Field Evaluation of Rapid "penside" Tests for FMDV antigen and FMDV-NSP antibody FAO-EUFMD pilot study in Erzurum, Turkey; 12-25 September 2004

[1]A. N. Bulut, [2]D. Sammın, [3]O. Mcdonagh, [4]U. Dinler and [1]N. Unal
[1]FMD (ŞAP) Institute, Ankara, Turkey; [2]EUFMD Secretariat, FAO, Rome, Italy; [3]Department of Agriculture and Food, Sligo, Ireland; [4]Veterinary Control and Research Institute, Erzurum

Introduction

At a training workshop held in Athens in December 2003 under an FAO technical co-operation project (TCP/RER/2903), participants from Turkey and other countries discussed the steps that need to be taken when investigating FMD outbreaks and the information that needs to be captured for tracing dangerous contacts. A set of revised guidelines and forms for use during outbreak investigation was eventually drafted. It was decided at the 70th Session of the EUFMD Executive Committee (Dublin, June 2004) that the Secretariat proceed with the first phase of a proposed pilot study to "field-test" the revised guidelines and forms in Eastern Anatolia. This was also seen by the Secretariat as an opportunity to conduct a field evaluation of rapid "penside" diagnostic tests (for FMD viral antigen and NSP antibody) during an outbreak investigation, thereby following up one of the recommendations of the 2003 Closed Session of the EUFMD Research Group (Gerzensee, September 2003).

Materials and methods

Implementation of revised guidelines - field investigation of an FMD outbreak

An investigative team (comprising local veterinary officers and veterinary expertise from Ankara and from overseas) was assembled in Erzurum and a local disease control centre (LDCC) was established at the Veterinary Control and Research Institute (VCRI). When a suitable FMD outbreak was identified, a field investigation was conducted at the outbreak location following the steps laid down in the revised guidelines mentioned above; clinical and epidemiological information was recorded on redesigned forms. Follow-up investigations in neighbouring villages and at more remote locations were then prioritised based on a logical assessment of the risk associated with proximity to the index village and different types and timing of contacts. In each village the same procedures were followed: epidemiological information was gathered by interviewing the Muthar (Head of the village) and livestock owners, a sample of animals were clinically examined and specimens were collected for diagnostic testing.

Clinical specimens:

A total of 144 sera were collected for testing by rapid test. These were collected from 81 cattle, which were either clinically-affected or in close contact with affected animals; 63 of these cattle were resampled 5-7 days later for retesting. Oesophago-pharyngeal (OP) fluid or "probang" specimens were collected from 38 cattle, all of which were either clinically-affected or in close contact with affected animals. Vesicular Fluid was obtained from two clinically-affected cattle with early lesions. Such intact vesicles were infrequently observed even in recently-infected groups of animals and when seen were very easily ruptured on handling (Figure 1).

Diagnostic test kits

The rapid test devices for both viral antigen and NSP antibody were supplied by Princeton Biomedical Corporation. These were both immunochromatographic strip test devices, one of which was designed to test either whole blood or serum for FMDV-NSP antibody and the other to test either OP fluid or vesicular fluid for FMDV antigen.

Sera were tested for antibodies to FMD virus non-structural proteins (NSP) using an ELISA test-kit, *Ceditest FMD-NS (*CEDI Diagnostics BV, Netherlands). A liquid phase blocking ELISA (LPBE) was also used to test sera for antibodies to the structural protein antigens of FMD virus; this was performed for each of three serotypes (O, A and Asia 1) with reagents supplied by the Institute of Animal Health (IAH), Pirbright, UK.

Vesicular fluids were tested by an Indirect Sandwich ELISA which was performed with reagents supplied by IAH as described by Ferris and Dawson (1988).

Rapid testing for both antigen and antibody was conducted at VCRI, Erzurum. The same specimens were subsequently transported to the Şap Institute, Ankara and retested using laboratory-based ELISA test-kits. Each of the tests was performed according to the instructions provided by either the manufacturer (Rapid tests and Cedi-diagnostics test) or the supplier of reagents (LPBE and ACE).

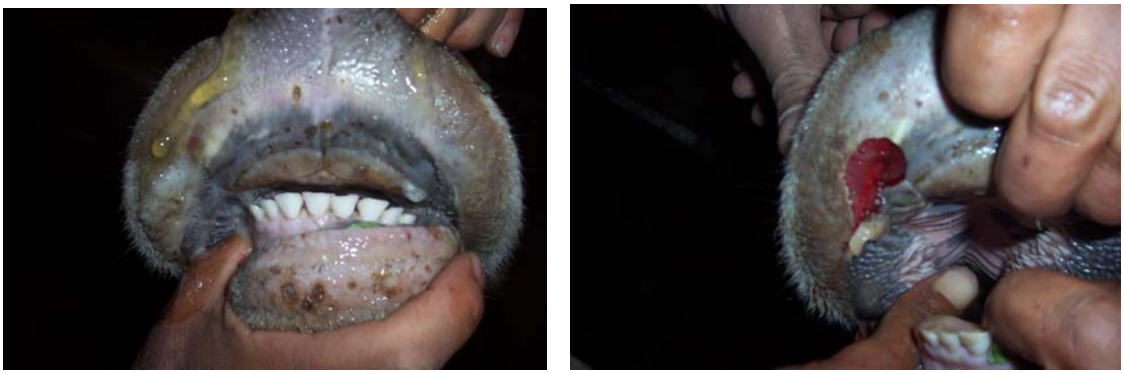

Figure 1: an intact vesicle (arrow in A) is ruptured during handling (B)

Results

Field investigation of an FMD outbreak

A suspected FMD outbreak in the village of Ozbek (which is located just over 20 km from the city of Erzurum) was reported on the day after the investigative team arrived in Erzurum province. A preliminary investigation confirmed the clinical suspicion of FMD. However a number of visits had to be conducted (over a four day period) to obtain the information required on each of the different grazing groups of animals in Ozbek. It was not until then that the neighbouring villages were investigated (the results of which are not presented in this paper).

The owner of a group of 40 calves which had recently been purchased, reported a suspicion of FMD. On clinical inspection of these calves, many were severely lame and some were recumbent and reluctant to rise. In addition to intra-oral lesions consistent with FMD, they had extensive ulceration of the interdigital space and underrunning of the bulbs of the heel in one or more feet which was complicated by secondary bacterial infection and in some cases blowfly myiasis. Thirty-one of the calves in this "index" group (Group 1) were examined in detail; 25 having FMD lesions estimated as between 4 and 10 days-old. Serum and OP fluid were collected to test for antibody (Table 1) and FMDV antigen (Table 2), respectively. Seven days later when most of the intra-oral lesions had healed; serum was again collected from this group to test for antibody.

Four other distinct epidemiological groups of cattle were identified in Ozbek:

Group 2 consisted of 17 "yearlings", 16 of which had FMD lesions ranging from 3 to 7 days-old. Serum was collected to test for NSP antibody by rapid test on that occasion and again six days later.

Group 3 was composed of more than 300 cattle in a single grazing group of which 60 were randomly-selected for examination. 22 of the 60 had oral lesions but only in three animals were these considered to be specific for FMD (in those animals the lesion age varied from 1 to 7 days). In the remaining 19 animals there were abrasions and scarring on the dental pad and gums suggestive of traumatic injury. Although serum was collected from all 60 animals, only in the case of the 22 animals with oral lesions was serum tested for NSP antibody by rapid test.

Group 4 also consisted of more than 300 cattle which were accompanied by 30 buffalo whilst at pasture. 42 cattle were sampled from this group, five of which had FMD lesions ranging from 1 to 7 days-old. Sera were collected from all 42 animals but only 11 were tested by rapid test; five days later nine of the 11 animals were rebled to be tested by rapid test for NSP antibody. In addition, OP fluid, collected from 10 of the 11 animals on the second sampling day, was tested for FMDV antigen by rapid test. Some of the animals in this group were reported by their owner to have shown clinical signs of FMD in May 2004. A separate subgroup of animals belonging to one owner were also exmained; these cattle had been grazing with Group 4 until housed approximately one week before they were examined (at the time that the owner first noticed one of his cattle to be ill); when first examined, 18 of 31 calves had early FMD lesions (0-3 days-old) and vesicular fluid was obtained from one of the calves; five days later yearling cattle were also severely-affected with swollen lips/muzzle, profuse salivation and 3-5 day-old intra-oral lesions.

Group 5 consisted of 38 housed, fattening bulls which when examined showed no clinical signs of FMD; these animals had been kept indoors for more than one month and had no contact in that time with any of the other cattle kept in the village; no specimens were collected from this group of cattle.

Evaluation of a rapid test for detection of antibody to FMDV-NSP

When first sampled, five of the 81 cattle tested by rapid test were strongly NSP seropositive whilst another 20 cattle were weakly seropositive (6-35 percent seropositive depending on whether or not weak positives are included). When sera from the same animals was tested by a laboratory-based ELISA for NSP antibody, 86% were seropositive. Repeat sera collected 4-7 days later from 63 of these animals were also tested by both methods. Nine of the resampled cattle were strongly NSP seropositive and 19 were weakly seropositive by rapid test (11-48% seropositive) whilst 98% were NSP-seropositive by laboratory-based ELISA. A breakdown of the serological results obtained from the different epidemiological groups of animals identified during the field investigation is provided in table 1. The test was relatively easy to perform and the result was easily read by the naked eye (Figure 2)

Table 1 % NSP seropositive in each group comparing rapid test and laboratory-based ELISA

GROUP	n	% with FMD lesions	Estimated age of lesions	RAPID TEST (day 0)	Lab ELISA (day 0)	resampling	RAPID TEST (retest)	Lab ELISA (retest)
1	31	81%	4-10 days	10-35%	100%	+7 days (n = 37)	14-48%	97%
2	17	94%	3-7 days	0-18%	94%	+6 days	19-44%	100%
3	22	12%	1-7 days	6-27%	73%	ND	-	-
4	11	0%	-	-	74%	+5 days (n = 9)	11-44%	100%

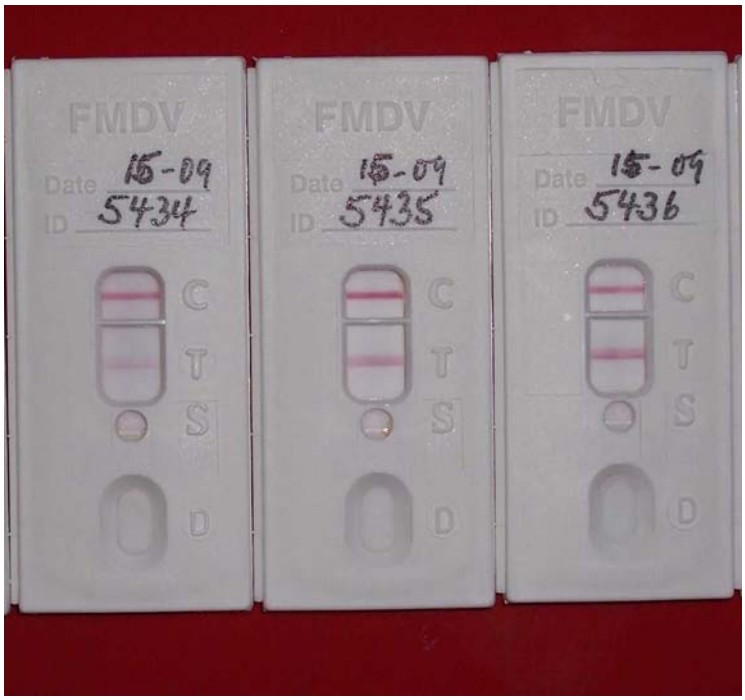

Figure 2: a weak positive test result (T) and two strong positive test results for NSP-antibody

Evaluation of a rapid test for detection of FMDV Antigen

Both vesicular fluid samples were positive for both SP and NSP antigens (Figure 3). Both were also positive when tested by laboratory-based antigen capture ELISA, one specimen for serotype O virus and the other specimen for serotype A virus.

Thirty-eight OP fluid specimens were tested using rapid test devices. Only three specimens gave any indication of positivity but this was a very weak, barely-visible or "trace" reaction on the membrane and in each case the control line did not develop properly on the test device such that the result had to be considered inconclusive. A similar problem was encountered with many of the OP fluid specimens that were tested; control lines did not develop presumably because the viscosity of OP fluid (even when diluted 50:50 with PBS) prevented capillary action or "wicking" through the membrane.

Table 2 Results of testing for viral antigen by rapid test and by laboratory-based ACE

Group	OPF or VF[1]	Rapid test for FMDV-Ag				ACE +ve
		Strong +	**Weak +**	**Inconclusive** (no control)	**Negative**	
Group 1	OPF (n=28)	0	0	13	15	ND
Group 3	VF (n=1)	1	0	0	0	1/1
Group 4	OPF (n=10)	0	0	0	10	ND
	VF (n=1)	1	0	0	0	1/1

[1]OPF = oesophago-pharyngeal fluid, VF = vesicular fluid; [2]the % positive values for the rapid test method ranges from a lower value where only the strong positives are considered "positive" to a higher value where weak/trace positives are also considered "positive"; ND = not done.

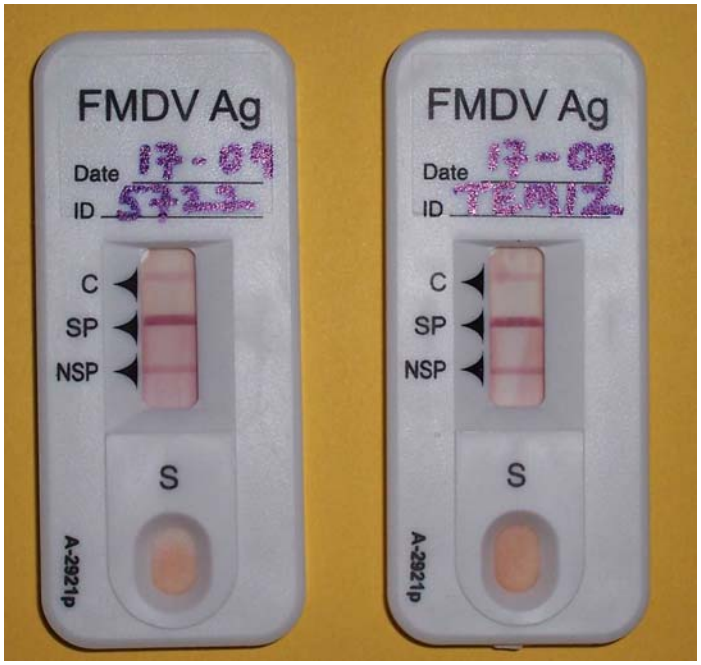

Figure 3: Vesicular fluid specimens gave a strong positive test result for both structural protein (SP) and non-structural protein (NSP) FMDV antigens.

Discussion

FMDV infection and clinical disease were widespread in the index village and all age groups were affected although the most severe disease, including lameness and the presence of interdigital lesions, was only evident in juvenile cattle. The onset of clinical FMD in Ozbek in September 2004 would appear to be associated with the recent purchase of young cattle from Erzurum market, most probably because of introduction of virus with these animals. However, there was also both epidemiological and serological evidence that an FMD outbreak had occurred in the village in the recent past, approximately 5 months before the present outbreak. Therefore it is also possible that the bought-in calves which were the most severely-affected group in the present outbreak may have developed disease because of being exposed to virus which was already present in the village in persistently-infected or subclinically-infected animals.

For practical reasons neither of the rapid tests evaluated in this pilot study were actually applied "penside" but both were immediately used on return of the investigative team to the LDCC. Both tests were relatively easy to perform although a steady surface and pipetting were required.

The test for detection of FMD viral antigen worked very well when it was used to test vesicular fluid and both specimens were strongly positive for both SP and NSP antigens. However, intact vesicles from which vesicular fluid could be obtained are infrequently observed in field cases of FMD. Furthermore, when "classical" vesicles are recognisably present, the clinical diagnosis is relatively certain and there may be little reason to perform a rapid test. The test performed poorly with OP fluid specimens in that many of the test-strips did not register a positive control line even where the specimen was diluted to reduce its viscosity. Although three specimens gave a very weak positive reaction ("trace" positives), these results had to be considered inconclusive given the absence of a control line on the testing device in each case. In addition it should be remembered that probang-sampling is unlikely to be performed during an outbreak investigation and requires the ready-availability of probang devices and considerable experience on the part of the sampler.

The usefulness of a rapid test for FMD antigen, which is only designed to test vesicular fluids and OP fluids and which in fact is only effective in testing the former, must be questioned. To be of use in field investigation it should be possible with a rapid testing method to use epithelial fragments from the edge of lesions as the clinical specimen under test; this would require that a suspension could be prepared from such specimens under field conditions that would be capable of diffusing through the membrane of an immunochromatographic test device. In addition the test devices would have to be sufficiently sensitive to detect the much smaller concentrations of FMD viral antigen that might be expected in such test materials derived from FMDV-infected animals.

Compared with results obtained when the same sera were retested using a laboratory-based ELISA test-kit the rapid test devices for detection of NSP-antibody did not detect very many seropositive cattle. The rapid test was therefore much less sensitive for detection of NSP antibody than the laboratory-based ELISA. However these devices may still be of some use during initial epidemiological investigations at an infected premises or village. In such a situation it is important to estimate the time elapsed since introduction of infection for the purposes of tracing the most likely source of the infection and also to determine the risk of spread associated with different contacts. Establishing which was the first group of animals in a herd or village to become infected can be attempted by estimating the age of lesions in different epidemiological groups within the herd/village and thus identifying the oldest lesion present. This option is no longer available if lesions have healed. However, as a stronger serological response might be expected from animals with healed lesions (due to earlier exposure to the virus) a random-sample could be selected from each group of animals and their serum tested for the presence of NSP antibody. In addition, healing intra-oral lesions which are observed during an outbreak investigation may be caused by trauma or something else other than FMDV infection, as suspected in one of the groups examined during the present study. If the healed lesions were caused by FMD virus some indication of a serological response would be expected whilst no such response would be expected if the injury arose otherwise. Sera from such suspect animals could be collected and tested "penside" for this purpose.

Conclusions

- Rapid tests may be a useful tool during FMD outbreak investigation but they are NOT a substitute for careful clinical and epidemiological investigation
- Available tests need further field evaluation and their use "penside" should be attempted.

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