at the site of inoculation and recumbancy for 3 days, then gradually the symptoms disappeared and the animals became normal after one week.

DISCUSSION

In the present investigation the protective power of 3 blackleg vaccines, namely, alum precipitated vaccine, aluminium gel with saponin adsorbed vaccine and an oil adjuvant vaccine were studied. In guinea-pigs the immune response of the3 vaccines had been tested by challenge with Cl.chauvoei spore suspension. Results obtained indicated hat oil adjuvant vaccine gave the best response in immunized guinea-pigs as after 10 days of the first dose, it protected 91.6 percent of immunized guinea-pigs, while the alum precipitated and aluminium gel with saponin adsorbed vaccine protected only 33.3 and 50 percent of vccinated animals respectively. After 20 and 30 days of vaccination the three vaccines were equal in their power of protection. These results indicated that protection with oil adjuvant vaccine developed earlier in comparison to the other two vaccines.

Table (5)

Immune response of bovines infected with 3 types of blackleg vcines during aperiod of one year

types of vaccines	No. of dose	Average agglutination titre (µL) in different intervals ter immunization														
		prevaccination	10 dy	21 dy	35 dy	2 m	3 m	4 m	5 m	6 m	7 m	8 m	9 m	10 m	11 m	12 m
aluminium gel adsorbed	2	**	0.45	0.42	0.25	0.4	1.0	1.5	2.5	4.0	6.3	10.0	40.0	**	**	**
alum precipitated	2	**	1.26	1.16	0.26	0.35	0.7	0.8	1.9	3.8	7.6	16.3	**	**	**	**
oil adjuvant	1	**	0.3	0.16	0.05	0.11	0.17	0.25	0.4	0.45	0.5	0.5	0.62	1.2	2.0	4.0

dy = days **m** = months ** = no detectable agglutinins.

THREE BLACKLEG VACCINES

Evaluation of blackleg vaccines in cattle or sheep by challenge is an expensive procedure as approximately 50 percent of the animals must die. Therefore, the need for a laboratory method is obvious, and the present investigation was undertaken to evaluate plate agglutination test in the immune response to blackleg vaccines.

Results of the agglutination test in guinea-pigs indicated that the agglutintion titre obtained from testing individual guinea-pigs sera was parallel to protective immunity against challenge with Cl.chauvoei spore suspension. Moreover, it was found that an agglutination titre of 2 uL of serum or less produced full protection against challenge. Therefore, the serum plate agglutination test could possibly provide a useful laboratory means for measuring circulating antibodies and thus replace challenge in guinea-pigs in ssaying blackleg vaccines.

The results obtained from vaccination of sheep with the alum precipitated, aluminium gel with saponin and oil adjuvant vaccines gave a high level of Cl.chauvoei aglutinins after 10 days of vaccination, but the oil adjuvant resulted in the highest agglitination titre. After the second dose of either alum precipitated or aluminium gel adsorbed vaccine the agglutination level increased and was parallel to that obtained by one dose of the oil adjuvant vaccine until about the fifth month, after which the level of the alum precipitated and aluminium gel vaccines subsided gradually until the end of the year when the agglutination titre dropped to a low level (4.3 uL of the serum in animals vaccinated with alum precipitated vaccine while it was 4.0 uL of serum in animals vaccinated with aluminium gel adsorbed vaccine. At the same time animals vaccinated with one dose of oil adjuvant vaccine remained with a good agglutinin level until the end of the year (0.3 uL). Agglutination titre in animals vaccinated with one dose of either alum precipitated vaccine and aluminium gel adsorbed vaccine dropped to a negligible level after 5 months of vaccination. This finding indicates that in Egypt alum precipitated vaccine when used in one dose cannot give protection for more than 5 months and to extend the protection for one year, second dose is needed within 6 weeks from the first one. Therfore, we recommend the use of a single dose oil adjuvant vaccine in sheep as its power of protection even exceeds one year.

Results of the agglutination tests in sheep indicated that the agglutintion titre obtained from testing individual sheep sera was parallel to protective immunity against challenge with Cl.chauvoei culture. It was found that an agglutination titre of 1 and 2 uL of serum resited challenge although the animals shwoed slight lamness. An egglutination titre of 0.5 uL of serum or less indicated full protection against challenge. This result would confirm that obtained with guinea-pigs, that agglutination titres were found to exist in parallel with protective immunity and thus can possible replace challenge in evaluation blackleg vaccines. The present findings agree with those of GRIGORIU et al. (1963), CLAUS & MACHEAK (1972) and MACHEAK et al. (1972).

Comparing the power of protection of the three blackleg vaccines in bovines results obtained indicated that the agglutination titre 10 days after vaccination was higher in animals vaccinated with oil adjuvant vaccine and aluminium gel vaccine than in those vaccinated with alum precipitated vaccine. After the second dose of alum precipitated vaccine, the titre showed a demonstrable increase and was equal to that obtained by animals vaccinted with aluminium gel with saponin vaccine, although lower than the obtained by animals vaccinated with one dose of oil adjuvant vaccine. From 2 months onward there was a gradual decline in the agglutination titres in all groups which was more rapid in animals vaccinted with the alum precipitated and aluminium gel adsorbed vaccines as the titres were very low at 6 months, while the titre in the group vaccinated with a single dose of the oil adjuvant vaccine remained at satisfactory level until 9 months post-vaccination. This results confirmed the superiority of oil adjuvant vaccine as did previous experiments.

Comparing the immune response afforded by the 3 vaccines in bovines with that of sheep, it was observed tht the agglutination titre was much less in bovines than in sheep vaccinated with the same vaccines. The level of antibodies in ovines vaccinated with any of the 3 vaccine, declined more rapidly, than in sheep. This variation had been explained by HUBER and KRANEVELD (1955) who suggested that guinea-pig and sheep are unsuitable test animals for a vaccine to be used in cattle as guinea-pigs are relatively slow in acquiring immunity, and sheep acquire immunity more rapidly than cattle.

In conclusion, our findings indicate the superiority of oil adjuvant vaccine in sheep and bovines, and its practicability is well appreciated, therefore, we venture to suggest that in Egypt we should use a single dose of oil adjuvant vaccine in ruminants, repeated yearly in blackleg areas. Our findings supports those of STERNE et al. (1962), THOMSON and BATTY (1967), IOUSTAU (1968) and THOMSON et al. (1969).

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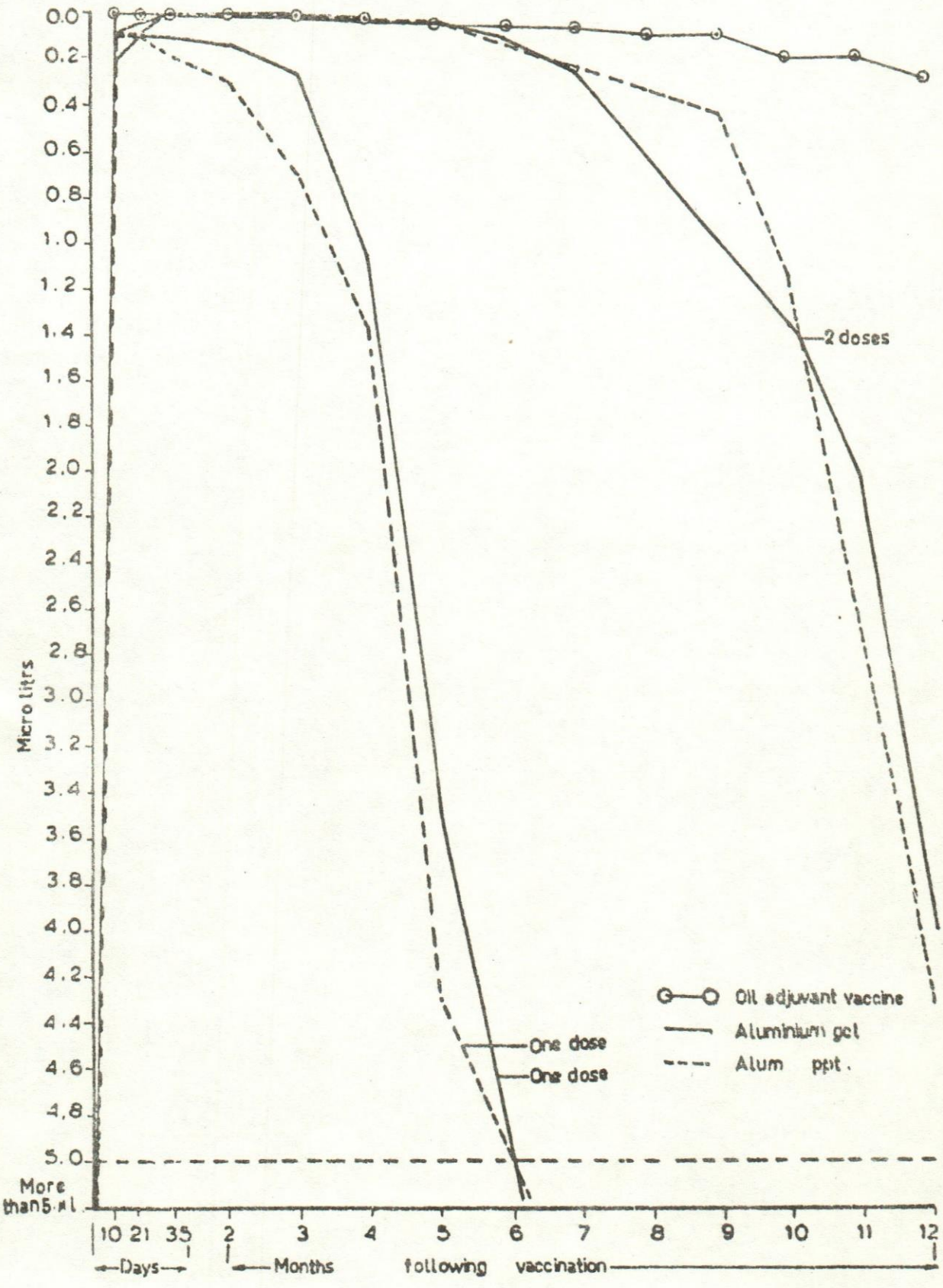


Fig. (1): Average Cl.chauvoei agglutinin response to 3 blackleg vaccines in sheep during a period of one year.

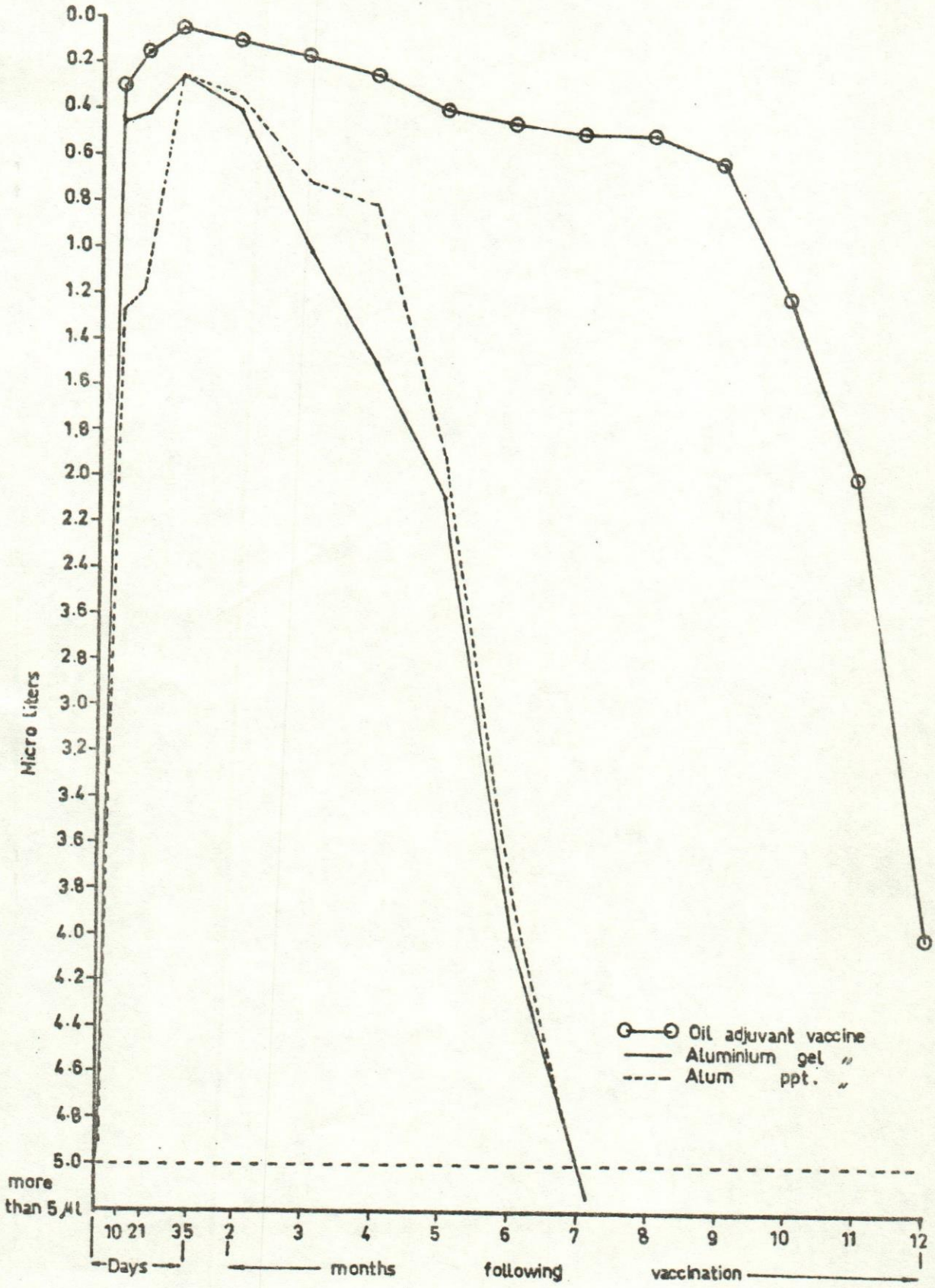
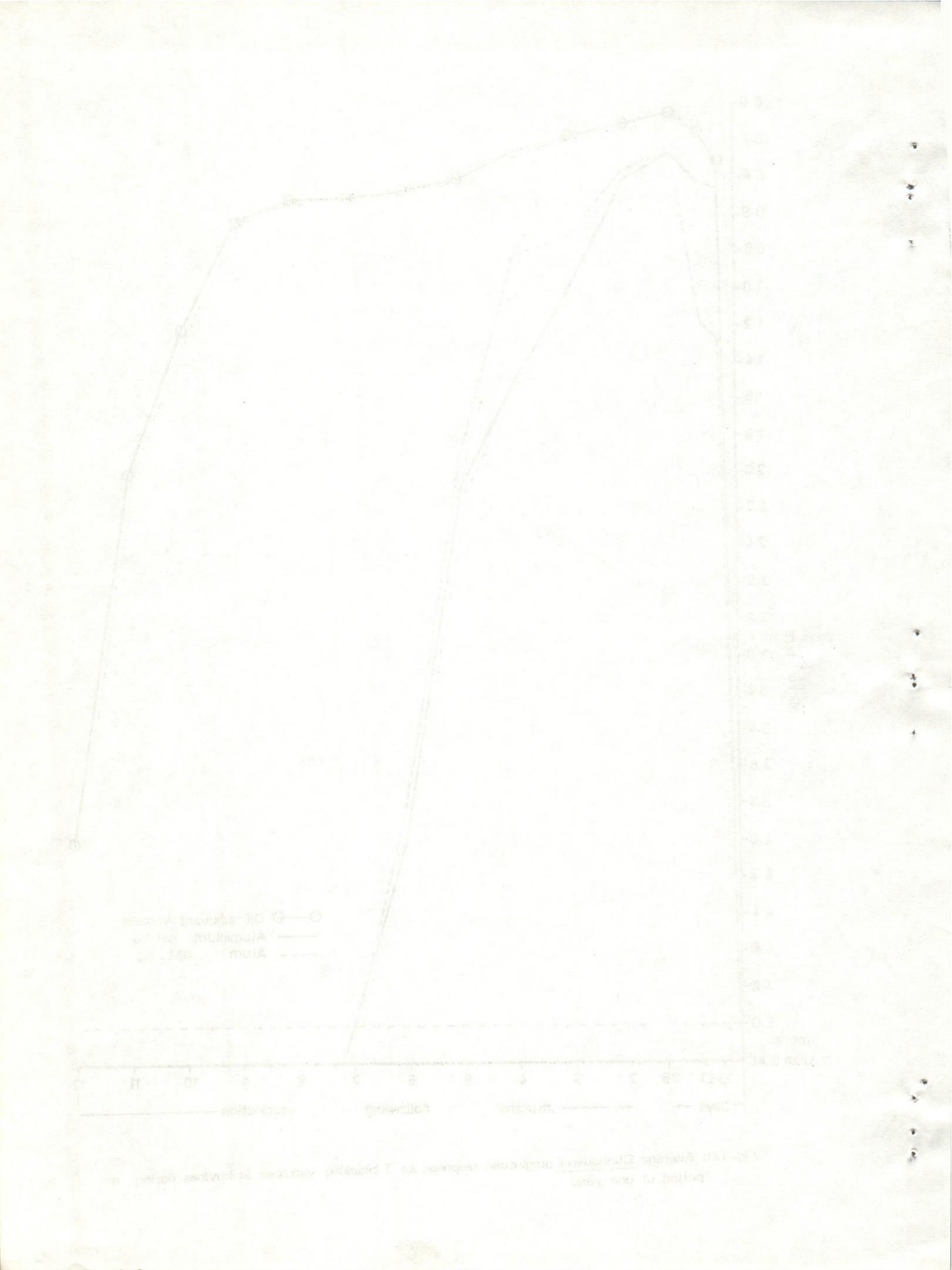


Fig. (2): Average Cl.chauvoei agglutinin response to 3 blackleg vaccines in bovines during a period of one year.



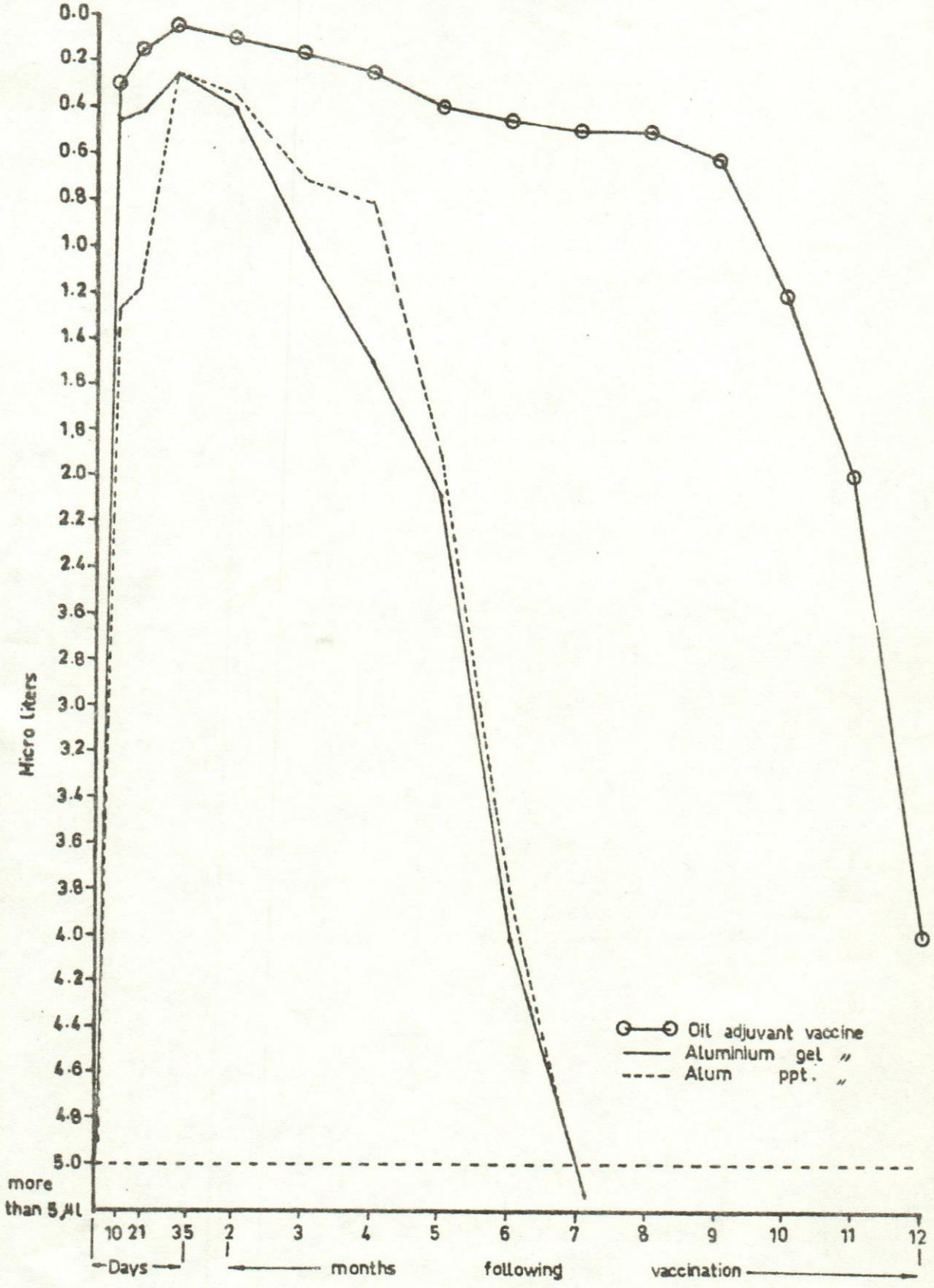


Fig. (3): Average Cl.chauvoei aglutinin response to 3 blackleg vaccines in bovines during a period of one year.