INSTITUT D'ÉLEVAGE ET DE MÉDECINE VÉTÉRINAIRE

DES PAYS TROPICAUX

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END OF YEAR 1 AND 2 REPORT

IMPROVEMENT OF THE RINDERPEST VACCINE THERMOSTABILITY

I

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CONTENTS

INTRODUCTION

According to the agreement between the **Commission of the European Communities (DG VIII)** and **IEMVT**, the research programme on the improvement of the Rinderpest vaccine thermostability started in May 1987, for a three-year period.

From the very beginning, two different approaches were chosen:

1) the development of a thermostable "clone" of the classical Plowright's virus, even if some modifications had to be brought to the present production techniques;

2) the construction of a recombinant virus using a Poxvirus as a carrier.

This report deals only with the development of the thermostable clone, the second point being the subject of another report following the recommendations of the meeting held in August in O.I.E.

The laboratory work has been carried out by Mlle Calvez and the animal tests by Dr. A Diallo with the collaboration of the staff of the Farcha laboratory in Chad (Dr. Idriss Al Farouk and Dr. Bidgé)

I/ MATERIALS AND METHODS.

I-1) Development of a thermostable "clone"

A vaccine batch (98 BK) was left at 45°C and titrations carried out every week on Vero cells. The wells of the final dilution giving a clear CPE were used to produce a new batch on Vero cells (98 BK, 2 Vero) that was freeze-dried and incubated at 45°C as before. The operation was repeated three times consecutively (see figure 1). In the case of the 3rd batch, the virus was recuperated after 60 days at 45°C.

Cell culture : The whole work was done with Vero cells (Pasteur Institute, Paris) grown in disposable trays with 2 % FCS-MEM.

The batches were freeze-dried using the Weybridge medium (casein hydrolysate 2.5 %, saccharose 5 %, Na glutamate 1 % - pH 7.2) as a diluent (aa) and according to the following scheme in a 68-70 hours cycle :

Starting temperature - 45°C for 14 hours, then - 30°C for 4 hours, - 25°C for 4 hours, - 20°C for 14 hours, - 5°C for 4 hours, 0°C for 4 hours, 5°C for 14 hours, + 20°C for 4 hours and finally + 35°C for 6 hours. The freeze-dryer equipment comes from FTS System Inc. (N.Y.- U.S.A).
The vials were stoppered under vacuum and stored either at - 20°C or + 45°C.

"Cloning" was carried out in 24-wells plates using 5 dilutions (1/2, 1/5, 1/10, 1/100, 1/1000) and 4 wells per dilution. The cells were observed every day and the results noted on day 10 and 12.
All the cell cultures were incubated at 37°C in a 5 % CO_2 atmosphere.

The thermostability of the virus at 45°C was increased at each passage associated with a reduction of the time of apparition of the CPE:

- 1st passage (98 BK) : Day + 18 CPE in 15 days
- 2nd passage (98 BK, 2 Vero) : Day + 35 CPE in 10 days
- 3rd passage (98 BK, 4 Vero) : Day + 60 CPE in 6 days

I-2) Titration tests

Titrations were carried out to estimate the half-life of both viruses in 96-wells plates using 1 in 10 dilutions and 5 wells per dilution.

Each time, the CPE was noted at day 10 and the plates were fixed with aceton and an indirect immunofluorescent test practiced using a RP-rabbit hyperimmune anti-serum and a fluorescein labelled anti-rabbit IgG serum.

Titration tests on Vero cells in 24 wells plates

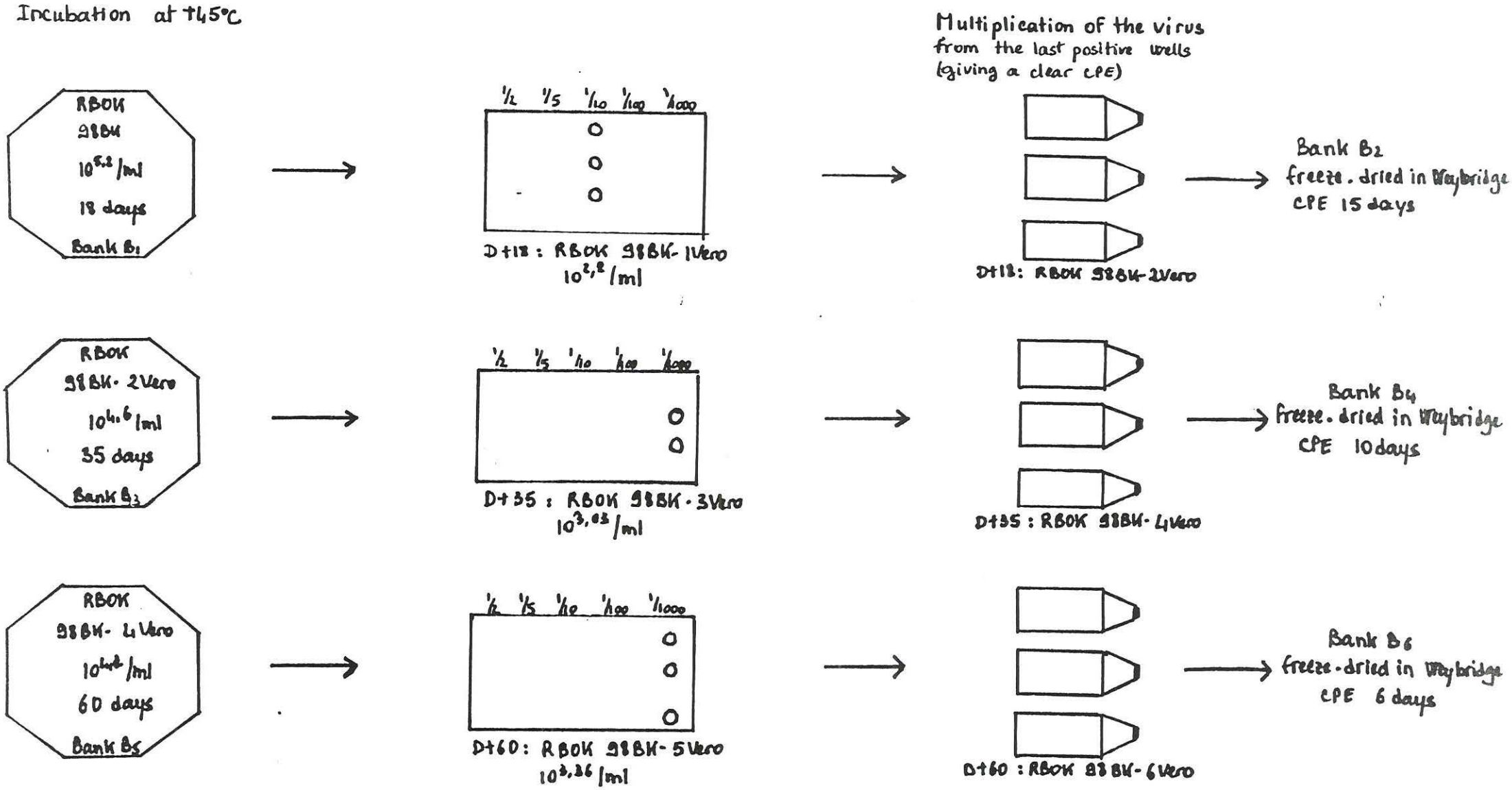


Figure 1 : Development of a thermostable "clone"

For each batch and on each date, three tests were performed on different Vero cell cultures and the mean titer calculated.

I-3) Virus-neutralisation tests

The constant virus-variable serum VN tests were performed on Vero cells in 96-wells plates. The titer of the virus bank was 10^3 $TCID_{50}$ per ml. 75 ul of the virus dilution is added to 75 ul of serum dilution, incubated 1 hour at 37°C and then 50 ul of a 10^5 cells per ml suspension are added. The results are read at 10 days.

I-4) Efficacy tests on animals

The tests were carried out in Chad (Laboratoire de Farcha) on 11 young zebu cattle (6 to 10 months old):
Before vaccination :

- 9 were serologically negative,
- 1 was positive 1/4,
- 1 was positive 1/2.

Among the 9 negative animals,

- 2 (N° 123, 167) received 50 dosis each,
- 4 (N° 153, 174, 187, 194) received 1 dosis each,
- 3 (N° 128, 165, 181) served as in-contact controls but only 2 were challenged afterwards.

The 2 positive animals (N° 114, 152) were also kept in contact with the vaccinated animals and were challenged.

For the challenge, three weeks after vaccination, the Saudi Arabia strain (2 BK, 7 Vero) was inoculated :

N° 167, 153, 174, 165, 114, 152 received 10^2 $TCID_{50}$

N° 123, 194, 181 received 10^3 $TCID_{50}$

N° 128 was not challenged.

After vaccination and after challenge, blood samples were taken to monitor 1) the viremia of the vaccine and virulent strains (using inoculation of lymphocytes on tissue culture and the cDNA probe), 2) the antibody response.

II/ RESULTS

II-1) Production of four batches

After the potential thermostable "clone" was obtained, a seed bank was produced (98 BK, 6 Vero).Three batches of vaccine were prepared from this bank (batch A, B and C - 98 BK, 7 Vero),freeze-dried and stored at + 45°C with a batch of vaccine prepared with the classical virus (99 BK).

Another batch (Th.Vac) was also prepared using the same seed bank but from vials kept for 60 days at +45°C.

II-2) Degradation curves at 45°C

The results of the titration tests are presented in Table 1 and Figures 2, 3, 4, 5 and 6.

It is clear that the difference between the classical strain and the thermostable one is very important although it is not possible with so few points to establish a regression line for each batch.

With the available results, two comments are possible:

1) The degradation process for the thermostable strain is comparable to that of the classical strain, i.e. a rapid initial decay in 7-14 days and a gradual decay that looks like a plateau for at least 60 days (batch A and Th.Vac.).

2) More titrations will be necessary to establish the complete degradation curve of the vaccine on a longer period (4 months and even more).

The first conclusion is that the population of the thermostable vaccine is not homogeneous and that the selection pressure will have to be maintained.

The second conclusion is that the thermostable vaccine as long as it is used with a certain number of precautions presents a great advantage on the classical vaccine.
The titer calculated at Day +14 (at 45°C) seems to remain the same for 1 to 1.5 month (Batch A and Th.Vac.) or to decrease very slowly (Batch B and C). More titration tests, over a longer period, will permit to establish the "k constant" or "slope" of the degradation curve.

NOTA : Preliminary results presented in the a previous report (half-life of 45 days) were indicative results. The curve drawn at that time was established on a batch prepared before the seed bank was completed and with a different free-

ze-drying procedure. With the current results, it seems that we are facing a more complex situation.

Due to the two-phases degradation curve, speaking of half-life does not mean much.

II-3) Animal trials and virulent challenge

All the classical tests recommended in the norms published by O.I.E for the R.P vaccine production were carried out to see if the selection of a thermostable vaccine had not modified the initial properties of the attenuated RBOK strain.

Safety tests : The 2 animals (N° 123 and 167) inoculated with 50 dosis showed no sign of clinical disease nor any significant rise in temperature.

Efficacy tests : The 4 animals (N° 153, 174, 187 and 194) inoculated with 1 dosis were protected when inoculated with the virulent strain while the negative controls (N°165 and 181) died of classical RP.

Excretion of the vaccine strain : The 3 control animals left in contact with the vaccinated ones remained serologically negative after 3 weeks.

Multiplication of the virulent strain in vaccinated animals : All the inoculations of lymphocytes on cell cultures were negative. The diagnostic tests, performed till now, using the cDNA probe were also negative while they were positive for the control animals.

Antibody levels : A significant rise of the antibodies in vaccinated animals was detected as soon as 9 days after vaccination:

N° 123 (50 dosis)	1/16
N° 153 (1 dosis)	1/64
N° 194 (1 dosis)	1/16
N° 187 (1 dosis)	1/64
N° 174 (1 dosis)	1/64

It can be noted that the level of antibodies does not depend on the number of dosis inoculated and that there is no booster effect after the challenge inoculation.
(See figures : temperature and antibodies).

CONCLUSION AND FUTURE TRENDS

The thermostable vaccine that was selected at 45°C and cloned by the final dilution method seems to be a good candidate for use in Africa :

1) After an initial decay during the first 2 weeks, the titer remains constant for 1.5 month at least.

2) The virus is effective and has kept all the properties of the initial RBOK strain.

However, more work is needed :

- on the genetic stability of the product when cultivated several times on cell culture without any selection pressure : A batch has been prepared after 3 consecutive passages in Vero cells and freeze-dried. The curve at 45°C will be established. The same operation will be performed after 10 and 15 passages.

- on the exact degradation curve at 45°C and on a longer period (4 or 5 months). More titration tests will be carried out so that regression procedure on the second phase of the degradation process will be possible.

In order to check the suitability of the vaccine in African conditions, vials of the seed bank could be sent to one or several National laboratories with recommendations for use and batches produced in local conditions.

The main recommendation should be, for the African laboratories, to prepare their own seed bank with 2 passages only and to keep this seed bank at 45°C for 2 weeks before using it for the production of a batch.

It is evident that the production technique will be more complicated than the current one but in exchange for more severe constraints on the laboratory, the field work becomes easier.

APPENDIX

TABLE 1 : Results of the titration tests :

Days at 45°C	BATCH A	BATCH B	BATCH C	ThV	BATCH99
Before lyo-philisation	ND	5.2	6.6	ND	ND
	ND	5,4	6.2	ND	ND
	ND	5.5	6.4	ND	ND
D 0	4.5	4.8	4.9	3.9	4.0
	4.6	4.6	5.2	4.2	4.2
	5.2	5.2	5.4	4.5	4.0
mean	4.76	4.87	5.16	4.2	4.1
D+7	4.2	4.4	4.9	3.9	2.8
	4.2	4.2	4.2	4.2	2.8
	4.2	4.4	4.9	3.6	2.5
mean	4.2	4.33	4.66	3.9	2.7
D+14	3.6	3.8	4.5	3.6	0
	3.8	4.2	4.4	3.6	0
	3.5	3.9	4.2	3.5	0
mean	3.6	3.96	4.36	3.56	
D+ 30	3.6	3.5	3.8	3.5	0
	3.8	4.2	4.2	3.6	0
	3.6	3.6	3.6	3.6	0
mean	3.66	3.76	3.86	3.56	
D+45	3.8	3.5	3.6	3.6	
	3.5	3.6	3.5	3.4	
	3.5	3.6	3.6	3.6	
mean	3.6	3.58	3.58	3.5	
D+60	3.6	3.3		3.5	
	3.6	3.5		3.5	
	3.5	3.3		3.4	
mean	3.56	3.41		3.46	

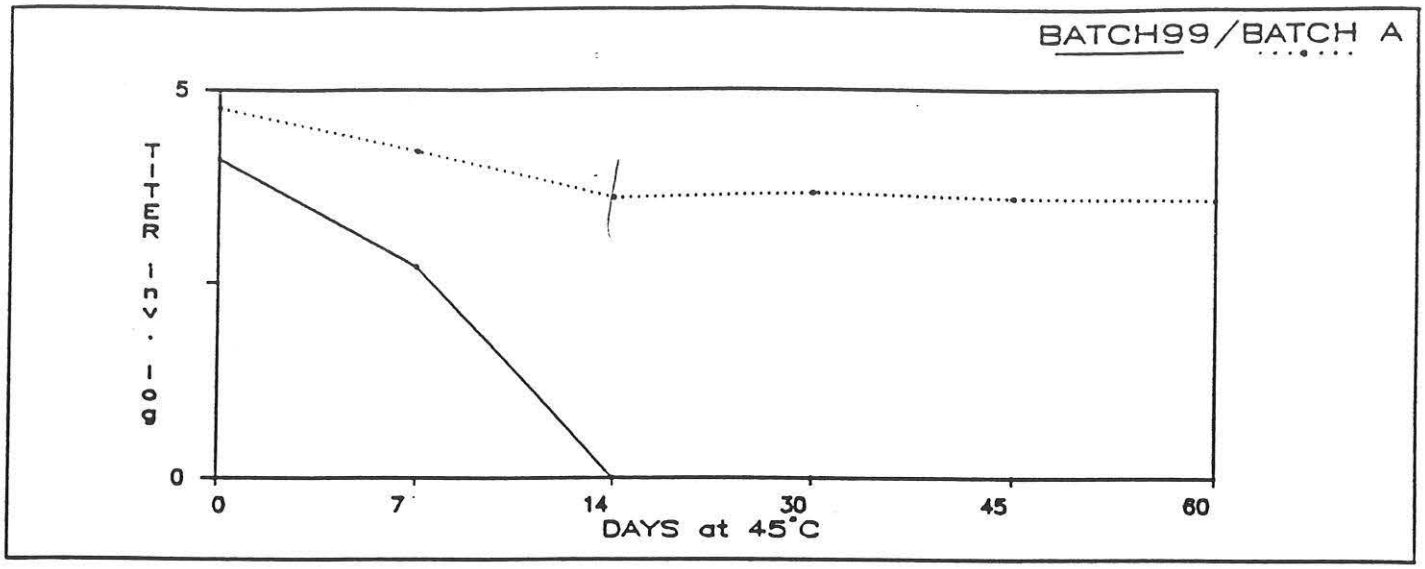
TABLE 2 : Residual moisture of the different batches on 20 vials per batch.

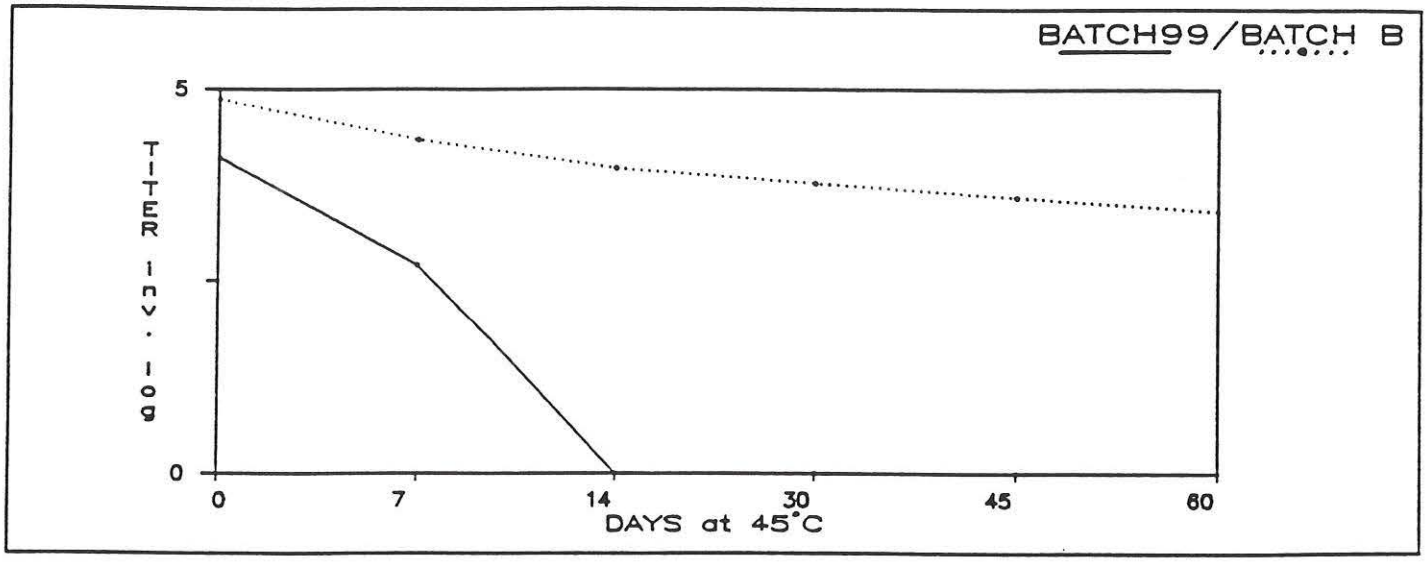
Batch A : mean = 0.99% (extremes = 0.8% and 1.5%)

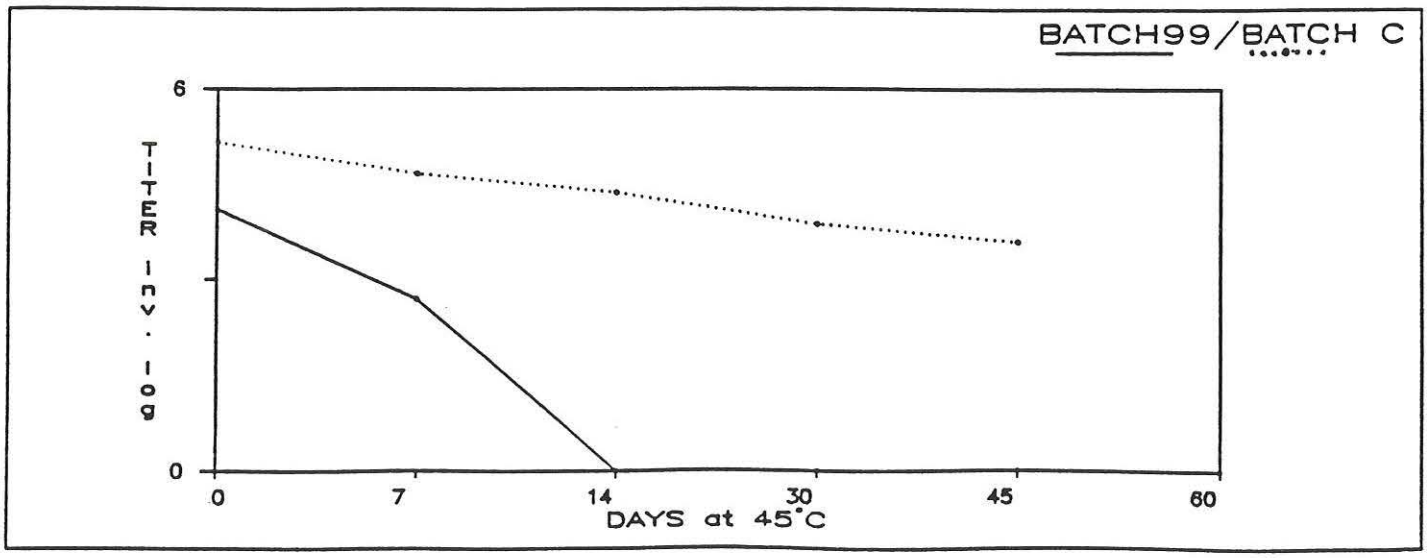
Batch B* : mean = 1.18% (extremes = 0.87% and 1.35%)
Batch B : mean = 1.17% (extremes = 0.9% and 1.6%)

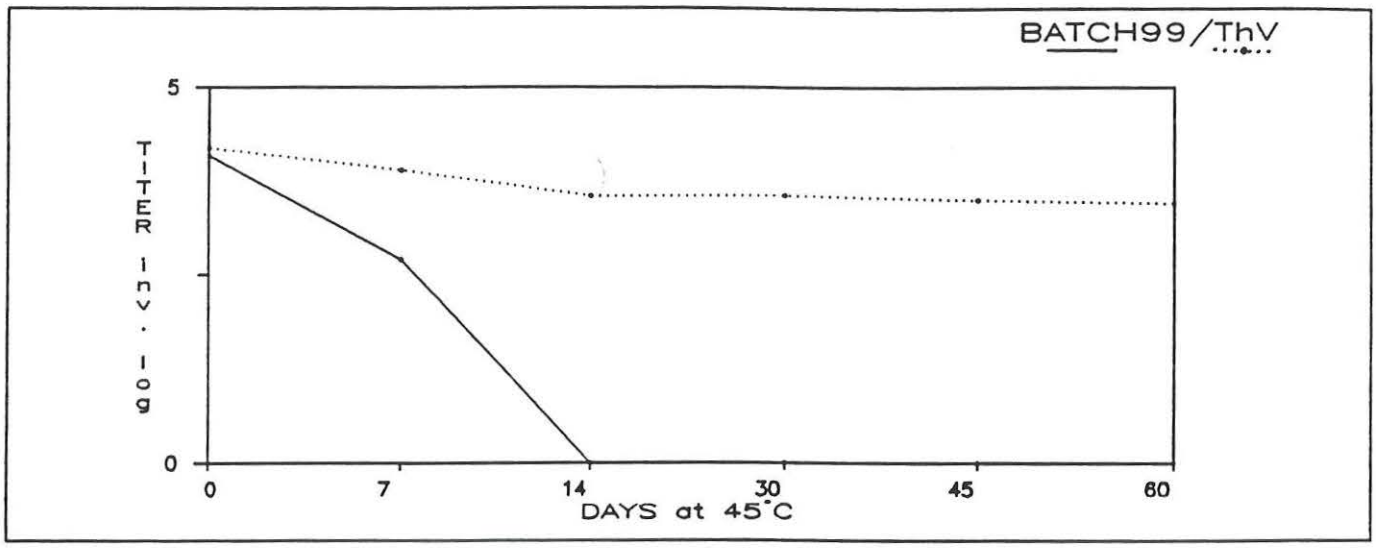
Batch C* : mean = 1.1 % (extremes = 0.66% and 1.43%)
Batch C : mean = 1.04% (extremes = 0.67% and 1.36%)

Batch Th.Vac.: mean = 0.99% (extremes = 0.64% and 1.43%)


BATCH99/BATCH A
TITER inv. log
5
0
0
7
14
30
45
60
DAYS at 45°C


BATCH99/BATCH B
TITER inv. log
5
0
0
7
14
30
45
60
DAYS at 45°C


BATCH99/BATCH C
TITER inv. log
6
0
0
7
14
30
45
60
DAYS at 45°C


BATCH99/ThV
5
0
TITER inv. log
0
7
14
30
45
60
DAYS at 45°C

HYPERTHERMIA AND ANTIBODY CURVES

OF THE VACCINATED AND CHALLENGED ZEBU CATTLE