ALLEGATO 8)

G. CAPORALE

Teramo, li 15 APR. 2002

Registered mail.

SETTORE General Affairs and Personnel

PROTOCOLLO: 4773

Commission of the European Communities
Health and Consumer Protection
Directorate-General
Rue de la Loi 200
B-1049 Brussels
For the attention of Dr. Bernard van Goethem

COPIA

European Commission
Health and Consumer Protection
Directorate
Financial Sector - B232 5/74
Rue de la Loi 200
B-1049 Brussels
For the attention of Ms. Guido De Clercq

OBJECT: contract relating to "*safety and potency testing of Bluetongue vaccines*".

Please find enclosed 2 copies of the final report of the above mentioned project. The results of the research have already been showed at the Health and Consumer Protection Standing Veterinary Committee on January 8th 2002

An invoice for the allocated amount of € 37.299,40 is also enclosed.
The sum should be paid to:
Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise
Account n. 900010
Bank: Cassa di Risparmio di Ascoli Piceno S.p.a.
Bank's address: P.zza Garibaldi, Teramo
cod. CAB 15300
cod. ABI 6080.

Kind regards,

IL DIRETTORE
Vincenzo Caporale

EDP/vmm

Enclos. n. 3

Sezioni: Avezzano: Contrada Caruscino - Tel. e Fax 0863 59177 • Isernia: Contrada Breccelle - Tel. e Fax 0865 41[illegible]
• Pescara: Via Raiale, 118/2 - Tel. e Fax 085 54306 • Campobasso: Via Garibaldi, 155 - Tel. e Fax 0874 31[illegible]
• Termoli (Rtica): Via L. Pilla, 26 - Tel. e Fax 0875 81343 • Lanciano: Viale S. Antonio, 8 - Tel. e Fax 0872 71[illegible]

ISTITUTO ZOOPROFILATTICO SPERIMENTALE
DELL'ABRUZZO E DEL MOLISE
"G.CAPORALE"
TERAMO

SAFETY AND POTENCY TESTING OF BLUE TONGUE VACCINES

TESTING OF MONOVALENT BLUETONGUE VACCINE (SEROTYPE 2) FOR INNOCUITY, POTENCY AND REVERSION TO VIRULENCE

CONTRACT DGSANCO/00/0127

FINAL REPORT

December 2001

SUMMARY

BLUETONGUE (BT) SEROTYPE 2 MONOVALENT VACCINE

The vaccine tested was the Bluetongue vaccine monovalent type 2 (BT2 monovalent vaccine), batch number 5, produced by the Ondersterpoort Biological Products, Ondersterpoort, South Africa, on the 18th of December, 2000, with expiry date: 1st of January, 2003

The vaccine was reconstituted with the diluent provided by the manufacturer: batch number 8027 with expiry date: 3rd of January 2004.

Sarda breed adult sheep – 1 to 3 year old - demonstrated to be highly susceptible to BT during the severe epidemic occurring in the Island, were used throughout the testing. Sheep originated from an area free of Bluetongue and tested negative to BTV Brucella, Chlamydia and Salmonella serology.

Testing was carried out in the maximum isolation unit of the Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise. Animals were introduced inside the unit a week before beginning of testing.

The Institute is accredited according to the ISO 17025:2000 and all testing carried out was under the quality system control.

All testing was carried out according to the programme laid down in the

Table 1 Innocuity testing in guinea pigs

BT2 monovalent vaccine - Batch 05 **Vaccination date: 08.05.2001** **Vaccination dose & route: *2 ml. intraperitoneally***							
Guinea pigs	*Control dates & Clinical signs recorded*						
	08.05.01	*09.05.01*	*10.05.01*	*11.05.01*	*12.05.01*	*13.05.01*	*14.05.01*
1	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
2	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
Guinea pigs	*Control dates & Clinical signs recorded*						
	15.05.01	*16.05.01*	*17.05.01*	*18.05.01*	*19.05.01*	*20.05.01*	*21.05.01*
1	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
2	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*

Table 2 Innocuity testing in mice

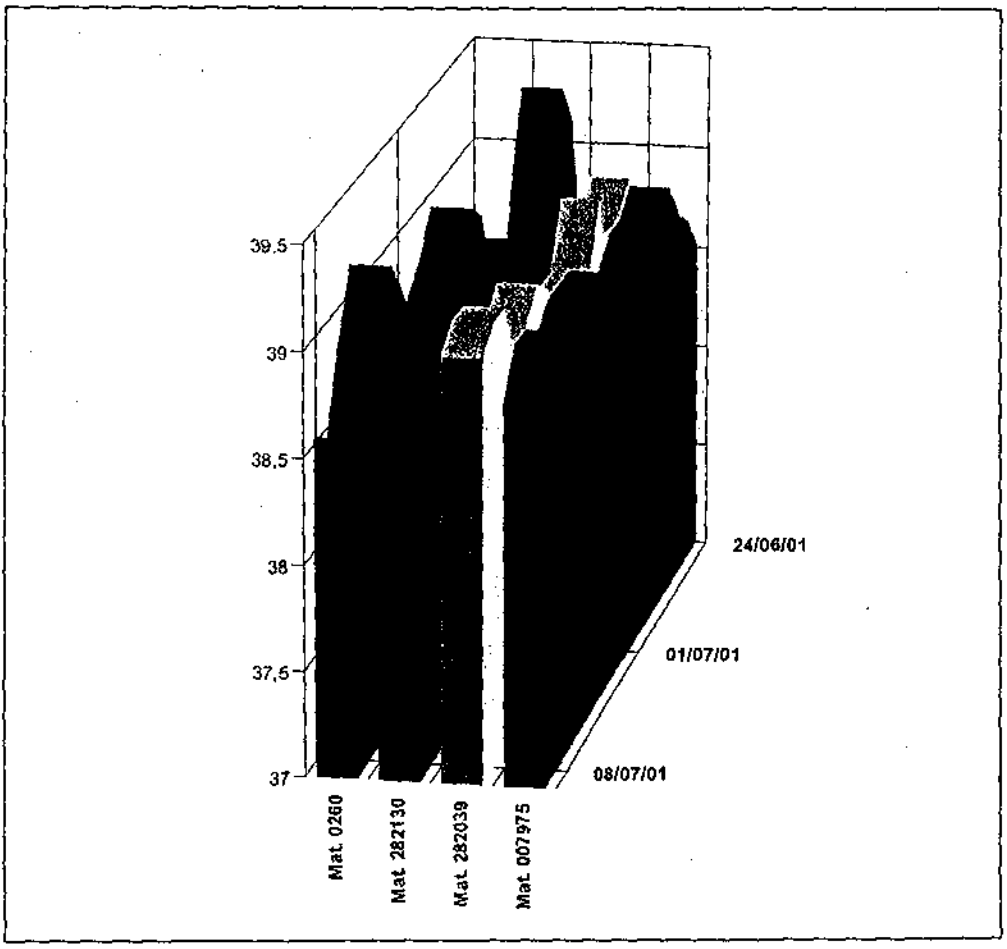
BT2 monovalent vaccine - Batch 05 **Vaccination date: 08.05.2001** **Vaccination dose & route: 0,25 ml intraperitoneally**							
Mice	*Control dates & Clinical signs recorded*						
	08.05.01	*09.05.01*	*10.05.01*	*11.05.01*	*12.05.01*	*13.05.01*	*14.05.01*
1	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
2	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
3	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
4	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
5	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
6	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
Mice	*Control dates & Clinical signs recorded*						
	15.05.01	*16.05.01*	*17.05.01*	*18.05.01*	*19.05.01*	*20.05.01*	*21.05.01*
1	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
2	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
3	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
4	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
5	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
6	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*

Table 3 Innocuity testing in sheep: clinical signs and rectal temperatures

BT2 monovalent vaccine – Batch 05 **Vaccination date: 25.06.2001** **Vaccination dose & route: 1.0 ml subcutaneously**								
Sheep	*Control dates and hour & Clinical signs recorded*							
	24.06.2001		25.06.2001*		26.06.2001		27.06.2001	
	h 9,0	h 16,0	h 9,0	h 16,0	h 9,0	h 16,0	h 9,0	h 16,0
Sheep n. 1 (Mat. 0260)	Nil 38,5	Nil 38,8	Nil 38,3	Nil 38,9	Nil 38,6	Nil 38,8	Nil 38,6	Nil 38,7
Sheep n.2 (Mat. 282130)	Nil 38,5	Nil 38,8	Nil 39,2	Nil 39,5	Nil 39,4	Nil 38,6	Nil 39,0	Nil 38,3
Sheep n. 3 (Mat. 282039)	Nil 38,4	Nil 39,1	Nil 38,9	Nil 39,0	Nil 38,8	Nil 39,1	Nil 38,3	Nil 38,8
Sheep n.4 (Mat. 007975)	Nil 38,5	Nil 38,8	Nil 38,7	Nil 39,2	Nil 38,8	Nil 39,1	Nil 39,0	Nil 39,3
	28.06.2001		29.06.2001		30.06.2001		01.07.2001	
	h 9,0	h 16,0	h 9,0	h 16,0	h 9,0	h 16,0	h 9,0	h 16,0
Sheep n. 1 (Mat. 0260)	Nil 38,9	Nil 39,0	Nil 39,0	Nil 38,9	Nil 38,8	Nil 38,6	Nil 38,7	Nil 38,5
Sheep n.2 (Mat. 282130)	Nil 38,3	Nil 38,5	Nil 38,7	Nil 38,6	Nil 38,6	Nil 38,5	Nil 38,8	Nil 38,9
Sheep n. 3 (Mat. 282039)	Nil 39,0	Nil 38,9	Nil 38,7	Nil 38,6	Nil 38,7	Nil 38,4	Nil 38,3	Nil 38,7
Sheep n.4 (Mat. 007975)	Nil 38,9	Nil 39,0	Nil 38,9	Nil 38,8	Nil 38,8	Nil 38,3	Nil 38,6	Nil 39,0
	02.07.2001		03.07.2001		04.07.2001		05.07.2001	
	h 9,0	h 16,0	h 9,0	h 16,0	h 9,0	h 16,0	h 9,0	h 16,0
Sheep n. 1 (Mat. 0260)	Nil 38,6	Nil 38,5	Nil 38,7	Nil 38,4	Nil 38,8	Nil 39,0	Nil 39,0	Nil 38,7
Sheep n.2 (Mat. 282130)	Nil 38,5	Nil 38,8	Nil 38,9	Nil 39,0	Nil 38,4	Nil 38,3	Nil 38,6	Nil 38,5
Sheep n. 3 (Mat. 282039)	Nil 38,6	Nil 39,4	Nil 38,9	Nil 38,9	Nil 39,0	Nil 39,0	Nil 38,9	Nil 39,1
Sheep n.4 (Mat. 007975)	Nil 38,2	Nil 39,3	Nil 39,0	Nil 39,1	Nil 39,0	Nil 38,9	Nil 39,0	Nil 38,9
	06.07.2001		07.07.2001		08.07.2001		09.07.2001	
	h 9,0	h 16,0	h 9,0	h 16,0	h 9,0	h 16,0	h 9,0	h 16,0
Sheep n. 1 (Mat. 0260)	Nil 39,2	Nil 39,0	Nil 38,9	Nil 38,8	Nil 38,5	Nil 38,6	Nil 38,6	Nil 38,7
Sheep n.2 (Mat. 282130)	Nil 38,7	Nil 38,6	Nil 38,8	Nil 39,0	Nil 38,8	Nil 38,9	Nil 38,9	Nil 39,0
Sheep n. 3 (Mat. 282039)	Nil 38,7	Nil 38,4	Nil 39,1	Nil 39,3	Nil 39,1	Nil 39,0	Nil 39,0	Nil 38,9
Sheep n.4 (Mat. 007975)	Nil 38,9	Nil 38,9	Nil 39,0	Nil 39,0	Nil 39,0	Nil 38,9	Nil 38,8	Nil 39,0

* Day of vaccination

Figure 1: Temperature values recorded in 4 sheep inoculated with Bluetongue vaccine monovalent type 2



POTENCY TESTING

MATERIALS AND METHODS

Two bottle of 100 dose vaccine were suspended with 100 ml of sterile diluent. Two different quantitative assays were used to titrate the vaccine by determining the Tissue culture cytopathic dose 50 ($TCID_{50}$) and the plaque forming units (PFU).

Both assays were performed using VERO cell lines and for each assay two replicates were carried out. Results are expressed as the average of the two replicates. Briefly, for the $TCID_{50}$ assay, 50 µl of vaccine dilutions - from 10^{-1} to 10^{-8} - were inoculated onto 96 flat- bottomed well microtitre plates. Approximately, 10^4 cells suspended in 100 µl of MEM containing antibiotics (Penicillin 100UI/ml, Streptomycin 100 µg/ml, Gentamycin 5µg/ml and Nystatin 50 UI/ml) and 3% Foetal Calf Serum (FCS), were

added to each well. Reading was carried out after incubation for 6 days at 37° C with 5% CO_2 supplement. End point was determined by the Reed and Muench method (1938).

For the PFU assay, two different media were used, 1% Agar Noble and Carboxymethylcellulose sodium salt (medium viscosity). When 1% Agar noble was used, a 6 flat-bottomed well plate containing confluent cell monolayers was infected with 0.8 ml of vaccine solution per well and incubated for 1 hour at 37° C. After adsorbtion and removal of the inoculum, monolayers were overlaid with 3.2 ml of MEM containing antibiotics, 3% FCS and 1% Agar. Conversely, when carboxymethylcellulose sodium salt was used, a 24 flat-bottomed microtitre plate containing confluent cell monolayers was infected with 0.2 ml of vaccine solution per well and kept at 37° C for 1 hour. After adsorbtion and removal of the inoculum, monolayers were overlaid with 0.8 ml of MEM containing antibiotics, 3% FCS and 0.7% carboxymethylcellulose. After 6 days medium was removed and staining with crystal violet was carried out.

RESULTS

The titre of the vaccine was $10^{5.15}$ /ml $TCID_{50}$ with CPE assay and 4.9×10^4 /ml PFU with 1% agar noble assay, respectively. A titre of 2.49×10^4 PFU was obtained when carboxymethilcellulose was used as overlay.

TESTING FOR IMMUNOGENICITY

MATERIALS AND METHODS

A bottle of 100 dose vaccine was suspended with 100 ml of sterile diluent. Four sheep were vaccinated by inoculating each animal with 1 ml of the vaccine subcutaneously. Starting from the vaccination day (T_0) they were bled at weekly intervals for 42 days. Antibody response was monitored by a commercial competitive ELISA test and by serum-neutralisation (SN). SN was performed according to the method described by the 2000 OIE Manual of standards for diagnostic tests and vaccines and in agreement with the method used by the Ondersterpoort OIE BT reference laboratory. BTV – 2 reference virus and serum were kindly supplied by the Ondersterpoort OIE BT OIE Reference laboratory. Sera with neutralization titres ≥ 1:10 were considered positive. The serum titre was considered the highest dilution capable of neutralising 50% of the virus activity.

The same method was used to monitor the 6 sheep used in the test for reversion to virulence (see next chapter).

RESULTS

SN and C-ELISA serology is reported in Table 4 and 5 respectively and figure 2.

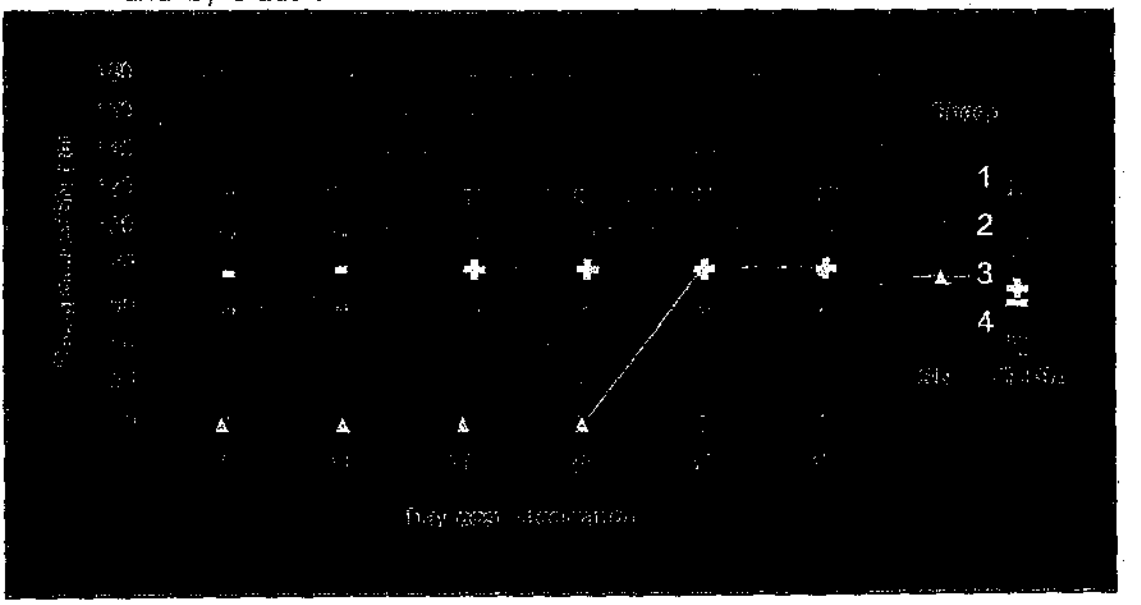
Table 4 Immunogenicity testing: antibody titres detected by serum-neutralisation

Animals & passage	Days post vaccine inoculation (dpi) & SN titres					
1st Passage	7	14	21	28	35	42
00260	-ve	-ve	-ve	1:80	1:160	1:160
282130	-ve	-ve	-ve	-ve	-ve	-ve
282039	-ve	-ve	-ve	-ve	1:80	1:80
007975	-ve	-ve	-ve	-ve	-ve (1:5)	-ve (1:5)

Table 5 Immunogenicity testing: antibody titres detected by C-ELISA

Animals	Days post inoculation (dpi)					
	7	14	21	28	35	42
00260	-ve	+ve	+ve	+ve	+ve	+ve
282130	-ve	-ve	-ve	-ve	-ve	-ve
282039	-ve	-ve	+ve	+ve	+ve	+ve
007975	-ve	-ve	-ve	-ve	-ve	-ve

Figure 2: Immunogenicity testing: antibody titres detected by serum-neutralisation and by C-ELISA



MATERIALS AND METHODS

Every two days, up until the end of the viraemia, 50 ml of blood with heparin were collected from each of the 4 animals used to test immunogenicity under sterile conditions and stored at 4° C for a maximum of 10 days before testing. To assess viraemia, 10 ml of the blood sample were centrifuged three times with PBS containing antibiotics. After the last washing, the sample was resuspended in MEM with antibiotics (1/10 v/v) and sonicated. Fifty µl of four ten fold dilutions of each of the 10 ml blood suspension (from 1:10 to 1:10.000) were inoculated into a 96 flat bottomed well microtitre plate according to the method described by the 2000 OIE Manual of standards for diagnostic tests and vaccines. Four replicates were made for each dilution. Approximately, 10^4 cells, in a volume of 100 µl of MEM plus antibiotics and 5% FCS, were added to each well and the plates were incubated at 37° C in presence of 5% CO_2. The plates were read after 6 days. The virus titre was determined by $TCID_{50}$ using the method of Reed and Muench (1938).

From each inoculated animal blood samples with the highest virus titre were selected for the second passage. In brief, 40 ml of each of these blood samples were pooled together to a total volume of 160 ml. Forty ml each were inoculated to 3 sheep subcutaneously. Animals were monitored for viremia titres as described. A further passage into 3 additional sheep was made with the same method described above.

All inoculated animals were observed daily for clinical signs and the rectal temperature was monitored and recorded twice daily for 14 days after inoculation. Temperatures above 39.5° C were considered non physiological.

RESULTS

One animal (034) at the third passage showed pyrexia on day 7 (40,3° C) and 8 (39.9° C) post-inoculation (dpi). Another animal (014) of this group showed temperature 7 dpi (39.7° C) but not 8 dpi. No other clinical signs was observed in any inoculated animal (Tables 6 and 7, Figures 4, 6 and 8).

Tables 8, 9 and 10 (figures 3, 5 and 7) show viraemia titres observed in the 3 groups of sheep inoculated.

Table 11 show antibody titres detected by serum-neutralisation in the group of sheep inoculated with pooled blood samples from vaccinated sheep (2nd passage)

Table 12 show antibody titres detected by serum-neutralisation in the group of sheep inoculated with pooled blood samples from vaccinated sheep (3rd passage).

Table 6: Reversion to virulence of the vaccine virus on sheep: clinical signs and rectal temperatures

BT2 monovalent vaccine: Sheep inoculated with the first passage of pooled blood samples Inoculation date: 25.07.2001								
Animals	Control date & time							
	24.07.2001		25.07.2001*		26.07.2001		27.07.2001	
	h 9,0	H 16,0	h 9,0	H 16,0	h 9,0	H 16,0	h 9,0	H 16,0
Sheep n. 5 (Mat. 0078)	Nil 38,6	Nil 39,2	Nil 38,8	Nil 38,8	Nil 38,6	Nil 38,3	Nil 38,8	Nil 39,0
Sheep n.6 (Mat. 0010)	Nil 38,8	Nil 39,2	Nil 39,0	Nil 39,0	Nil 39,0	Nil 39,2	Nil 38,8	Nil 39,1
Sheep n. 7 (Mat. 0133)	Nil 39,1	Nil 39,0	Nil 38,8	Nil 38,5	Nil 38,7	Nil 38,9	Nil 38,7	Nil 39,2
	28.07.2001		29.07.2001		30.07.2001		31.07.2001	
	h 9,0	h 16,0	h 9,0	h 16,0	h 9,0	h 16,0	h 9,0	h 16,0
Sheep n. 5 (Mat. 0078)	Nil 38,9	Nil 38,8	Nil 38,9	Nil 38,8	Nil 39,4	Nil 39,1	Nil 38,8	Nil 39,2
Sheep n.6 (Mat. 0010)	Nil 39,2	Nil 39,0	Nil 39,0	Nil 39,0	Nil 39,2	Nil 39,2	Nil 39,0	Nil 39,3
Sheep n. 7 (Mat. 0133)	Nil 39,0	Nil 38,9	Nil 38,7	Nil 38,9	Nil 39,1	Nil 39,5	Nil 39,0	Nil 39,2
	01.08.2001		02.08.2001		03.08.2001		04.08.2001	
	h 9,0	h 16,0	h 9,0	h 16,0	h 9,0	h 16,0	h 9,0	h 16,0
Sheep n. 5 (Mat. 0078)	Nil 38,8	Nil 39,1	Nil 38,9	Nil 39,3	Nil 38,7	Nil 39,3	Nil 38,7	Nil 38,8
Sheep n.6 (Mat. 0010)	Nil 38,9	Nil 39,0	Nil 39,0	Nil 39,0	Nil 38,9	Nil 39,2	Nil 39,1	Nil 39,0
Sheep n. 7 (Mat. 0133)	Nil 38,5	Nil 39,0	Nil 38,7	Nil 38,8	Nil 38,9	Nil 39,2	Nil 38,9	Nil 39,1
	05.08.2001		06.08.2001		07.08.2001		08.08.2001	
	h 9,0	h 16,0	h 9,0	h 16,0	h 9,0	h 16,0	h 9,0	h 16,0
Sheep n. 5 (Mat. 0078)	Nil 38,6	Nil 39,0	Nil 38,8	Nil 39,2	Nil 38,9	Nil 39,0	Nil 38,9	Nil 38,8
Sheep n.6 (Mat. 0010)	Nil 38,8	Nil 39,0	Nil 38,8	Nil 38,9	Nil 38,8	Nil 39,1	Nil 39,0	Nil 39,0
Sheep n. 7 (Mat. 0133)	Nil 39,0	Nil 38,9	Nil 38,6	Nil 38,8	Nil 39,0	Nil 38,9	Nil 38,7	Nil 39,0

* Day of vaccination

Table 7: Reversion to virulence of the vaccine virus on sheep: clinical signs and rectal temperatures

BT2 monovalent vaccine: Sheep inoculated with the second passage of pooled blood samples Inoculation date: 15.08.2001								
Animals	Control date & time							
	14.08.2001		15.08.2001*		16.08.2001		17.08.2001	
	h 9,0	h 16,0	h 9,0	h 16,0	h 9,0	h 16,0	h 9,0	h 16,0
Sheep n. 8 (Mat. 0034)	Nil 38,6	Nil 38,9	Nil 38,6	Nil 38,6	Nil 38,6	Nil 38,8	Nil 38,4	Nil 38,7
Sheep n.9 (Mat. 0220)	Nil 38,8	Nil 39,0	Nil 38,7	Nil 38,8	Nil 38,8	Nil 38,8	Nil 38,4	Nil 38,5
Sheep n. 10 (Mat. 0014)	Nil 38,9	Nil 39,1	Nil 38,4	Nil 38,5	Nil 38,3	Nil 38,4	Nil 38,6	Nil 38,5
	18.08.2001		19.08.2001		20.08.2001		21.08.2001	
	h 9,0	H 16,0	h 9,0	H 16,0	h 9,0	H 16,0	h 9,0	H 16,0
Sheep n. 8 (Mat. 0034)	Nil 38,8	Nil 38,9	Nil 38,6	Nil 38,5	Nil 38,9	Nil 39,2	Nil 39,3	Nil 38,9
Sheep n.9 (Mat. 0220)	Nil 38,7	Nil 39,0	Nil 38,8	Nil 38,9	Nil 38,9	Nil 39,1	Nil 38,9	Nil 38,9
Sheep n. 10 (Mat. 0014)	Nil 39,0	Nil 39,1	Nil 38,9	Nil 38,8	Nil 38,7	Nil 39,3	Nil 39,0	Nil 38,8
	22.08.2001		23.08.2001		24.08.2001		25.08.2001	
	h 9,0	h 16,0	h 9,0	h 16,0	h 9,0	h 16,0	h 9,0	h 16,0
Sheep n. 8 (Mat. 0034)	Nil 40,3	Nil 40,0	Nil 39,7	Nil 39,9	Nil 38,5	Nil 39,0	Nil 38,7	Nil 38,9
Sheep n.9 (Mat. 0220)	Nil 39,2	Nil 39,1	Nil 39,4	Nil 39,5	Nil 38,8	Nil 39,1	Nil 38,9	Nil 38,9
Sheep n. 10 (Mat. 0014)	Nil 39,7	Nil 39,6	Nil 38,4	Nil 39,0	Nil 38,5	Nil 38,8	Nil 38,6	Nil 38,7
	26.08.2001		27.08.2001		28.08.2001		29.08.2001	
	h 9,0	h 16,0	h 9,0	h 16,0	h 9,0	h 16,0	h 9,0	h 16,0
Sheep n. 8 (Mat. 0034)	Nil 38,9	Nil 38,7	Nil 38,6	Nil 38,5	Nil 38,9	Nil 39,1	Nil 38,6	Nil 39,0
Sheep n.9 (Mat. 0220)	Nil 39,0	Nil 39,0	Nil 38,4	Nil 38,7	Nil 38,8	Nil 38,7	Nil 38,8	Nil 38,7
Sheep n. 10 (Mat. 0014)	Nil 38,7	Nil 38,6	Nil 38,5	Nil 38,7	Nil 38,6	Nil 38,5	Nil 38,7	Nil 38,5

*Day of vaccination

1st Passage

Figure 3 : Viraemia titres

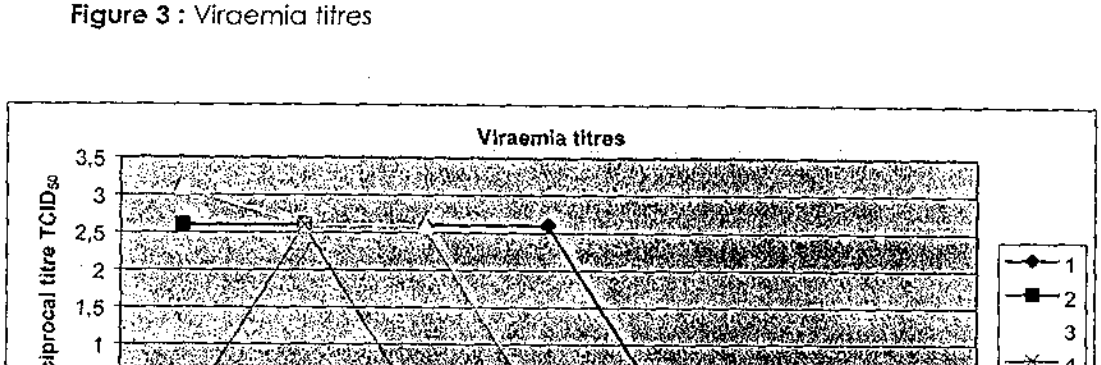
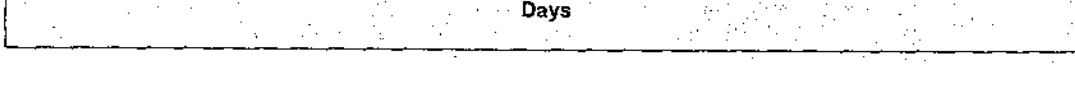


Figure 4 : Temperature values



TESTING FOR REVERSION TO VIRULENCE
2nd Passage

Figure 5 : Viraemia titres

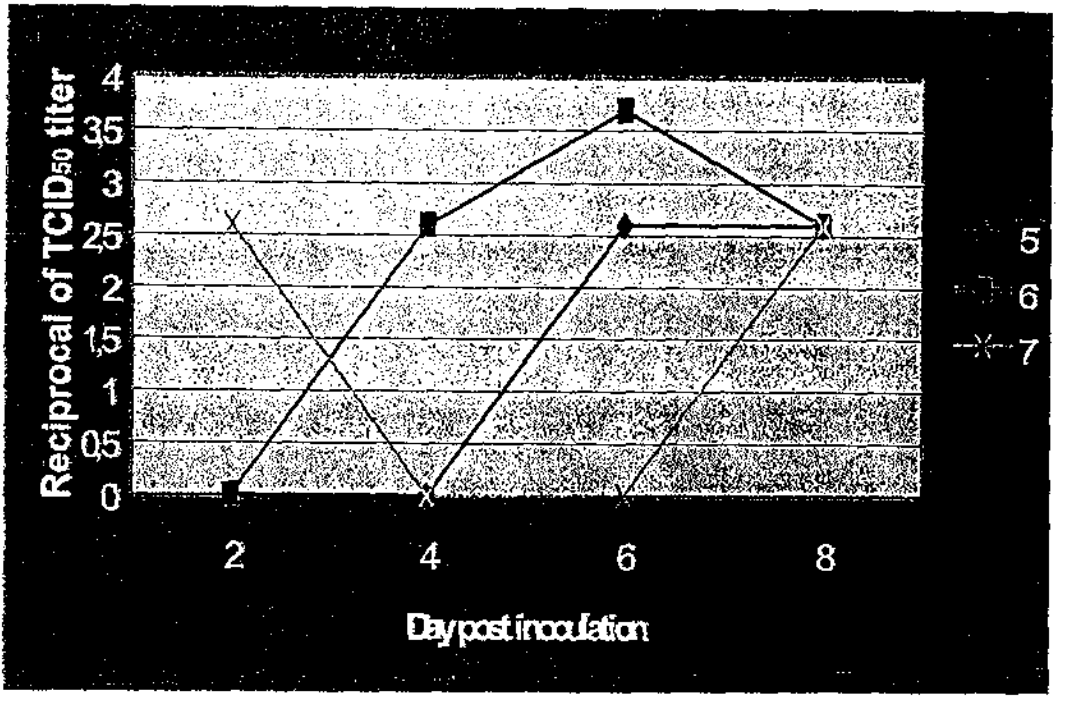
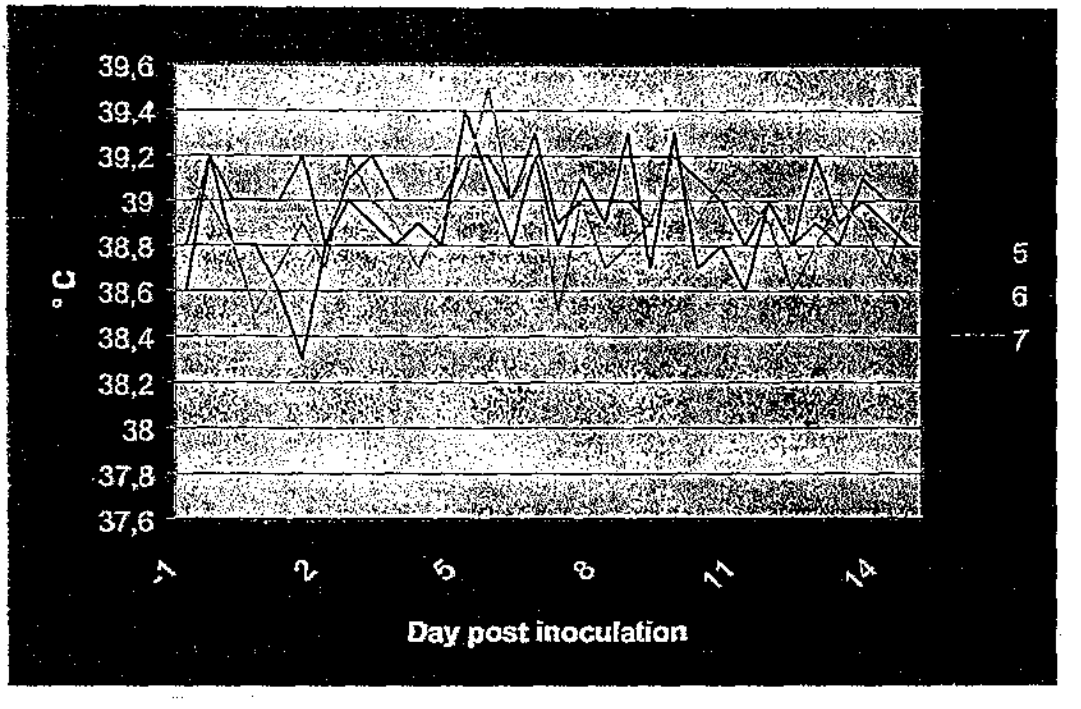


Figure 6 : Temperature values



TESTING FOR REVERSION TO VIRULENCE
3rd Passage

Figure 7 : Viraemia titres

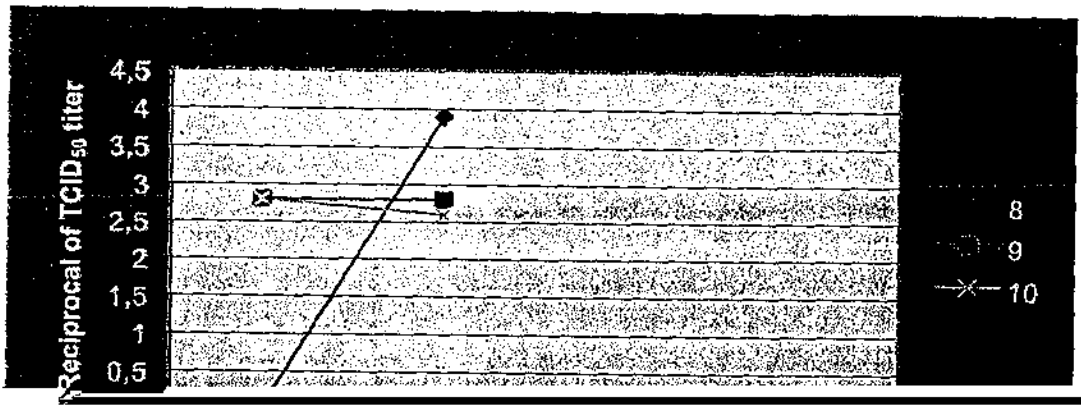


Table 8: Viraemia titres recorded in the group of sheep inoculated with BT2 monovalent vaccine

	Days post inoculation (dpi)						
Animals	**2**	**4**	**6**	**8**	**10**	**12**	**14**
00260	-ve	$<10^{2.6}$/ml	$<10^{2.6}$/ml	$10^{2.6}$/ml	-ve	-ve	-ve
282130	$<10^{2.6}$/ml	$10^{2.6}$/ml	-ve	-ve	-ve	-ve	-ve
282039	$10^{3.1}$/ml	$<10^{2.6}$/ml	$<10^{2.6}$/mll	-ve	-ve	-ve	-ve
007975	-ve	$<10^{2.6}$/ml	-ve	-ve	-ve	-ve	-ve

Table 9: Viraemia titres recorded in the group of sheep inoculated with pooled blood samples from vaccinated sheep (2nd passage)

	Days post inoculation (dpi)			
Animals	**2**	**4**	**6**	**8**
00078	-ve	$<10^{2.6}$/ml	$<10^{2.6}$/ml	$<10^{2.6}$/ml
00010	-ve	$<10^{2.6}$/ml	$10^{3.7}$/ml	$<10^{2.6}$/ml
00133	$10^{2.6}$/ml	-ve	-ve	$<10^{2.6}$/ml

Table 10: Viremia titres recorded in the group of sheep inoculated with pooled blood samples from vaccinated sheep (3rd passage)

Animals	**Days post inoculation (dpi)**	
	2	**4**
00034	-ve	$10^{3.9}$/ml
00220	$10^{2.8}$/ml	$10^{2.8}$/ml
00014	$10^{2.8}$/ml	$<10^{2.6}$/ml

Table 11: Antibody titres detected by serum-neutralisation in the group of sheep inoculated with pooled blood samples from vaccinated sheep (2nd passage)

2nd Passage	**Days post vaccine inoculation (dpi) & SN titres**					
	7	**14**	**21**	**28**	**35**	**42**
00078	-ve	-ve	1:10	1:160	1:80	1:80
00010	-ve	1:20	1:80	1:120	1:80	1:80
00133	-ve	1:10	1:20	-ve	1:80	1:80

Table 12: Antibody titres detected by serum-neutralisation in the group of sheep inoculated with pooled blood samples from vaccinated sheep (3rd passage)

3rd Passage	**Days post vaccine inoculation (dpi) & SN titres**					
	7	**14**	**21**	**28**	**35**	**42**
00034	-ve	1:80	1:30	1:80	1:80	1:80
00220	-ve	1:320	1:10	1:160	1:80	1:40
00014	-ve	1:320	1:80	1:160	1:80	1:20

CONCLUSIONS

The vaccine innocuity, stability and potency appeared satisfactory. Two of the 4 vaccinated sheep showed an antibody response to the BT2 monovalent vaccine using the methods indicated by the working programme of the contract. It induced viraemia when re-inoculated into susceptible animals.

Independently from the contract granted for the vaccine evaluation, we have, at our disposal, further data derived from experimental and field inoculations of the BT2 monovalent vaccine both in sheep and cattle.
Ninety eight negative sheep vaccinated during the current vaccination campaign were examined serologically eight weeks after vaccination. Of these 90,1% tested positive to serum neutralization test - with a median titre of 40 - and 79,4% to cELISA respectively (see Annex 1). Seventeen of 21 (81.0%) negative bovine animals vaccinated under field conditions produced serum neutralization antibody with a median titre of 80.

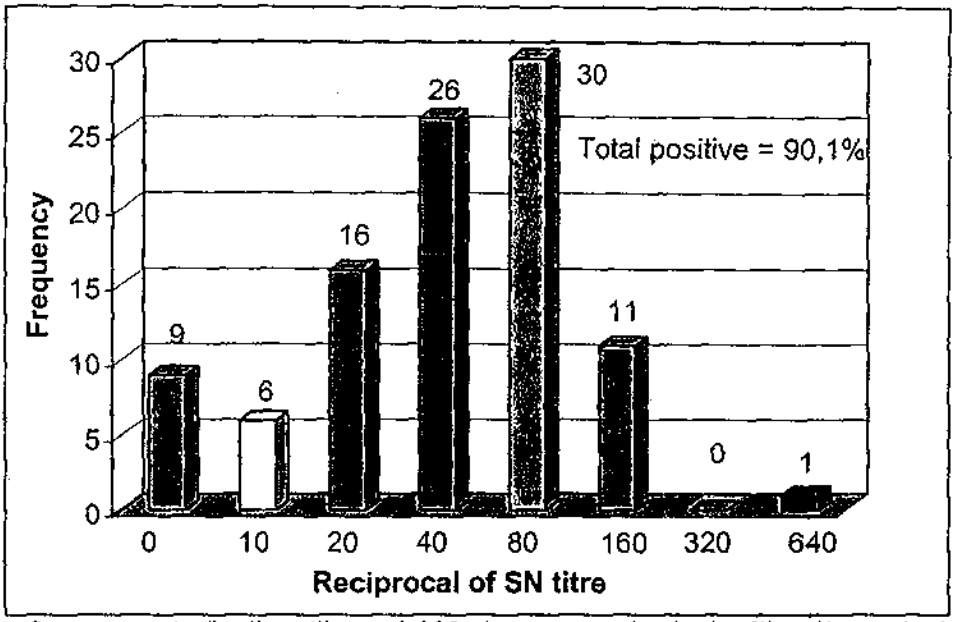
Twenty one pregnant cows at various pregnancy period were vaccinated with the same vaccine used in the trial reported. All animals tested positive for BT - 2 SN and ELISA antibody (see Annexes 2 and 3). Twenty of these animals gave birth to normal calves, but one stillbirth from which protozoon infection has been diagnosed. All calves that have been sampled before assumption of the colostrums have shown no SN antibody for BTV.

The protection of the vaccinated animals to challenge remains to be determined both for the sheep vaccinated under experimental condition and the bovine animals whose serology has been reported in the Annexes. Challenge trials are under way [redacted] will be presented as soon as they will be available.

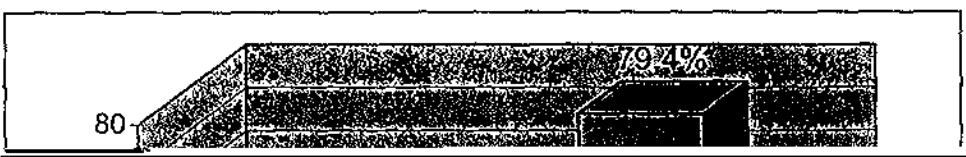
REFERENCES

- Contract DGSANCO/00/0127 "Annex 1 – Testing methods"
- European Pharmacopoeia Third edition, paragraph 2.6.1
- 2000 OIE Manual of standards for diagnostic tests and vaccines
- IZS TE B2.1.4 SOP046 "Isolamento ed identificazione del virus delle Blue tongue"
- IZS TE B2.1.4 SOP047 "Sieroneutralizzazione per virus della Blue tongue"
- IZS TE B2.1.6 SOP013 "Diagnosi sierologica Blue tongue mediante AGID ed ELISA"
- IZS TE B3.1.2 SOP042 "Controllo di sterilità dei prodotti ad azione immunologica e dei prodotti diagnostici per profilassi di stato"
- IZS TE B4.2.1 SOP007 "Sistema informatico per la gestione dei campioni"
- IZS TE B5.1.2 SCM001 "Controllo di tossicità anormale"

ANNEX 1



Serum neutralization titres of 100 sheep vaccinated with attenuated blue tongue type 2 vaccine in field conditions at approximately eight weeks after vaccination



ANNEX 2

BOVINE ANIMALS VACCINATED WITH BLUE TONGUE TYPE 2 ATTENUATED VIRUS UNDER EXPERIMENTAL CONDITIONS

DAYS POST - VACCINATION & SERUM NEUTRALIZATION AND cELISA RESULTS

BOVINE IDENTIFICATION	0		2		4		6		8		10		13		15		17		20		22		24		27		31		34		38		41		45		48		52		58		65		78		84	
	cELISA	SN*	cELISA	SN	cELISA	SN	cELISA	SN	cELISA	SN	cELISA	SN	cELISA	SN	cELISA	SN	cELISA	SN	cELISA	SN	cELISA	SN	cELISA	SN	cELISA	SN	cELISA	SN	cELISA	SN	cELISA	SN	cELISA	SN	cELISA	SN	cELISA	SN	cELISA	SN	cELISA	SN	cELISA	SN	cELISA	SN
C023	-	-	-	-	-	-	-	-	+	1:5	+	-	+	-	+	-	+	-	+	-	ND	ND	+	40	+	80	+	40	+	160	+	60	+	120	+	160	+	240	+	80	+	80	+	480	ND	ND	+	320
C030	-	-	-	-	-	-	-	-	+	1:10	+	-	+	-	+	-	+	-	+	10	+	10	-	40	+	160	+	80	+	240	+	120	+	160	+	120	+	80	+	160	+	160	+	80	ND	ND	+	120
1822	-	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-	+	-	+	40	+	80	+	120	+	240	+	160	+	240	+	160	+	120	+	80	+	320	+	160	+	160	+	160	ND	ND	+	160
1823	-	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-	+	-	+	10	+	10	+	20	+	80	+	80	+	160	+	160	+	160	+	160	+	80	+	160	+	160	+	160	ND	ND	+	40
16777	-	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-	+	-	+	20	+	40	+	80	+	640	+	320	+	160	+	240	+	240	+	240	+	120	+	80	+	80	+	120	+	40	Dead	Dead

*Reciprocal of SN titer
**Parturition of cow 16777

ANNEX 3

VACCINATION OF BOVINE ANIMALS WITH BTV 2 ATTENUATED VACCINE

HERD 1

VIRUS ISOLATION, ELISA & SERUM NEUTRALIZATION RESULTS AT VARIOUS TIME AFTER VACCINATION

BOVINE IDENTIFICATION	DATE OF SAMPLING VIRUS ISOLATION & SEROLOGY RESULTS																																
	16/03 (day 0)			23/03			30/03			06/04			13/04			20/04			27/04			04/05			11/05			18/05			25/05	01/06
	VIRUS	ELISA	SN*	VIRUS	ELISA	SN	VIRUS	ELISA	SN	VIRUS	ELISA	SN	VIRUS	ELISA	SN	VIRUS	ELISA	SN	VIRUS	ELISA	SN	VIRUS	ELISA	SN	VIRUS	ELISA	SN	VIRUS	ELISA	SN	SN	SN
C001	-	-	-	-	+	-	-	+	-	-	-	-	+	+	10	-	+	100	-	+	80	-	+	240	-	+	240	-	+	480	120	160
NC	-	-	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	ND	ND	ND	ND	ND	ND	ND	ND	-	ND	ND	-	-	-
C004	-	-	-	-	-	-	-	-	-	-	+	-	-	+	Neg	-	+	10	-	+	320	-	+	320	-	+	160	-	+	400	160	240
C005	-	-	-	-	-	-	-	-	-	+	-	-	-	+	10	-	+	160	-	+	120	-	+	160	-	+	160	-	+	640	160	240
C010	-	-	-	-	-	-	-	-	-	-	-	-	-	+	5	-	+	20	-	+	80	-	+	80	-	+	120	-	+	160	40	40
C011	-	-	-	-	-	-	-	-	10	+	+	10	-	+	20	-	+	160	-	+	160	-	+	240	-	+	120	-	+	160	80	40
C012	-	-	-	-	-	-	-	-	-	+	-	-	-	+	40	-	+	40	-	+	80	-	+	240	-	+	320	-	+	480	80	120
2874	-	-	-	-	+	40	-	+	40	-	+	40	-	+	60	-	+	40	-	+	40	-	+	160	-	+	100	-	+	30	40	20
2875	-	-	-	-	+	80	-	+	80	-	+	80	-	+	80	-	+	120	-	+	120	-	+	240	-	+	160	-	+	80	160	120
7244	-	-	-	-	-	-	-	-	-	+	+	-	-	+	320	-	+	780	-	+	160	-	+	640	-	+	480	-	+	480	160	80

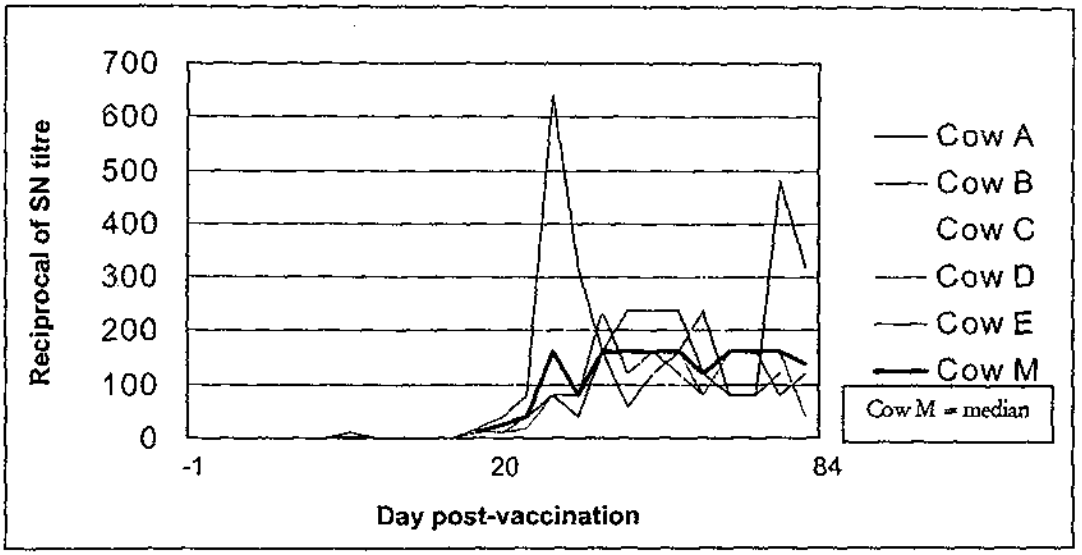
LEGEND: NC = normal control; ND = not done
*Reciprocal of serum neutralization titre

HERD 2

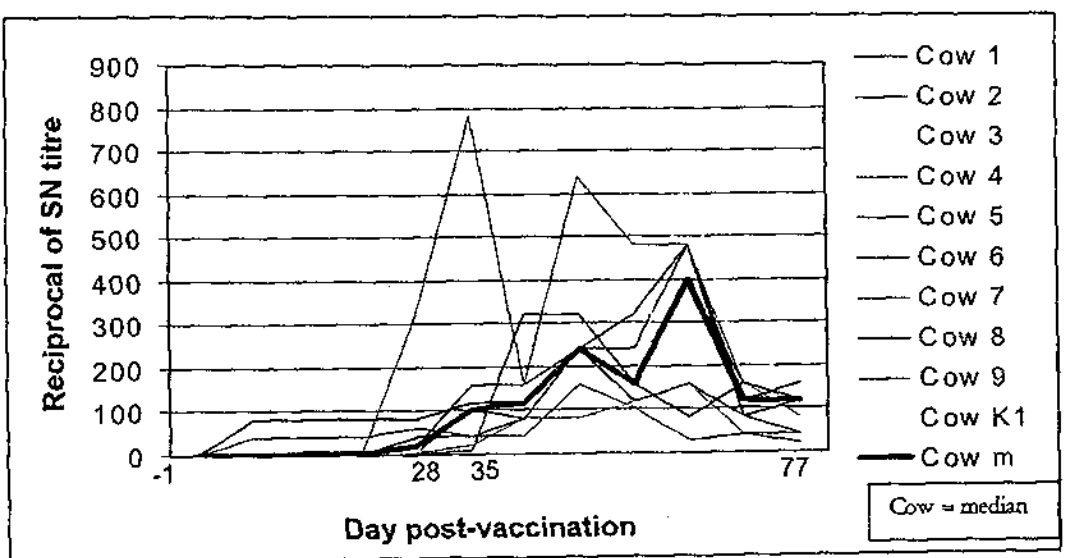
VIRUS ISOLATION, ELISA & SERUM NEUTRALIZATION RESULTS AT VARIOUS TIME AFTER VACCINATION

BOVINE IDENTIFICATION	DATE OF SAMPLING VIRUS ISOLATION & SEROLOGY RESULTS																													
	16/03 (day 0)			23/03			30/03			06/04			13/04			20/04			27/04			04/05			11/05			18/05			25/05	01/06
	VIRUS	ELISA	SN*	VIRUS	ELISA	SN	VIRUS	ELISA	SN	VIRUS	ELISA	SN	VIRUS	ELISA	SN	VIRUS	ELISA	SN	VIRUS	ELISA	SN	VIRUS	ELISA	SN	VIRUS	ELISA	SN	VIRUS	ELISA	SN	SN	SN
C261	-	-	-	-	-	-	+	+	Neg	-	+	160	-	+	15	-	+	160	-	+	80	-	+	320	-	+	160	-	+	120	60	40
C270	-	-	-	-	-	-	+	+	40	-	+	20	-	+	40	-	+	320	-	+	160	-	+	320	-	+	120	-	+	160	160	160
NC	-	-	-	-	-	-	ND	ND	ND	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C279	-	-	-	+	-	-	+	-	Neg	+	+	40	-	+	10	-	+	160	-	+	40	-	+	-	-	+	160	-	+	40	80	60
C280	-	-	-	-	-	-	+	+	10	-	+	160	-	+	160	-	+	120	-	+	120	-	+	240	-	+	640	-	+	240	160	80
C282	-	-	-	-	-	-	+	+	Neg	+	+	80	-	+	160	-	+	40	-	+	160	-	+	160	-	+	320	-	+	320	160	80
C283	-	-	-	-	-	-	+	+	Neg	+	+	40	-	+	40	-	+	40	-	+	60	-	+	80	-	+	100	-	+	120	80	160
1283	-	-	-	-	-	-	+	+	10	-	+	160	-	+	80	-	+	160	-	+	80	-	+	120	-	+	120	-	+	160	120	160

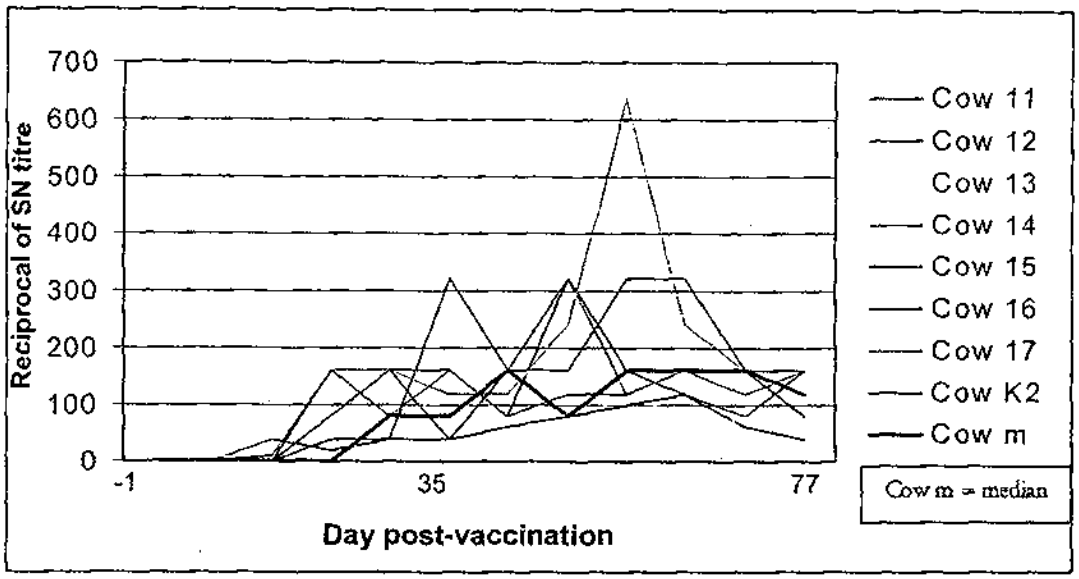
LEGEND: NC = normal control; ND = not done
*Reciprocal of serum neutralization titre



Serum neutralization antibody curve in five pregnant cows vaccinated in experimental condition with BTV-2 live attenuated vaccine



Serum neutralization antibody curve in nine pregnant cows vaccinated in field condition with BTV-2 live attenuated vaccine (Herd 1)



Serum neutralization antibody curve in seven pregnant cows vaccinated in field condition with BTV-2 live attenuated vaccine (Herd 2)