

## SURVEILLANCE OF FMD IN ITALY DURING THE YEAR 2001

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This report concerns results of the FMD surveillance performed during the year 2001 at the CERVES (Italian Reference Centre for Vesicular Diseases), Brescia, Italy.

The period taken in consideration lasts from 21 February till 18 May, when restrictive regulations on animal trade were enforced by the E.U.

Surveillance was extended also to consignments of FMD susceptible animals imported in Italy since 1<sup>st</sup> February 2001.

### Materials and Methods

Materials and Tests used at the CERVES are as follows:

#### Serology

FMD viruses in use were the O Manisa and O Switzerland subtypes. The screening ELISA employed inactivated antigens.

Screening ELISA The test was a monoclonal antibody-based LPBE. The cut-off is the titre corresponding to the Weak Positive Reference Serum.

SN The test was performed according the "Manual of Standards for diagnostic Tests and Vaccines", OIE, 2000.

3ABC ELISA The test was a trapping-indirect ELISA for the detection of antibodies to the NS polypeptide 3 ABC of the FMD Virus. Results were evaluated as T/P (Test Serum/Positive Serum) Ratio.

#### Virology

Materials submitted to virological tests were the homogenates of epithelial tissues (from tongue, gum and lips), tonsils, bone marrow and scabs collected in the field or at slaughterhouses.

Tissue Culture IBRS-2 and BHK are the cell lines currently in use. The sample was scored as negative after three blind TC passages without showing CPE.

Antigen detection The test currently in use is a sandwich ELISA performed with a combination of rabbit immune sera and MAbs specific for O, A, C and Asia types.

RT-PCR The test was performed using two sources of primers, namely:

- F17 and F21 (21-40 and 210-228 of the 3D gene), (Rodriguez A. , Virology 1992)
- LD2 and LR2 (209-226 and 403-420 of the 3D gene), (Lomakina N., unpublished, 1998)

TEST "in vivo" Suckling mice (3-5 days old) were inoculated "in peritoneum" and kept under observation for 5 days.

### Results and Discussion

#### Serological Examinations

A final 43 166 serological tests have been performed during the emergency period.

Sera have been collected from 29 721 animals among which cattle (14 515), sheep and goats (11 775) and swine (3 421), officially submitted for examination.

The origin of consignments was from E.U countries as well as from Italy. Species involved were bovine, ovine and swine. Very few samples originated from other countries (East Europe, Uganda) or species (elephant, camelids).

All sera received during the emergency period have been submitted to the screening ELISA (1<sup>st</sup> sampling).

Positive and/or doubtful reactors were sampled again (2<sup>nd</sup> sampling). Sampling was usually extended to further animals in contact with reactors and submitted again to the screening ELISA.

All doubtful and/or positive sera in the screening ELISA were examined by the 3 ABC test.

In order to improve the knowledge of the performances of the 3ABC test, besides the examination of sera identified as reactors by the screening ELISA, also all the sera received from 18<sup>th</sup> February to end of March (n = 10 487 sera) have been submitted to this test.

In **Table 1** results of 1<sup>st</sup> and 2<sup>nd</sup> samplings are reported

Titres of reactors of the 1<sup>st</sup> sampling resulted unchanged or decreased at the 2<sup>nd</sup> sampling and never an increase of titres has been observed.

All reactors identified during the two sampling phases with the screening ELISA (n =782) resulted negative with the 3 ABC ELISA . On the contrary, within sera negative in the screening ELISA (n =10 487), 26 reacted in the 3 ABC test. Among them 5 scored clearly positive whilst 21 resulted borderline. Twenty-five out of 26 sera originated from cattle (n = 7468), one (borderline) from a sheep (n =1976) and none from pig (n = 1049). The follow up of these herds demonstrated that any peculiar FMD sign has not been observed onwards so it is reasonable to consider that positive titres detected in 782 sera with the screening ELISA and in 26 sera with the 3 ABC ELISA were not specific.

Finally 586 out of 782 reactors in the screening ELISA were submitted also to SN.

Thirty-six among these reactors were able to neutralise to some extent the infectivity of O Manisa strain (6.2%).

The distribution in classes of titres of 586 sera positive in the screening ELISA and the number of SN reactors found in each class is shown in **Table 2**.

The summary of serological tests performed during the emergency period is reported in **Table 3**.

## **Virological Examinations**

**Table 4** reports the results of virological tests performed to clear suspicions due to observation of clinical signs or after positive serological results or because herds in contact with the above mentioned ones.

Often the occurrence of vesicles was claimed but never confirmed at receiving of suspect samples.

Suspicions for positive serology confirmed at re-sampling was usually followed by slaughter of the reactor animals and collection of tonsils for virological studies.

All (n = 26) of such cases has been examined in tissue culture resulting negative: 13 out of them were negative also in RT-PCR tests.

Tonsils from one cattle (origin Poland) with questionable serology showed CPE in both cell lines currently used. A positive reaction was obtained in an RT-PCR test using the primers described by Rodriguez but not with the ones described by Lomakina. The CPE was unaffected by treatment at pH 5 so it is unlikely to be an FMD virus. TC fluids resulted negative to all other virological tests.

Strong alarm (serological findings and clinical signs) was connected to 3 consignments from different places of France: alarm became panic when the first outbreak was declared in France. A "parapox" virus was immediately seen by Electron Microscopy in pathological samples from two out of three consignments, whilst all tests for FMD virus resulted negative.

Electron microscopy gave again a powerful help on two other similar occasions due to "Ectima" in sheep and "Papular Stomatitis" in cattle.

In one clinical suspicion due to the presence of vesicles on cattle tongue a BVD virus was demonstrated.

All other samples from clinical or serological suspicions resulted negative to virological tests.

**TABLE 1 : Serological results with the MABs-based LPB-ELISA and the 3ABC-ELISA**

ORIGIN	SPECIES (*)	LPB-ELISA				3ABC ELISA				
		1st sampling		2nd sampling		1st + 2nd sampling POS LPB-ELISA		1st + 2nd sampling NEG LPB-ELISA		
		NEG	POS (%)	NEG	POS (%)	NEG (%)	POS	NEG	POS (%)	
ITALY	Cattle	68	9 (13,2)	0	0	9	0	nd		
	G and S	9742	112 (1,1)	519	28 (5,4)	140	0			
	Swine	102	0	0	0	0	0			
<b>TOTAL</b>		9912	121 (1,2)	519	28 (5,4)	149	0			
E.U.	Cattle	12532	345 (2,7)	1915	256 (13,3)	601	0			
	G and S	664	21 (3,1)	850	9 (1,0)	30	0			
	Swine	3319	2 (0,06)	0	0	2	0			
<b>TOTAL</b>		16991	368 (2,1)	2765	265 (9,6)	633 (100)	0			
<b>TOTAL</b>	Cattle	12600	354 (2,8)	1915	256 (13,3)	610	0		7471	25 (0,33)
	G and S	10406	133 (1,2)	1369	37 (2,7)	170	0		1967	1 (0,005)
	Swine	3421	2 (0,05)	0	0	2	0	1049	0	
<b>TOTAL</b>		26437	489 (1,8)	3284	293 (8,9)	782 (100)	0	10487	26 (0,25)	

Legenda: (\*) G and S = Goat and Sheep

**TABLE 2 : Distribution in classes of titres of 585 sera positive in the LPB-ELISA.  
In brackets: number of sera resulted positive to Serum Neutralisation.**

>20-50	> 51-100	> 100-200	> 201
300 (22)	223 (13)	43 (2)	20 (1)

**TABLE 3 : Summary of serological tests performed during the FMD emergency period  
(February-May 2001)**

LPB-ELISA	30.503
3ABC-ELISA	12.077
Serum neutralisation	586
<b>TOTAL</b>	<b>43.166</b>

**TABLE 4 : Tests on animals with clinical and/or epidemiological and/or serological suspicions of FMD.**

HOLDING PROVINCE	NUMBER SPECIES	ORIGIN (clinical suspicions)	SEROLOGICAL TESTS ELISA FMDV O Man		VIROLOGICAL TESTS						
			POS/ N°samples	RANGE	SAMPLE	Tissue culture		PCR		ME	other tests
						IBRS 2	BHK 21	primers A	primers B		
DL Pescara	sheep	France (c.s.)	9 / 9	20-70	tonsils	neg		nd			
DL Pescara	262 sheep	France (c.s.)	8 / 56	20>270	tongue vesicles	neg		para pox	nd		
DL Pescara	369 sheep	France (c.s.)	1 / 360	60	tongue vesicles	neg		para pox	nd		
ZM Varese	49 goats	France (c.s.)	0 / 49	nd	lips epith.	neg		para pox	nd		
PR Modena	cattles	Italy (c.s.)	0 / 20	nd	foot and mouth ep.	neg		para pox	neg		
AO Piacenza	cattles	Italy (c.s.)	0 / 1	nd	gum epith.	neg		neg			
TFG Pisa	332 sheep	France (c.s.)	0 / 12	nd	tonsils	neg		nd			
TFG Pisa	323 sheep	France (c.s.)	0 / 1	nd	bone m. foot ep.	neg		nd			
LM Novara	cattles	Italy (c.s.)	0 / 2	nd	tongue vesicles	neg		nd	BVD		
TL Ravenna	pigs	Italy (c.s.)	0 / 20	nd	vesicles	neg	nd	nd	neg		
BP Como	cattles	Italy (c.s.)	nd		gum epith.	neg	nd	nd			
Bioparco Roma	elephant	Italy (c.s.)			vesicle	neg	nd	neg			
SF Pordenone	pigs	Italy (c.s.)			vesicle	neg	nd	nd			
MD Ravenna	pigs	Italy (c.s.)			scabs	neg	nd	nd			
N° 27 holdings	cattles, sheep, goats	Ita, Fra, Ger, Pol (*)	positive serology		tonsils	neg	13 neg (*)		nd		
	cattles	Poland	1 / 31	40	tonsils	pos	pos	neg	nd		

Legenda:

Primers A – Rodriguez et al., Virology 1992; Primers B – Lomakina, unpublished, 1998.

(°) Ita = Italy, Fra = France, Ger = Germany, Pol = Poland

(\*) Number of examined holdings