TRANSMISSION AND VIRULENCE OF RINDERPEST VIRUS

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FINAL REPORT

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1. INTRODUCTION

The project entitled 'Transmission and virulence of rinderpest virus' has been approved and financed by the European Economic Community, General Direction XII (EEC-GD XII – STD2). It comprises a total of four laboratories:

- Institute for Animal Health, Pirbright Laboratory, Great Britain;
- Laboratoire de virologie et d'immunologie, [Virology and immunology laboratory] of the Faculty of Veterinary Medicine of the Université de Liège, Belgium;
- Institut d'élevage et de médecine vétérinaire (IEMVT), [Livestock and Veterinary Medicine Institute], Maison-Alfort, France;
- Laboratoire de recherches vétérinaires et zootechniques de Farcha, [Laboratory for veterinary and Zootechnical Research of Farcha], N'Djamena, Tchad.

The purpose of this project is to study some rinderpest's viral strains of different pathogenecity, which will firstly give us the opportunity to understand the basics of those variations, to assess if this phenomenon can be associated to the virus's persistence and, finally, to bring to the fore factors, badly understood until then, which influence the virus's pathogenecity. Secondly, the following aspects of those strains will be studied:

- genes sequences of their F and H proteins;
- incubation period;
- duration of the virus's excretion in case of an infection;
- minimum time required for an infecting contact;
- number of animals infected, over a given period of time, by an animal excreting the virus.

The results obtained in the course of those experiments will lead to the development of a mathematical model of rinderpest infection.

Rinderpest constitutes a disease which is acute, febrile, inoculable, very contagious and mainly transmissible through direct contact. It affects almost all species belonging to the Artiodactyles family among which ruminants and swines are the most sensitive (PLOWRIGHT, 1968). With rabies, it is the longest known affection and perhaps the most deadly for cattle. It is still affecting Africa, India and the Near East (PLOWRIGHT, 1985). The disease is known since the fifth century, but it is in 1902 that NICOLE and BEY demonstrated the filterability of the virus.

As a result of a virus belonging to the Paramyxoviridae family, of the morbillivirus type, this disease is characterised by a more or less apparent typhic condition, an inflammation of mucous membranes, that of the digestive tractus in particular, with erosion and necrosis.

Although there is only one antigenic type of rinderpest virus, whatever its geographic origin, many isolated strains fundamentally differ over the world because of its level of virulence. As a result, they were coarsely divided into three categories (TAYLOR, 1986). The first category comprises those called hypervirulent which cause 100% mortality in animals. Strains of the second category have an average virulence resulting in a mortality rate of 33%. The third one encompasses hypovirulent strains leading to a mortality rate from 0 to 5% which result however in abortive forms, with truncated clinical expression.

The duration of the incubation period, the infection and the mortality noticeably vary according to the virus's virulence, the receptivity or the natural resistance of animals, the intensity of contacts, the number of viral units excreted by an animal and finally, to a lesser extent, to the environment in which contact is made. Indeed, those factors play a crucial role in the transmission speed of the virus from one animal to another and, consequently, influence the spread of the disease in a given population.

The existence of atypical forms, indeed asymptomatic (subclinical) makes the possible dissemination of the virus in herds even more dreadful and, by way of consequence, renders the clinical diagnosis almost impossible. This complicates even more the epidemiological study of the disease (PROVOST et al., 1973).

Several in vivo experiments in relation to the pathogenic power of isolated strains were undertaken (COOPER, 1932; PROVOST, 1958; ROBSON et al., 1959; PLOWRIGHT, 1963, 1964; LIESS et al., 1964; PROVOST, 1972; TAYLOR et al., 1965, 1986). But those results are fragmentary and only provide information on the isolated virus.

To date, there is no information in literature about the compared study of rinderpest virus's strains of different pathogenecity in the transmission through close contact over variable periods of time.

The part played by the laboratory of Farcha in this project is to achieve a compared study of three rinderpest virus's strains of variable virulence in sensitive animals in order to address the abovementioned problems. Samplings and data are collected with a view to forward them to European laboratories which are partners involved in the said project.

2. EQUIPMENT AND METHODS

2.1 Experimented animals

A total of 45 bullocks of the Arab Zebu breed approximately aged between 10 and 17 months were purchased on the market of N'Djamena. Their sera were tested for neutralising antibodies against rinderpest virus. Only animals without rinderpest antibodies and apparently sensitive are used in the experiment as PLOWRIGHT and FERRIS (1961) clearly demonstrated that there is a correlation between the resistance or the receptivity of cattle to rinderpest virus and the presence or absence of neutralising antibodies in them. The food is based on straw supplemented with 2.3 kg of cotton cattle-cakes per animal and per day. None of those animals was administered medical treatment, i.e. neither disinfestation or antibiotherapy; nor vaccination.

2.2 Virus

Three rinderpest virus's strains of different pathogenecity were used:

- the Saudi strain isolated in 1981 in Saudi Arabia (TAYLOR, 1986), considered as hypervirulent
- the Sokoto strain which is a strain of average virulence, isolated in 1964 in Nigeria (TAYLOR, 1986)
- the RBT/1 strain, hypovirulent, isolated in Tanzania (PLOWRIGHT, 1963).

The three strains were kindly offered by the Service de pathologie infectieuse, [Infectious Pathology Service], of the EMVT-CIRAD[1] (France). They are meticulously titred in Vero cells according to the method described by this laboratory. Whatever the strain, the titre used for experiments is about 10^4 $DICT_{50}$.

2.3 Process of experiments

Experimental procedures for a year are presented in Table 1. Experimental infections are produced by inoculating the virus subcutaneously to an animal isolated from the others. Its rectal temperature is recorded daily at the same time (morning) until the first clinical sign appears which represents the beginning of hyperthermia. The animal infected experimentally is only regarded as excretor of the virus from the second day of pyrexia. The contact of the diseased animal with others takes place this way: the animal excreting the virus is introduced in a pen with four other animals for a period of time determined in conformity with the planned protocol; it is then withdrawn from the group and the other animals are then locked in individual pens separated by partitions surmounted by wire fence in order to prevent any contact between those animals (see Diagram in Annex). During the whole observation period which lasts 21 and 30 days for the Saudi, Sokoto and RBT1 respectively (two first years of experiment) and only 30 days (for all strains) the third year, the clinical examination takes place daily and the characteristic symptoms of rinderpest are recorded on an individual sheet displayed in each pen (see Appendix). All clinical signs such as hyperthermia, discharge, tear production, buccal lesions and diarrhea are called clinical score. The latter is given a value of 10 points; 2 points for each sign. In this context, a rectal temperature of 39.3° and more is considered as a beginning of hyperthermia. The duration of the incubation period is calculated from the first contact up to the apparition of hyperthermia.

Nasal, buccal and ocular smears as well as a blood sampling on anticoagulant (EDTA) for the collection of lymphocytes are performed every day. On the other hand, samples of sera are taken every three days to search for neutralising antibodies. Except for sera which are analysed on the spot, all other samplings collected in the course of those experiments are forwarded to the

[1] Centre de coopération internationale en recherche agronomique pour le développement

EMVT-CIRAD (France) to be treated by hot probe, a specific test which only detects the RNA of rinderpest virus.

The technique used for obtaining pure lymphocytic suspensions is that of Ficoll-Hypaque@ density gradients (Pharmacia):

1) Take a sample of venous blood on anticoagulant
2) Pour 3 ml of Ficoll-Hypaque@ (d=1.077) in a centrifugal tube
3) Smoothly add 4 ml of blood half-diluted in PBS on top of the Ficoll-Hypaque@ while making sure not to mix up both phases
4) Centrifuge at 1750 rpm for 35 min. at +4°c. The layer of lymphocytes is then separated from red corpuscles and polynuclears (see Appendix).
5) Remove the layer of lymphocytes with a pipette. Wash 3 times in PBS (1300 rpm for 10 min.). Lymphocytes are taken back in 0.5 ml of sodium thiocyanate.

The method used to search for neutralising antibodies is that described by PLOWRIGHT and FERRIS (1961) with a slight modification which consists in replacing veal kidney cells with Vero cells.

The second technique used to analyse serum is the ELISA competition test well-adapted to rinderpest serology. It is much more sensitive and specific that the Indirect ELISA test. The equipment was delivered in the form of a KIT by the joint FAO/IAEA division in the framework of the Pan African Rinderpest Campaign Project (PARC). The analysis protocol in the KIT is applied to test sera which are only 20% diluted and the reading is done with MULTISKAN (MCC/340) at a wavelength of 492µ.

After each series of experiments, faecal matters are treated with quicklime. All pens are disinfected twice with commercial 10% formalin. The duration of the pause left between two experiments is about 7 days. The disinfection takes place according to WAMWAYI's recommendation (1989).

Table 1

EXPERIMENTAL PROCEDURES

YEAR	BATCH	VIRAL STRAINS	No OF ANIMALS		CONTACT DURATION	DURATION OF OBSERVATION
			EXPERIMENTALLY INFECTED	CONTACT		
1990	1	SAUDI $VERO_5 / 10^4 DICT_{50}$	1	4	24 HOURS	21 DAYS
	2	SOKOTO $BK_2\text{-}VERO_2 / 10^{3.75} DICT_{50}$	1	4	24 HOURS	21 DAYS
	3	RBT_1 $VERO_5 / 10^4 DICT_{50}$	1	4	72 HOURS	30 DAYS
1991	1	SAUDI $VERO_2 / 10^{3.75} DICT_{50}$	1	4	8 HOURS	21 DAYS
	2	SOKOTO $BK_2\text{-}VERO_2 / 10^{3.75} DICT_{50}$	1	3	48 HOURS	21 DAYS
	3	RBT_1 $BK_8\text{-}P_1\text{-}V_2 / 10^4 DICT_{50}$	1	4	24 HOURS	30 DAYS
1992	1	SAUDI $VERO_2 / 10^{3.75} DICT_{50}$	1	4	8 HOURS	30 DAYS
	2	SOKOTO $BK_2\text{-}VERO_2 / 10^{3.8} DICT_{50}$	1	4	72 HOURS	30 DAYS
	3	RBT_1 $BK_9\text{-}VERO_1 / 10^4 DICT_{50}$	1	4	72 HOURS	30 DAYS

3. RESULTS

3.1 Clinical aspects

Results of clinical observations are listed in Tables 2, 3 and 4. For the Saudi strain (Table 2), the data analysis indicates that, after a contact of 24 hours, 4 bovines out of 4 reacted positively to the infection (100%). All diseased animals died later (100% of mortality).

By reducing the contact duration to 8 hours, the following results are obtained: only one animal (no 382) was subject to a classic form (12.5%) and then died, and 5 other animals only presented hyperthermia varying between 39.5° and 40.2° without any other clinical sign. As for the animal no 379, a minor and temporary hyperthermia (39° - 39.3°) in 3-4 periods was observed. The incubation period lasts between 7 to 16 days for 24 hours of contact and it varies from 2 to 19 days for 8 hours of contact.

As regards the Sokoto strain (Table 3), we see that the infection reaches 50% with an atypical abortive form for the animal no 1089 for a contact of 24 hours. No mortality was recorded. By increasing the duration up to 48 hours, the atypical form limited to a mere and moderate hyperthermia is observed in three animals (100%). For a contact duration of 72 hours, 2 animals out of 4 (50%) reacted positively to the disease with a classic form; no mortality was recorded. The incubation period lasts between 3 and 16 days for a contact of 24 hours; between 6 and 12 days for 48 hours and, finally, it varies between 5 and 12 days for 72 hours of contact.

Over a contact of 72 hours for the RBT/1 strain (Table 4), we observed a typical form of the disease in only one animal (no 383), which corresponds to 12.5%; 25% of bovines only suffered temporarily from a minor hyperthermia in 2-3 periods; no mortality was recorded in the course of those experiments. The incubation period varies between 3 and 24 days. For a contact of 24 hours, 4 animals out of 4 (100%) temporarily suffered from a minor and temporary hyperthermia as in the previous case. This minor hyperthermia is fragmented in 2-3 periods. Except for that, no other clinical sign or mortality was recorded. The incubation period lasts between 4 and 16 days.

Table 2

Influence of contact duration on the transmission of the Saudi strain over an observation period from 21 days (Batches 1 and 2) to 30 days

BATCH	CONTACT DURATION	ANIMAL No	HYPERTHERMIA (*)	INCUBATION PERIOD (days)	CLINICAL SCORE (;10)	SERONEUTRALISATION (**)	MORTALITY
1	24 HOURS	984	40.3 – 41.0°	7	10	1/20	100%
		980	39.3 – 40.5°	10	10	1/5	
		977	40.2 – 41.0°	16	10	1/5	
		974	39.8 – 40.4°	14	10	1/5	
2	8 HOURS	391	39.1 – 40.2°	2	2	0	25%
		388	39.2 – 40.2°	16	2	0	
		382	39.9 – 40.8°	2	10	1/20	
		381	39.0 – 39.5°	2	2	1/20	
3	8 HOURS	377	-	0	0	0	0%
		379	39.0 – 39.2 ; 39.0 – 39.1 ; 39.0 – 39.1 ; 39.0°	1 ; 10 ; 19 ; 25	2	0	
		481	-	-	0	0	
		378	39.0°	19	2	0	

(*) Minimum – maximum

(**) Maximum titre before death

Table 3

Influence of contact duration on the transmission of the SOKOTO strain over an observation period from 21 days (Batches 1 and 2) to 30 days

BATCH	CONTACT DURATION	ANIMAL No	HYPERTHERMIA (*)	INCUBATION PERIOD (days)	CLINICAL SCORE (;10)	SERONEUTRALISATION (**)	MORTALITY
1	24 HOURS	283	–	-	-	-	0%
		1089	39.5 – 41.3°	3	2	1/320	
		1088	–	-	-	0	
		1031	39.8 – 41.2°	16	10	1/40	
2	48 HOURS	976	39.1 – 39.3°	12	2	1/5	0%
		1029	39.1°	6	2	1/5	
		1791	39.0°	10	2	0	
3	72 HOURS	398	39.0 – 41.2°	5	10	1/160	0%
		396	39.3 – 41.1°	12	8	1/320	
		482	-	-	-	0	
		400	-	-	-	0	

(*) Minimum – maximum

(**) Maximum titre before death

Table 4

Influence of contact duration on the transmission of the RBT_1 strain over an observation period of 30 days

BATCH	CONTACT DURATION	ANIMAL No	HYPERTHERMIA (*)	INCUBATION PERIOD (days)	CLINICAL SCORE (;10)	SERONEUTRALISATION (**)	MORTALITY
1	72 HOURS	1039	-	-	-	0	0%
		985	-	-	-	0	
		971	39.1 – 39.5 ; 39.1 - 39.5 ; 39.4°	3 ; 10 ; 24	2	0	
		983	-	-	-	1/5	
2	24 HOURS	372	39.0 ; 39.2 – 40.0 ; 39.1°	7 ; 10 ; 17	2	0	0%
		371	39.2 ; 39.0 – 39.5 ; 39.1 – 39.2 ; 39.4 – 39.8°	4 ; 7 ; 11 ; 16	2	0	
		365	39.0 – 39.4°	16	2	0	
		364	39.0 ; 39.2°	7 ; 16	2	0	
3	72 HOURS	363	-	-	-	1/10	0%
		387	-	-	-	0	
		383	39.8 – 40.4°	7	8	1/320	
		389	39.0 – 39.2 ; 39.2°	4 ; 7	2	0	

(*) Minimum – maximum

(**) Maximum titre before death

Table 5 gives us observation results of clinical signs after subcutaneous inoculation of the three different strains abovementioned. The analysis shows that all experimentally infected animals showed characteristic clinical signs of rinderpest. On average, the incubation period is 3.6 days for the Saudi strain; 4 days for the Sokoto strain and 5 days for the RBT_1 strain. The duration of pyrexia varies between 6 and 18 days (11.3 on average) for the RBT_1 strain; 3 to 8 days for the Sokoto strain (5.3 days on average); it is not much variable as regards the Saudi strain (5-6 days or 5.3 on average). The recorded mortality is 100% for the Saudi strain; 33.3% for both Sokoto and RBT_1 strains.

The minimum duration of pyrexia is 5 days for the Saudi strain; 3 days for the Sokoto and 4 for the RBT_1. This data is only valid for animals which presented the classic form of the disease (Table 6). As for animals which suffered from an atypical form, this period only lasted 1-2 days. Clinical observations showed that, for the three strains, buccal lesions and diarrhea appeared almost at the same time; either one day before or a few days the one after the other. The Sokoto strain did not cause diarrhea in a contact animal (animal no 1089); the animal no 996 experimentally infected with the RBT_1 strain did not have diarrhea. Tear production were absent in the animals no 1098 (Sokoto) and no 383 (RBT_1). In general, the congestion of ocular and nasal mucous membranes accompanied by serous and then mucopurulent discharge is observed after 2-3 days after the thermic reaction (see Temperature sheet, Table 6). Nasal lesions slightly appeared before or simultaneously with that of the buccal cavity.

3.2 Serological aspect

The serology of sera collected during the experiments provides the following results: whatever the strain's origin and the transmission mode of the virus, all animals which reacted positively to the infection (classic form) usually had sustained a seroconversion between 7 and 8 after inoculation. Some animals suffering from an atypical form (no 381, 976 and 1029) were seropositive at the end of the observation. But the titres of those serical antibodies are relatively low and stable at least for this experimental period (1/5 and 1/10). Among animals which only presented a moderate and temporary hyperthermia without any other clinical sign, 10/36 animals (27.7%) always remained seronegative. Faced with this situation, a virulence test was carried out for all animals in this position. This way, it was observed that all animals of which sera are positive with 20% and 1/10 resisted the test.

Table 5

Compared study of three rinderpest virus's strains of variable virulence in sensitive animals (subcutaneous inoculation)

STRAINS	ANIMAL No	INCUBATION PERIOD	CHARACTERISTIC SYMPTOMS OF RINDERPEST					DECEASED	SLAUGHTERED
			TEMPERATURE	DISCHARGE	TEAR PRODUCTION	BUCCAL LESIONS	DIARRHEA		
SAUDI	959	4	40.3 – 40.4°	+	+	+	+	+	-
	390	3	39.2 – 39.7°	+	+	+	+	+	-
	476	4	39.6 – 40.5°	+	+	+	+	+	-
SOKOTO	1098	5	39.4 – 40.4°	+	-	+	+	-	+
	975	4	39.4 – 39.7°	+	+	+	+	+	-
	392	3	39.3 – 40.7°	+	+	+	+	-	+
RBT_1	996	6	39.4 – 40.4°	+	+	+	+	-	+
	361	5	39.3 – 40.4°	+	+	+	+	-	+
	490	4	39.2 – 40.7°	+	+	+	+	+	-

On the other hand, those with negative sera reacted, indeed, positively to reinoculation. The duration of the incubation period for this phase is relatively stable (3-4 days).

For the Saudi strain, 2 out of 8 bullocks (25%) had experienced a seroconversion after a contact of 8 hours and 100% of them if the contact duration is 24 hours. As for the Sokoto strain, it resulted in 50% of seropositive animals for contact durations of 24 and 72 hours and 66.6% over 48 hours. As regards the RBT_1 strain, results are as follows: if no seroconversion is observed after 24 hours of contact, it reaches 37.5% after 72 hours.

Results of the serological kinetic study are shown in Table 7. Over the 21 and 30 days of observation which followed the infection through direct contact, serical antibodies were generally not subject to a major variation. However, it has to be said that there is progression in the production of antibodies in animals infected with the Sokoto and RBT_1 strains; this shows especially in animals which suffer from a classic form of rinderpest.

Table 8 shows results of the ELISA competition test. It can be seen that all sera positive in seroneutralisation beyond the dilution of 1/20 are also positive in ELISA. Although the clinical score is 10 in seven animals, their sera remained negative in ELISA but positive in seroneutralisation and all those animals died as a result of the disease (after observation with the Saudi strain).

3.3 Epidemiological aspect

Based on clinical and serological results, we see that, with the Saudi strain, the transmission reaches 100% after a contact of 24 hours and 25% if the duration is reduced to 8 hours. With the Sokoto strain, the observed variation is low: the infection is 50% after a contact lasting between 24 and 72 hours, then 66.6% for 48 hours. As for the RBT_1 strain, still in the context, if no viral transmission took place after a contact of 24 hours, it reaches 37.5 after 72 hours of contact.
The simplified descriptive model summarising all fundamental characteristics of rinderpest's evolution is presented in Figure 1. Nevertheless, in the course of our experiments, the obtained results led to another descriptive model where the evolutive cycle of the disease is considerably reduced. It starts from the latent period to the immune animal.

Table 6

Relationship between the apparition of neutralising antibodies and clinical signs

STRAIN	No ANIMAL	INCUBATION (days)	PYREXIA (days)	DAYS AFTER THE BEGINNING OF HYPERTHERMIA				SEROPOSITIVITY	
				DISCHARGE	TEAR PRODUCTION	BUCCAL LESIONS	DIARRHEA	DILUTION	P. I. D.
SAUDI	974	14	5	2	3	3	3	1/5	13
	977	16	5	2	2	2	2	1/5	16
	980	10	6	2	1	2	3	1/5	10
	984	7	6	1	1	4	4	1/10	10
	959 e. i.	4	5	1	3	2	3	1/10	9
	476 e. i.	4	5	3	2	3	2	ND	-
	390 e. i.	3	6	2	4	4	2	1/20	6
	381	2	2 : 2	0	0	0	0	1/10	21
	382	2	8	5	1	5	6	1/20	9
SOKOTO	1031	16	4	2	2	4	3	1/5	19
	976	12	2	0	0	0	0	1/5	14
	396	12	8	2	0	4	4	1/40	18
	1029	6	1	0	0	0	0	1/5	14
	398	5	10	4	7	5	5	1/40	18
	1098 e. i.	5	5	3	0	7	4	1/80	8
	975 e. i.	4	3	1	3	4	3	ND	-
	392 e. i.	3	8	3	5	3	4	1/160	12
	1089	3	10	5	0	0	0	1/160	10
RBT_1	383	7	4	2	0	4	3	1/10	12
	996 e. i.	6	18	4	6	5	0	1/5	10
	361	5	13	3	4	4	6	1/80	9
	490	4	6	2	4	4	5	1/80	10
	363	0	0	0	0	0	0	1/10	15
	983	0	0	0	0	0	0	1/2	21

e. i. = experimentally infected animals

ND = not done

P. I. D. = post-infection days

Table 7 KINETICS OF ANTIBODIES PRODUCTION

STRAIN	CONTACT DURATION	No ANIMAL	DATE OF BLOOD SAMPLING AFTER CONTACT											
			D + 3	D + 6	D + 9	D + 12	D + 15	D + 18	D + 21	D + 24	D + 27	D + 30	D + 33	D + 36
SAUDI 1990	24 HOURS	959 e. i.	ND	ND										
		984	0	0	1/10	1/20	-	-	-					
		974	0	0	1/5	1/5	1/5	1/5	-					
		977	-	0	1/5	1/2	1/5	1/5	-					
		980	0	0	1/5	1/5	-	-	-					
SAUDI 1991	8 HOURS	390 e. i.	ND	1/20		-	-	-	-					
		382	0	0	1/20	-	-	-	-					
		381	0	0	0	0	0	0	1/10					
		388 / 391	0	0	0	0	0	0	0					
SAUDI 1992	8 HOURS	476 e. i.	ND	ND	ND	-	-	-	-	-	-	0		
		377/379/378/481	0	0	0	0	0	0	0	0	0	0		
SOKOTO 1990	24 HOURS	1098 e. i.	ND	1/80	1/80	1/160	1/160	1/160	1/160	1/160				
		1089	0	0	1/160	1/320	1/80	1/80	1/160					
		1031	0	0	0	0	0	1/10	1/40					
		283	0	0	0	0	0	0	0					
		1088	0	0	0	0	0	0	0					
SOKOTO 1991	48 HOURS	975 e. i.	-											
		976	0	0	0	1/5	1/5	1/5	1/5					
		1029	0	0	0	1/5	1/5	1/5	1/5					
		1791	0	0	0	0	0	0	0					
SOKOTO 1992	72 HOURS	392 e. i.	ND	ND	ND	1/160	1/160	1/160	1/160	1/80	1/80	1/80	1/160	1/160
		396	0	0	0	ND	1/40	1/160	1/320	1/160	1/320	1/320		
		398	0	0	ND	0	1/40	1/160	1/160	1/80	1/160	1/160		
		400 / 482	0	0	0	0	0	0	0	0	0	0		
RBT 1 1990	72 HOURS	996 e. i.	ND	ND	1/5	1/5	1/5	1/5	1/5	1/5	1/10	1/10	1/20	1/20
		983						1/2	1/5	1/5	1/5	1/5		
		985 / 971 / 1039	0	0	0	0	0	0	0	0	0	0		
RBT 1 1991	24 HOURS	361 e. i.	ND	ND	1/180	1/160	1/320	1/160	1/80	1/80	1/80	1/80	1/80	1/80
		364 / 371	0	0	0	0	0	0	0	0	0	0		
		372 / 365	0	0	0	0	0	0	0	0	0	0		
RBT 1 1992	72 HOURS	490 e. i.	ND	ND	1/80	1/160	-	-	-	-	-	-		
		383	0	0	1/10	1/80	1/80	1/160	1/160	1/160	1/320	1/320		
		363	0	0	0	1/10	1/10	1/10	1/10	1/10	1/10	1/10		
		387 / 389	0	0	0	0	0	0	0	0	0	0		

e. i. = experimentally infected 0 = seronegative (-) = deceased animal

Table 8 Comparative results between clinical score, seroneutralisation and ELISA competition

STRAIN	YEAR	No ANIMAL	CLINICAL SCORE	SEROLOGY	
				SN positive at dilution	ELISA (dilution at 1/5)
SAUDI	1990	984	10	1/20	-
		980	10	1/5	-
		977	10	1/5	-
		974	10	1/5	-
		959 i	10	1/10	-
	1991	391	0	-	-
		388	0	-	-
		382	10	1/20	-
		381	2	1/10	-
		390 i	10	1/20	-
	1992	377	0	-	-
		379	2	-	-
		378	2	-	-
		481	0	-	-
		476 i	0	deceased before BS	deceased before BS
SOKOTO	1990	283	0	-	-
		1089	2	1/320	+
		1088	0	-	-
		1031	10	1/40	+
		1098 e. i.	10	1/160	+
	1991	976	2	1/5	-
		1029	2	1/5	-
		1791	2	-	-
		975 e. i.	10	deceased before BS	deceased before BS
	1992	398	10	1/160	+
		396	8	1/320	+
		482	0	-	-
		400	0	-	-
		392 e. i.	10	1/160	+
RBT 1	1990	1039	0	-	-
		985	0	-	-
		971	2	-	-
		983	0	1/5	-
		996 e. i.	10	1/20	+
	1991	372	2	-	-
		371	2	-	-
		365	2	-	-
		364	2	-	-
		361 e. i.	10	1/320	+
	1992	363	0	1/10	-
		387	0	-	-
		383	8	1/320	+
		389	2	-	-
		390 e. i.	10	1/160	+

e. i. = experimentally inoculated BS = blood sampling SN=seroneutralisation

EVOLUTIVE CYCLES OF RINDERPEST ACCORDING TO THE TRANSMISSION MODE AND THE VIRULENCE OF VIRAL STRAINS

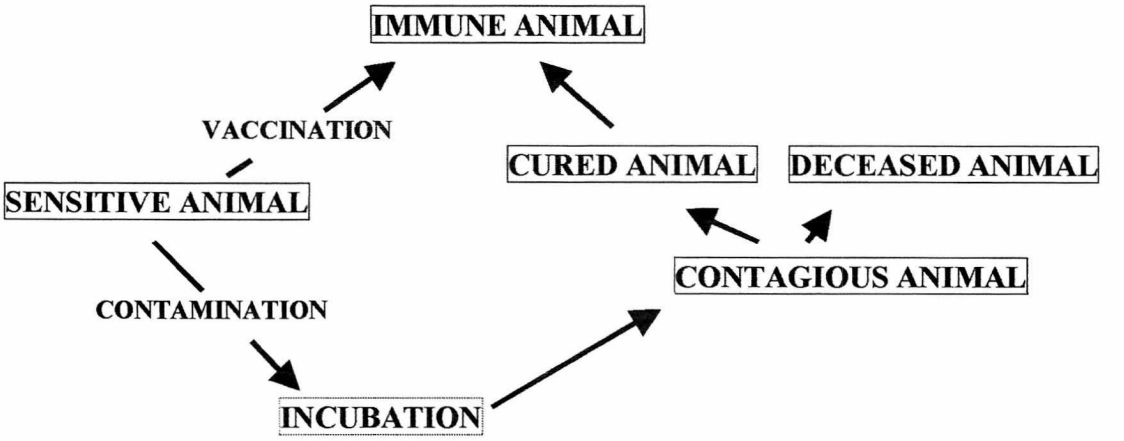


Fig. 1 **Long cycle (normal virulence or hypervirulence)**
*** contamination takes place by inoculation**
*** contact duration is extended**

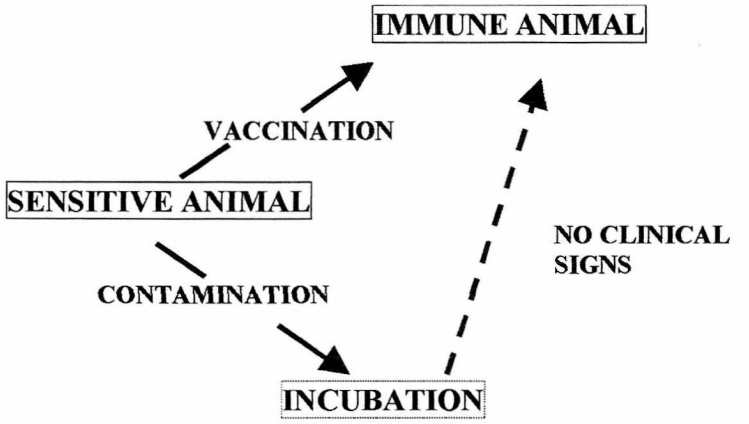


Fig. 2 **Short cycle (low virulence)**
*** contamination through direct contact**

4. ANALYSE ET CONCLUSION

Under our experimental conditions, we observed great irregularity in the transmission of rinderpest virus, whatever the contact duration, except for the Saudi strain for which the infection reaches 100% after 24 hours of close contact. The same observations were made by COOPER (1932), BROTHERSTON (1951), PLOWRIGHT (1952), MACOWAN (1956), ROBSON et al. (1959), PLOWRIGHT et al. (1959), TAYLOR et al. (1965) and RWEYEMANU et al. (1974). In this respect, JACOTOT (1935) writes that it is impossible to draw accurate conclusions on the reaction of animals exposed to contagion because, in this case, the number of virulent units which come effectively into play is very variable. Indeed, several factors can cause this irregularity:

- the receptivity or natural resistance of animals,
- the level of pathogenecity of used viral strains,
- the contact duration between sensitive and diseased animals which are thus excreting the virus,
- the condition of the animal transmitting the virus at the moment of the first contact,
- the number of virulent units which come effectively into play.

As for experimentally infected animals, the mortality rate (100% for the Saudi strain and 33.3% for the Sokoto strain) recorded in the course of the experiments corroborates the rate obtained by other researchers cited by TAYLOR (1986). The observed difference between the mortality rate with the RBT_1 strain (33.3%) and that mentioned by TAYLOR (1986), 0 to 5%, can be explained by the variations of genetic resistance on one hand and the limited number of animals used in our experiment on the other hand.

Contrarily to expected results with the Saudi strain which usually kills animals before they reached the stage of diarrhea, all animals, experimentally infected as well as those infected through contact (which reacted positively), died after showing all clinical signs and often over 2 to 3 days.

Durations of incubation periods observed in experimentally infected animals confirm findings by MAURER et al. (1955), SCOTT and BROCON (1961), LIESS and PLOWRIGHT (1964) who noted that, under experimental conditions, the incubation period lasts 2 to 3 days, whereas it

varies between 3 and 9 days under natural conditions. On the other hand, in contact animals, the variation in the incubation period is very high; our results do not differ much from those obtained by the abovementioned researchers and are similar to those observed by PLOWRIGHT et al. (1959). In our opinion, the variation in the incubation period under those working conditions depends on the virus's pathogenic power and the quantity of virions excreted by the diseased animal, on the receptivity or natural resistance of contact animals and, finally, on the contacts' intensity. Furthermore, LIESS and PLOWRIGHT (1964) mentioned that the incubation period varies according to the transmission mode: after subcutaneous inoculation, it lasted 3 to 5 days for 8 animals; after intranasal inoculation, from 3 to 4 days for 6 animals and, after close contact, this period varies between 8 and 11 days. For MACADAM (1968), the incubation period rather depends on the viral dose whatever the way of inoculation.

During our experiments, three forms of the disease were observed: the characteristic typical classic form of rinderpest; the atypical abortive form and the asymptomatic subclinical form. The classic form is obtained, above all, with the hypervirulent Saudi strain in 41.66% of the cases against 27.27% with the Sokoto strain and 8.33% with the RBT_1. However, the corrected calculation of chi^2 (alpha = 5% and ddl = 1) shows that there is no difference statistically significant between Saudi strains and RBT_1 ($chi^2 = 2$), Sokoto and RBT_1 ($chi^2 = 0.43$), Saudi and Sokoto ($chi^2 = 0.08$). This contradiction can be explained by the fact that the experiment only involved a small number of animals.

The asymptomatic form was only obtained with the RBT_1 (16.66%). Animals only presenting hyperthermia without any other clinical sign or seroconversion cannot be considered as animals suffering from rinderpest. In other respects, TAYLOR (1986) reports that, in the course of an experiment with the Egypt/82 strain and during pyrexia of normal duration, the disease can be clinically diagnosed. As a result, a high reduction of viral antigen in tears makes the diagnosis difficult to confirm. It is this way that hypervirulent strains circulate undetected in cattle populations.

RECEVEUR quoted by PROVOST (1958) notes that, in Chad, the thermic reaction can never be used to appreciate the organic reaction to rinderpest virus. Besides, RECEVEUR (1938) had already signalled that atypical forms of rinderpest are the most common in Chad.

Animals that are vaccinated and then infected, naturally or experimentally, by rinderpest virus resist the disease more or less whatever the strain. On the other hand, during a virulent experience, the organism's reaction depends on the number of virulent units involved.

All experimentally infected animals sustained a seroconversion, except for animals no 476 and 975 which died before the first blood sampling. Our serological results are similar to those of PLOWRIGHT (1962), SINGH et al. (1967) who noted that after infection by virulent strains, the neutralising antibodies appear in the blood from the fourth to the sixth day after the thermic reaction and increase the titre to reach the maximum from the twelfth to the fourteenth day of the disease. Nevertheless, we observed that in animals which presented a subclinical form of the disease, antibodies' titres are very low and stable. This phenomenon can be linked to the dose-effect relationship. Those results corroborate those of PLOWRIGHT et al. (1959, 1962) which demonstrated that, with an attenuated strain of rinderpest virus, a small dose cannot induce a high titre of neutralising antibodies.

To conclude, we can think that the infection level depends on the dose-effect relationship which, in turn, depends on the contacts' intensity.

The serological study revealed that the evolutive cycle of rinderpest is considerably reduced when the contact duration is insufficient to ensure normal transmission of the virus from an excreting animal to other sensitive animals. In this case, we go straight from the latent period to the immune condition of the animal.

This experiment clearly showed that transmission speed and pathogenecity of viral strains are two different parameters.

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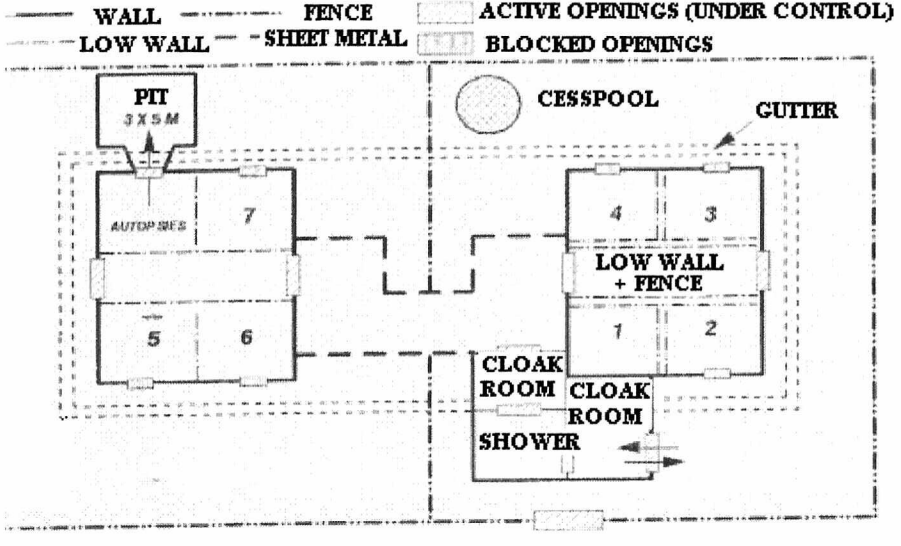
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SKETCH OF EXPERIMENTAL STABLES *ANNEX 6.1.*



LRVZ/FARCHA

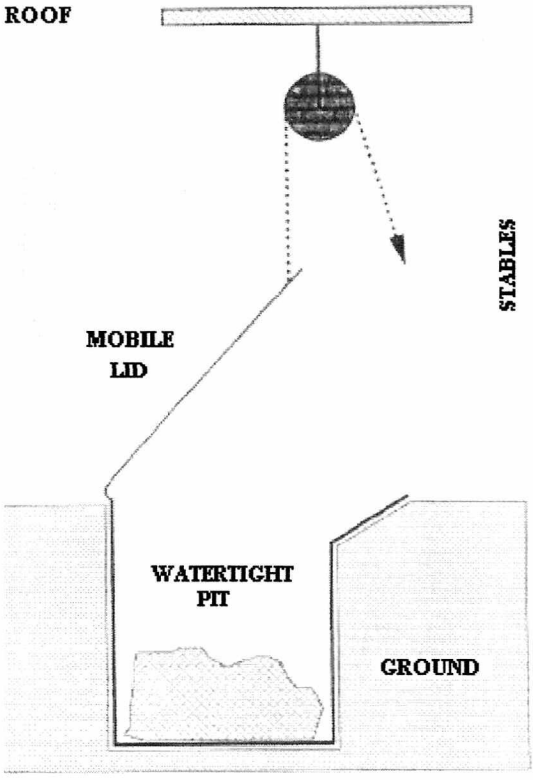
ANNEX 6.2

ANIMAL No ☐ **YEAR** ☐

INOCULATED ☐ **CONTACT** ☐ **STRAIN** ☐

INDIVIDUAL SHEET

DATE	D1	D2	D3	D4	D5	D6	D7	D8	D9	
TEMPERATURE										
GENERAL CONDITION: NORMAL SLAUGHTERED LYING										
APETITE: NORMAL REDUCED NONE										
OCULAR MUCOUS MEMBRANE: NORMAL CONGESTIONNED										
TEAR PRODUCTION										
DISCHARGE: SEROUS MUCOPURULENT										
SALIVATION										
BUCCAL LESIONS										
COUGH										
FAECES: NORMAL CONSTIPATION										
DIARRHEA: MINOR PROFUSE BLOOD MUCUS										
DECEASED: NATURALLY SLAUGHTERED										



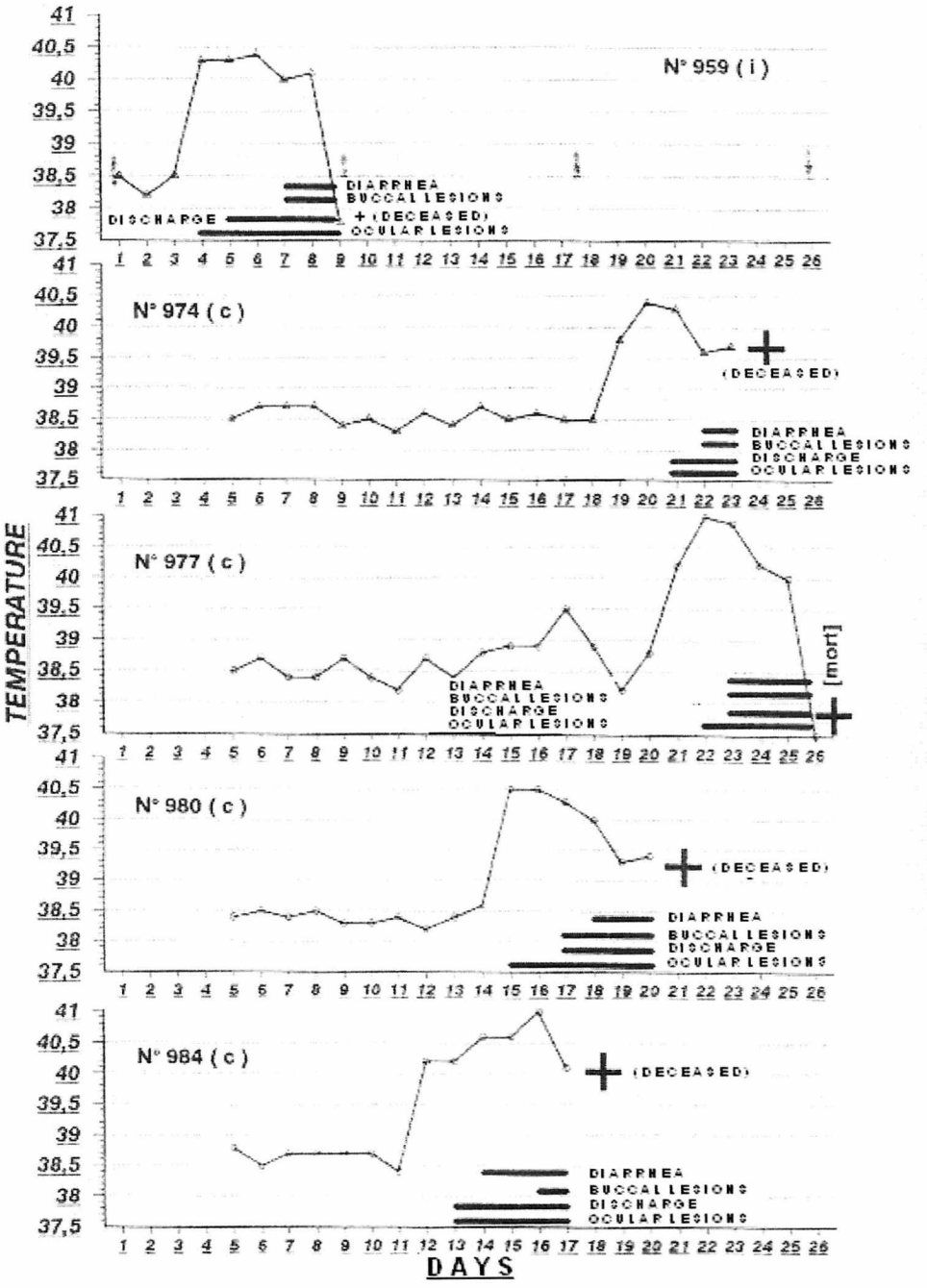
SKETCH OF DECONTAMINATON PIT

LRVZ/FARCHA RINDERPEST

RELATIONSHIP TEMPERATURE / OTHER CLINICAL SIGNS

****SAUDI STRAIN/90****

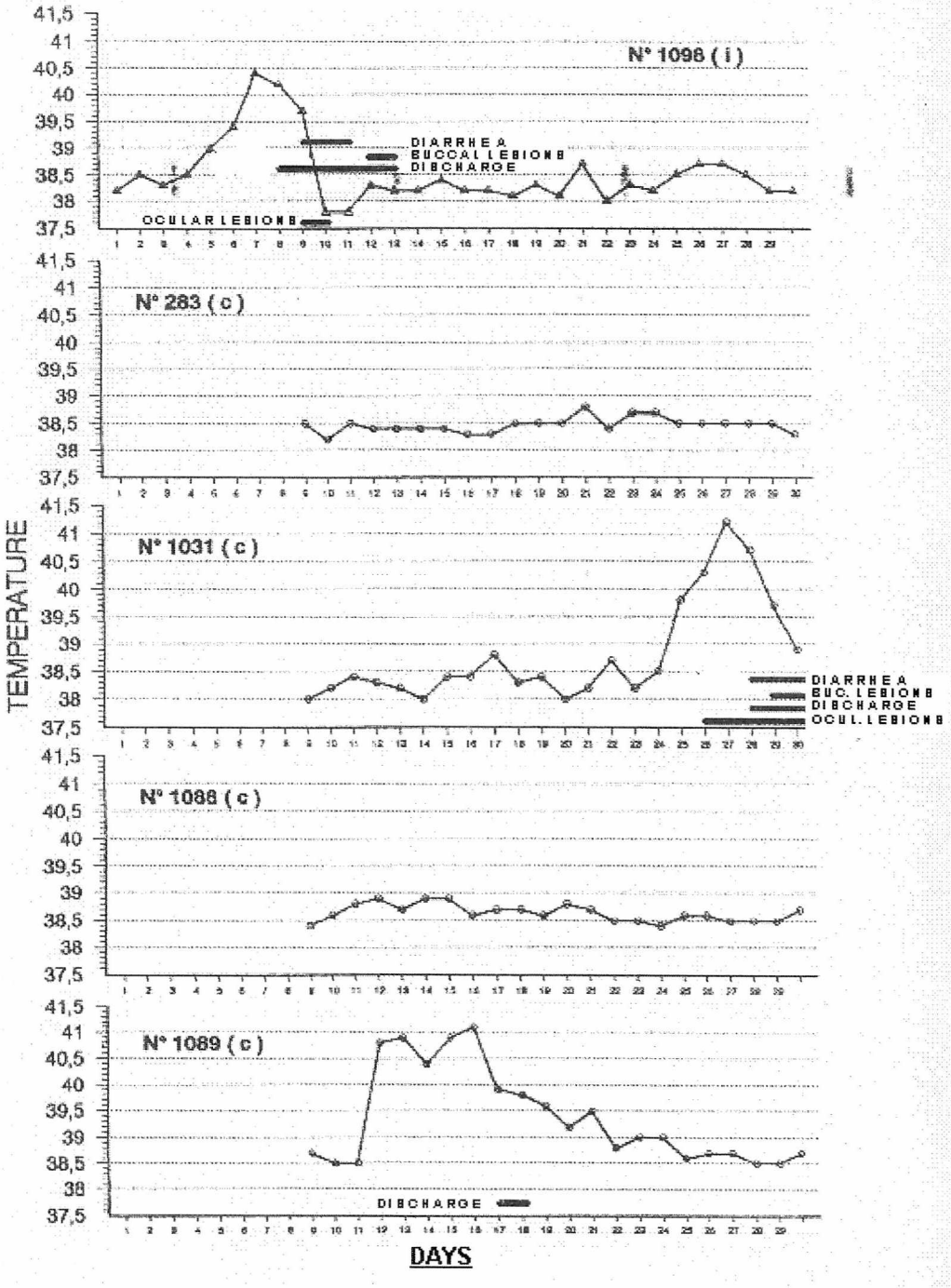
ANNEXE 6.4
[9 pages]



LRVZ / FARCHA RINDERPEST

RELATIONSHIP TEMPERATURE - OTHER CLINICAL SIGNS

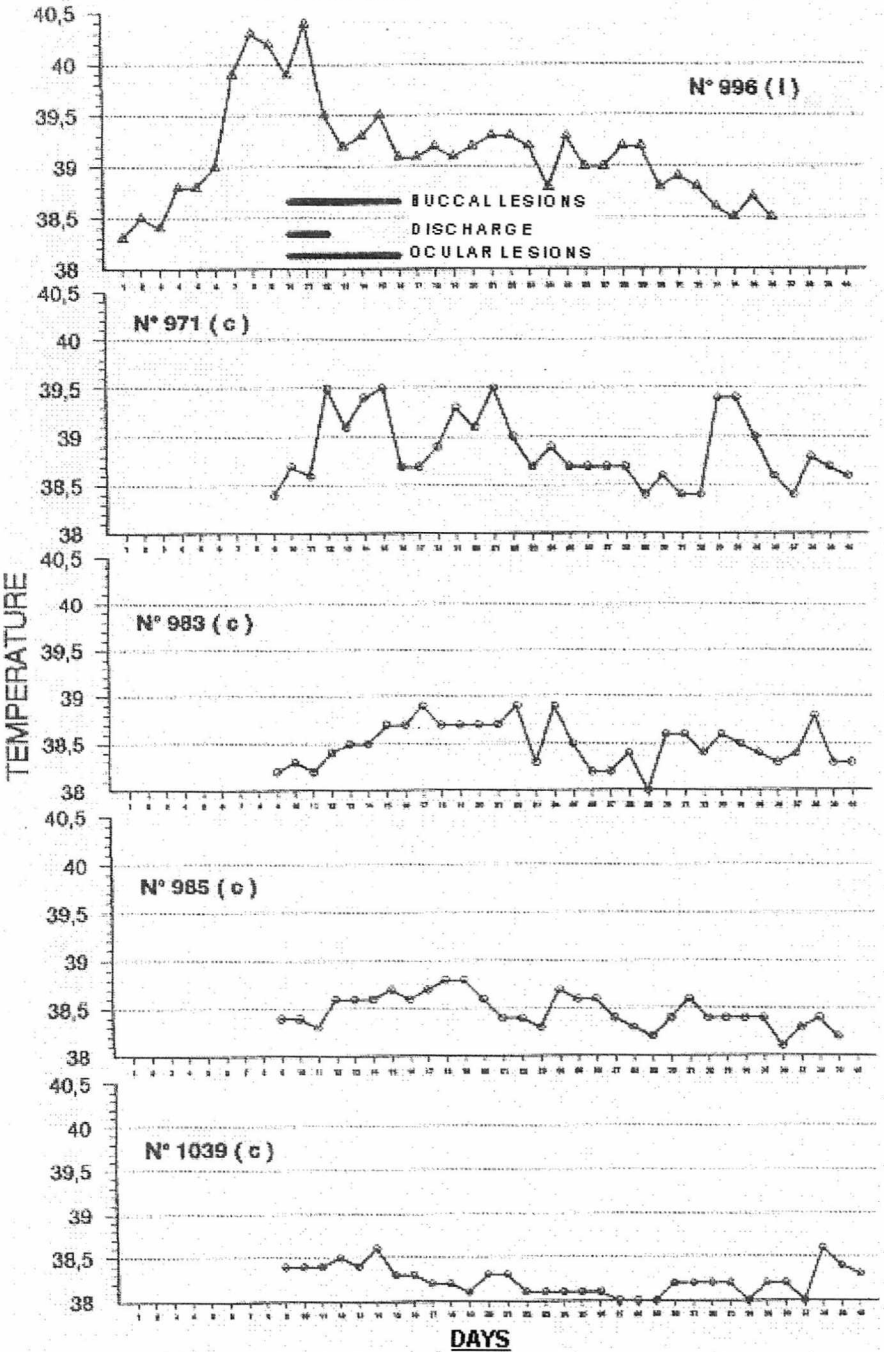
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LRVZ / FARCHA RINDERPEST

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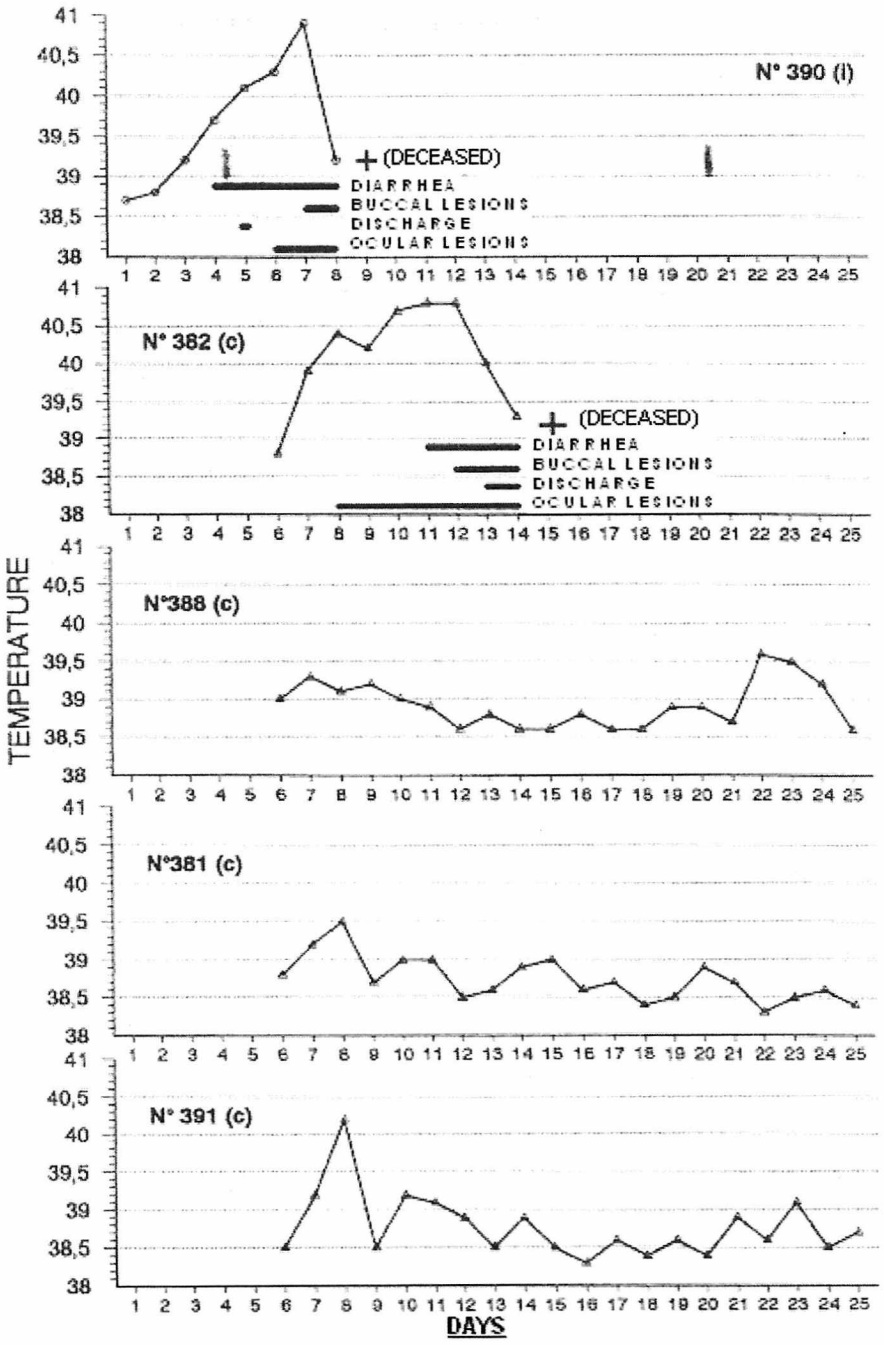
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LRVZ / FARCHA RINDERPEST

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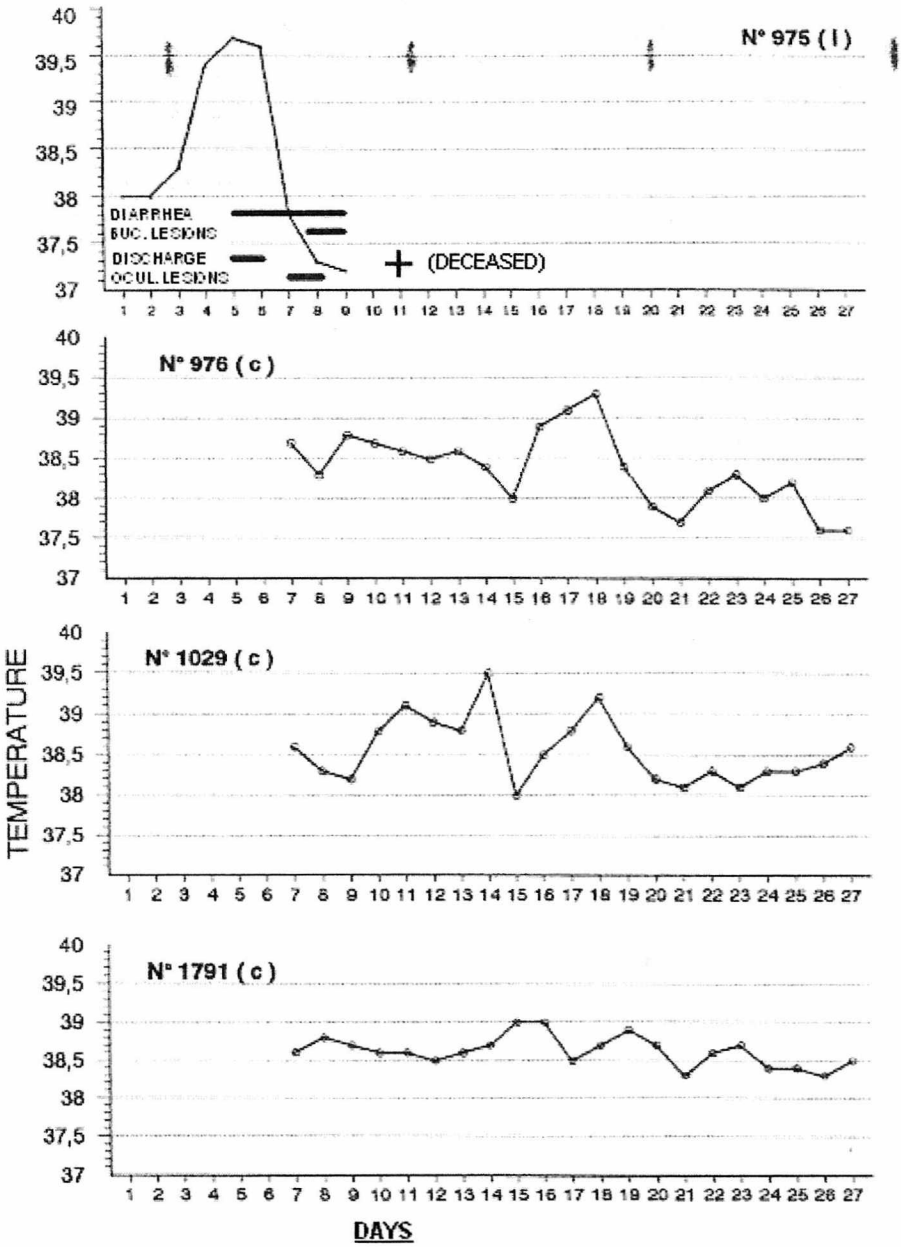
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LRVZ / FARCHA RINDERPEST

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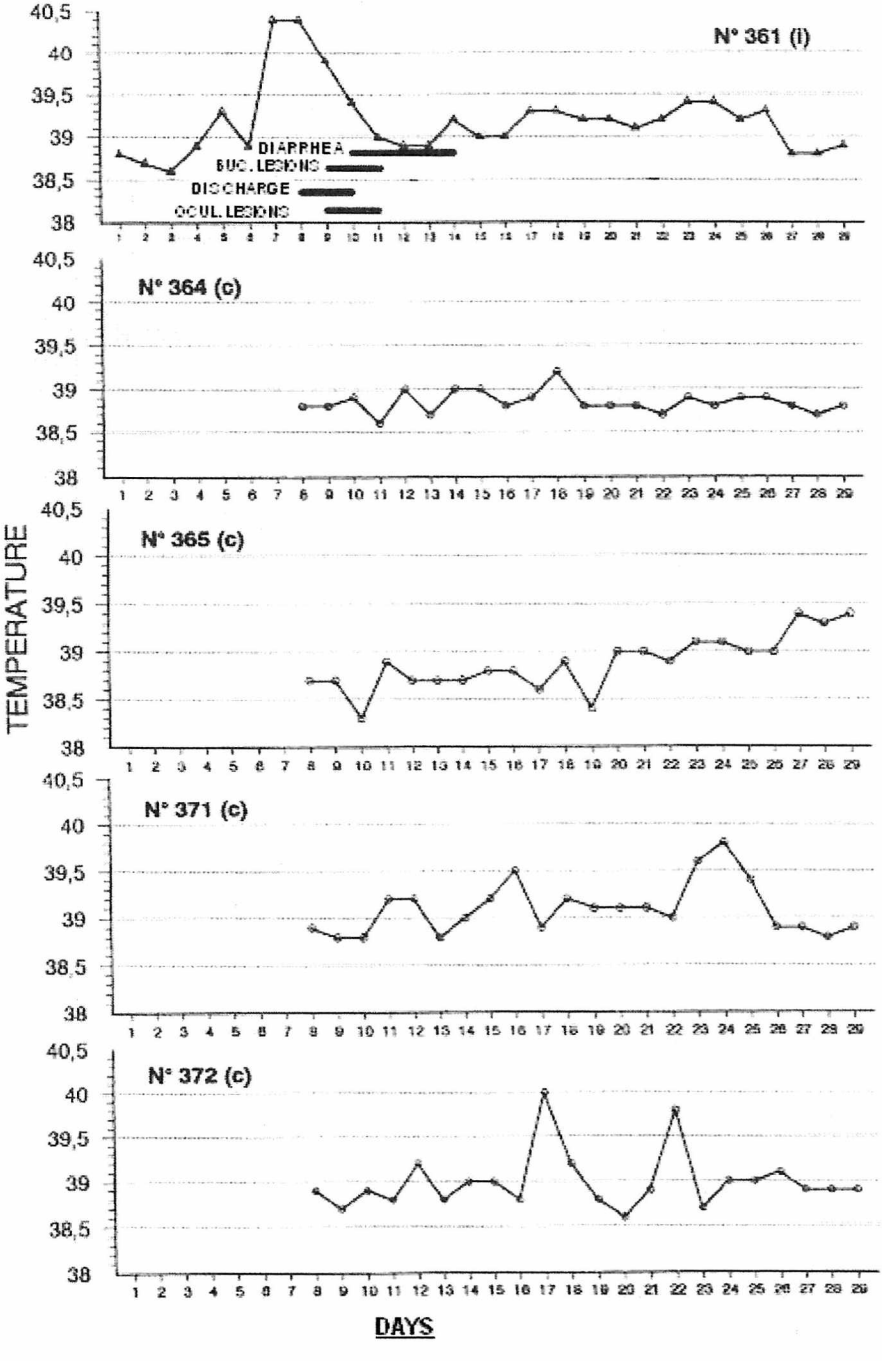
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LRVZ RINDERPEST

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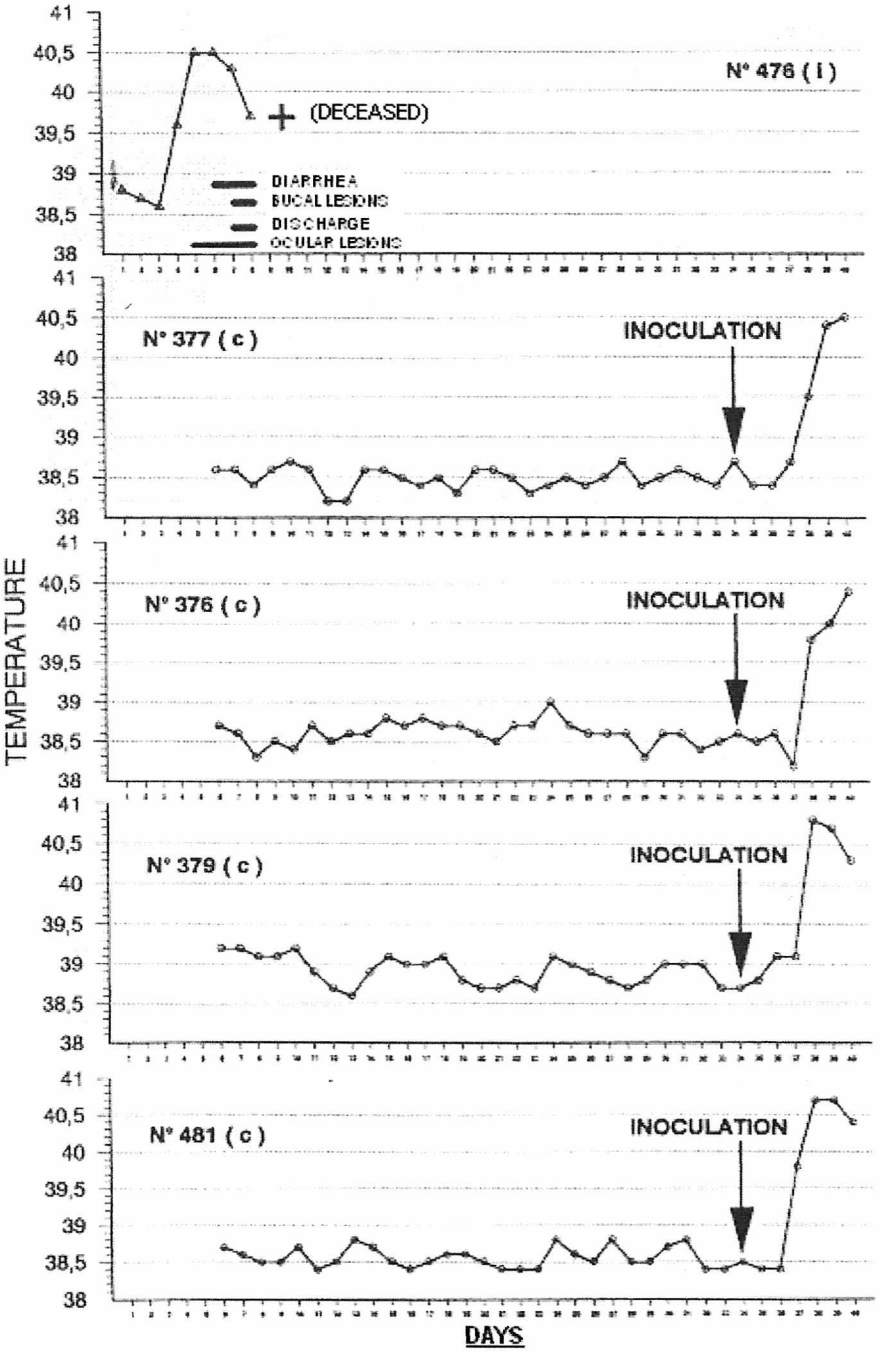
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LRVZ RINDERPEST

RELATIONSHIP TEMPERATURE - OTHER CLINICAL SIGNS

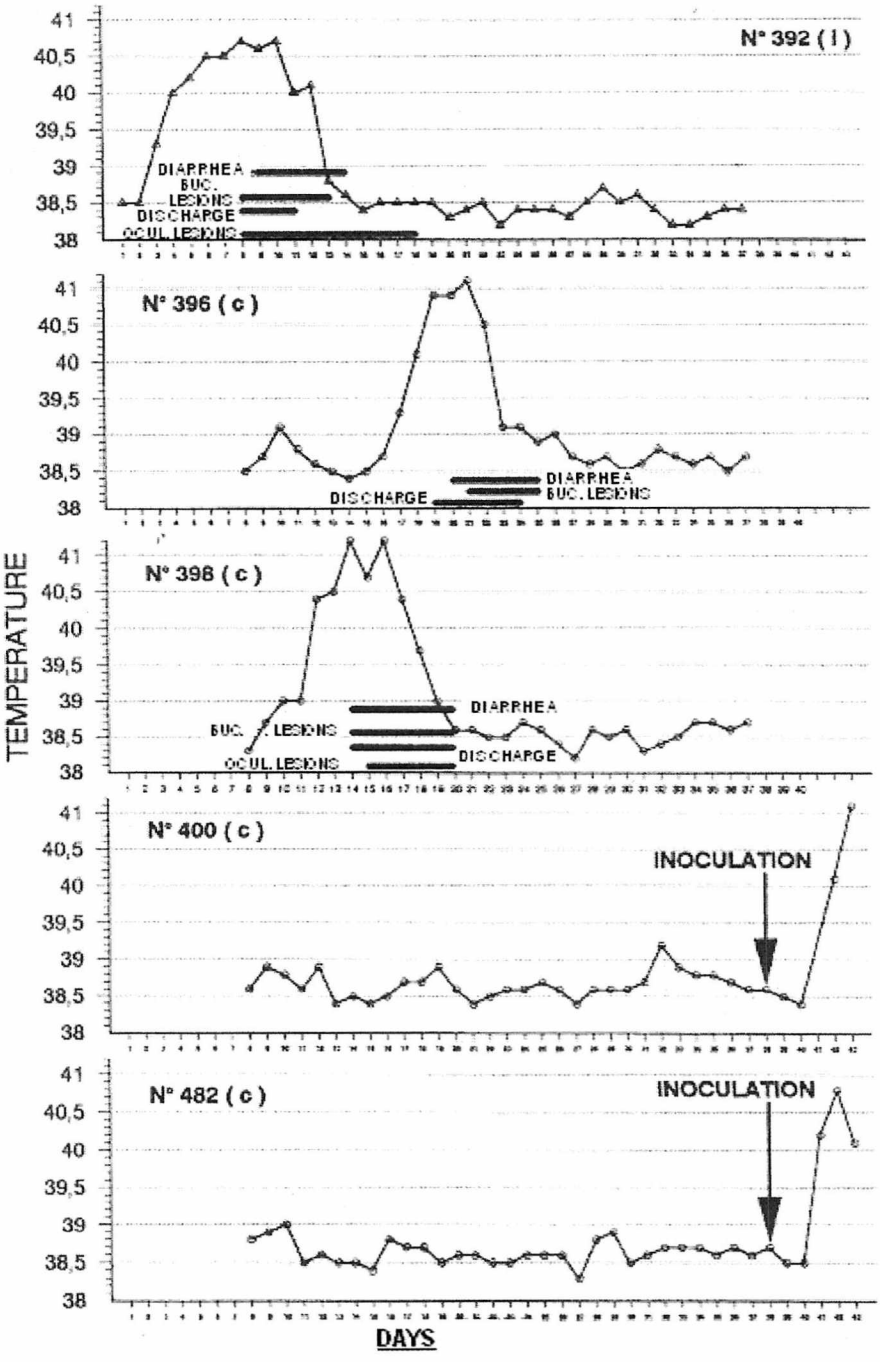
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LRVZ / FARCHA RINDERPEST

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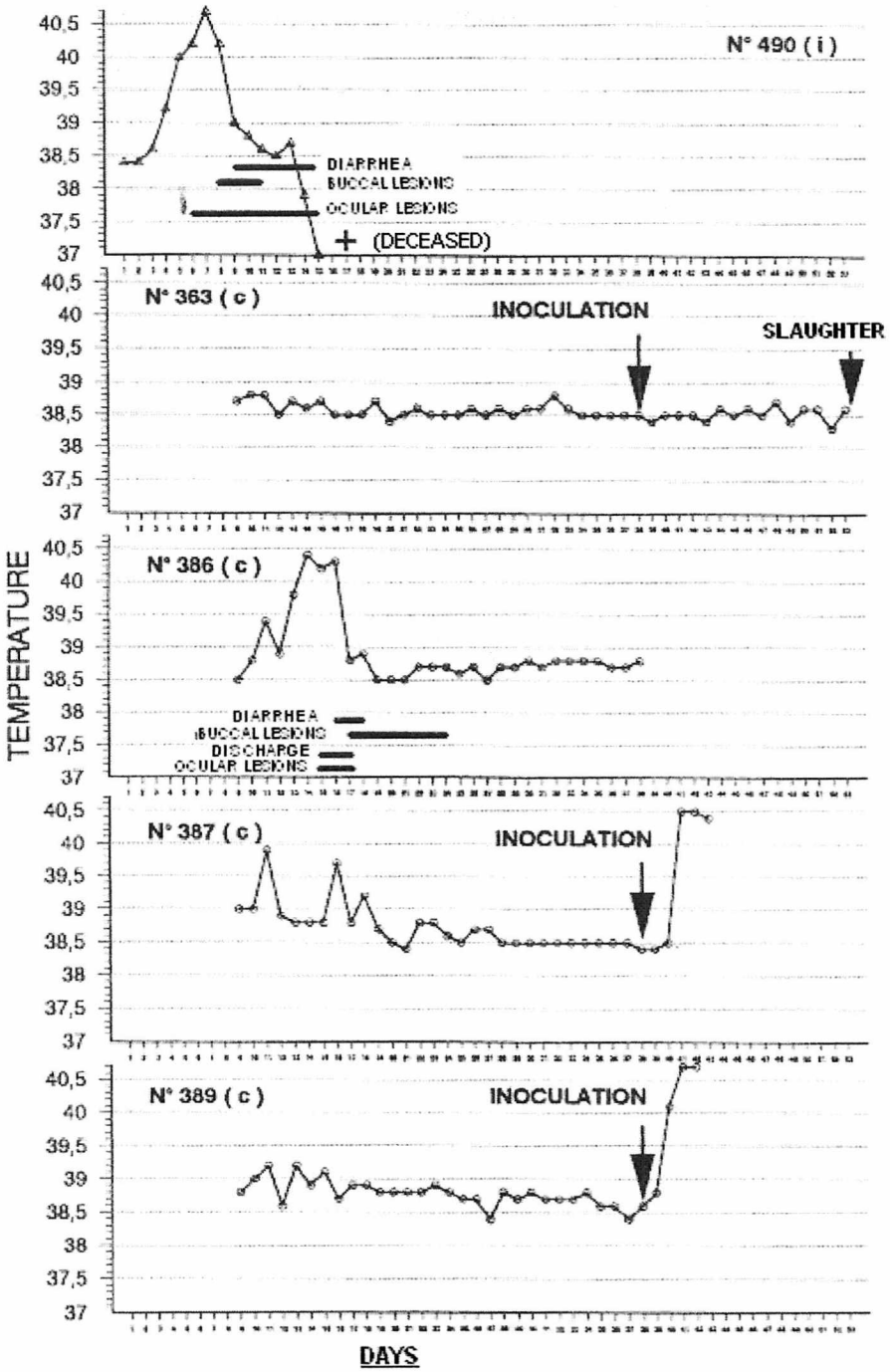
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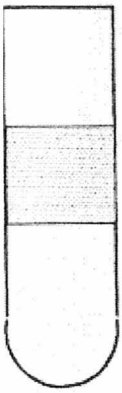
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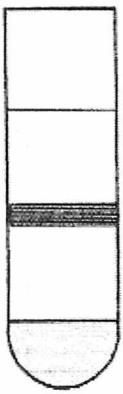


TECHNIQUE FOR OBTAINING LYMPHOCUTES
(according to PHARMACIA LKB)



BLOOD

FICOLL-HYPAQUE@



PLASMA

LYMPHOCYTIC LAYER

FICOLL-HYPAQUE@

GRANULOCYTES
ERYTHROCYTES

LYMPHOCYTES

