Using NSP ELISA (Chekit-FMD-3ABC Bommeli-Intervet) as a Tool for FMDV Serosurveillance in Bulgaria

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Abstract:
Bulgaria has adopted a non-vaccination strategy for the control of FMD since 1993 and is free country from the disease since 1996. For us is deeply important to have a high sensitive and exact laboratory method for early detection of FMDV infection and for serum-surveillance purposes. Therefore Bulgaria has more 300 km. common border with Turkey which use vaccination policy against FMDV in Thrace and a couple of FMD outbreaks caused by more than one and often some exotic FMDV strains each year have been reported in Anatolia. So, each serum sample should be tested minimum against 3 or 4 different serum types of FMDV currently circulating in the neighbouring countries. The commercial 3ABC NSP ELISA tests are available since 2001 on the market. We performed Bommeli-Intervet CHEKIT-FMD-3ABC ELISA test bov-ov in Bulgaria during 2 years period (2002-2003). On this paper we are going to summaries the results of evaluation of the test's sensitivity on the basis of the investigated serum samples. The CHEKIT-FMD-3ABC ELISA was used according to the manufacturers instruction (SOP), accompanied the test-kit 4797 serum samples in 2002 and 5754 serum samples in 2003 have been tested using CHEKIT-FMD-3ABC ELISA Bommeli according to the Bulgarian National monitoring and surveillance FMDV program. Only 4 serum samples from cattle origin have been determined as NSP FMDV antibody positive on the basis of estimation of OD % value and confirming the results using additional NSP ELISA tests. The correlation between the number of non-conclusive and positive evaluated samples have been shown. CHEKIT-FMD-3ABC ELISA Bommeli-Intervet is easy to perform, reproducible and specific. The final result can be obtain in real time and the test can be use with success as a tool in FMDV surveillance programs in FMDV free countries bordering with endemic regions. The non-conclusive serum samples have to be retested using the same or the second confirmation test up to the full determination.

Introduction

Foot-and-mouth disease (FMD) is highly contagious viral and economically devastating disease of cloven-hoofed animals. The causative agent is aphtovirus belonging to the Picornaviridae family for which seven serum types have been described. The detection of antibodies to the nonstructural 3ABC poly protein is the single most reliable indicator of the infection and confirmation of serum conversion of these antibodies is evidence of infection with wild FMD virus (FMDV) (3, 4). Antibodies directed to the capsid proteins of FMDV are induced by both – inactivated (from vaccines) and live viruses (infection, carrier animals) therefore it is not possible to differemtiate the origin of the antibodies using routine Liquid Phase (LPBL) or Solid Phase ELISA tests. The non-structural proteins (NSP) of FMDV have received considerable attention in recent years with a search for improved serological tests for FMDV (5). For countries using vaccination in their strategy for the control of FMD outbreaks, it is of a great importance to differentiate post vaccine from post infection derived antibodies in order to discriminate FMDV which is circulated in the field (1). Bulgaria has adopted a non vaccination strategy for the control of FMD since 1993 and has a free country status from the disease since 1996. For us is important to have a high sensitive and exact laboratory method for early detection of FMDV infection and for serum-surveillance needs. Moreover Bulgaria has more than 300 km. common border with Turkey, which use vaccination policy against FMD in Thrace and each year reported of a couple of FMD outbreaks caused by more than one and often some exotic FMD viral strains. There also reports for presence of NSP FMDV serum positives in Thrace as a result of low, but still circulation of wild FMDV in that part of the country (1). The commercial 3ABC NSP ELISA tests are available on the market since 2001. In this paper we present the results of use of Bommeli FMDV 3 ABC NSP ELISA CHEKIT-test (bov-ov) as a tool for FMDV serum – surveillance programs in Bulgaria during 2 years period (2002-2003). We also would like to summary the results of evaluation of the test specificity.

Material and Methods

CHEKIT-FMD-3ABC Bommeli ELISA bov-ov

This ELISA test-kit was used according to the manufacturer's instruction (SOP).
The estimated serum samples were diluted previously 1:100 and added in duplicate to the wells of a 96 well microtitre plates pre-coated with the vector expressed FMD viral 3ABC antigen. The ELISA reaction was made according the SOP. The degree of color that developed was proportional to the amount of antibody complexes on the plate surface and read at 450 nm and reference filter at 492 nm. The final reading for the sample was calculated as follows using the means of the pairs of samples and the median of the 2 positive and 2 negative controls on each plate:

$$Value\% = \frac{OD\ sample - OD\ neg}{OD\ pos - OD\ neg} \times 100$$

Interpretation of the results: if a %OD of less than 20% is negative, 20-30% is ambiguous and greater than 30% is positive.

3 ABC NSP ELISA submitted by WRL FMD Laboratory (WRL – IAH);
3 ABC NSP ELISA submitted by IZSP – Brescia, Italy and
3 ABC NSP ELISA antigen, kindly submitted by CISA Valdeolmos (Spain):
Recombinant 3 ABC polypeptide from FMDV, expressed in E. coli was used to perform NSP ELISA. Plates were coated with capture anti 3A NSP protein monoclonal antibodies of FMDV and then the plates have been sensibilized by 3ABC NS protein. Detection of antibodies bound to 3ABC in investigated serum samples was performed by addition of anti-sheep or protein – A (for cattle samples) conjugate to horseradish peroxidase (Sigma).

LPHBL FMDV ELISA - WRL FMD Laboratory Pirbright - England:
Monotype LPBL ELISA for O (O-1 Manisa), A (A-5,A-22 and A-24) and ASIA – 1- Samir was used for estimation the type specific FMD antibodies in sera showed positive results in FMDV NSP Bommeli CHEKIT ELISA test (30% cut off). The ELISA tests were performed according the SOP's prepared by the producers. The cut off was established following standard procedures (2).

SERUM SAMPLES:
We made our FMDV serum-surveillance investigations in accordance to the National FMD monitoring programs, approved by the Minster of Agriculture of Bulgaria for 2002 and 2003 years. In 2002 - **4797** serum samples (4293 ovine, 360 caprine and 144 bovine) have been tested. In 2003 – **5754** serum samples have been tested (5435 – ovine, 266 – caprine, 32 - bovine, 15 – from buffaloes, 2 - from deer, 2 – from iah, 2 - from camel).

Results

The CHEKIT Bommeli NSP FMDV ELISA results (4293 from ovine, 360 from caprine and 144 from bovine origin**)** from serum samples tested during 2002 are shown on Table 1.

The FMDV serum-surveillance program started from 01.04 and finished at 30.11.2002. The 35 sentinel groups of animals were located in a 10-th kilometre border strip zone in Bourgas, Yamblol, Haskovo and Kardjali districts of Bulgaria (Map 1.) and bordering with Republic of Turkey and Republic of Greece. In May 2002 when 110 regular samples from (30 cattle, 45 goat and 35 sheep) derived from sentinel village Rezovo (Bourgas District) were negative for presence of antibodies to 3ABC FMDV by NS proteins by ELISA test, while 4 samples from cattle showed positive result by this test. All the samples were retested again with FMDV NS 3ABC Bommeli CHEKIT ELISA test and the same sample from cattle were positive for presence of antibodies against NS proteins of FMDV. The clinical and epidemiological investigations were negative for any evidences for presence of FMDV clinical symptoms or the disease in the village and the surrounding region.

Then we tested the same bovine serum samples positive by Bommeli CHEKIT-FMD-3ABC ELISA using the reagents for detecting NSP FMDV antibodies from three different sources - WRL-IAH Pirbright, England, IZPS – Brescia, Italy and CISA Valdeolmos, Spain with the same result. All 4 positive serum samples by CHEKIT Bommeli were positive for FMDV

antibodies to NS proteins by these 3 ELISAs (Table 2). In that time the suspected animals have been destroyed.

Further investigations using LPBL monotype ELISA were negative and we haven't a success to detect FMDV antibodies to structural FMDV proteins and to determine the type specificity of the antibodies. The additional serological and clinic investigations with the samples and animals of the Rezovo village region showed constant negative results.

The evaluation of the specificity of CHEKI-FMD-3ABC on the basis of distribution of % OD value for 2002 correlated with the NSP serum samples found positive. As shown on Fig 1. only 65 tested samples had range between 20-20% OD or non-conclusive statues. 25 another samples have been evaluated with positive result by CHEKIT-FMD-3ABC ELISA. This is well demonstrated using the distribution of % OD value for the suspected samples from Rezovo cattle, investigated in May 2002 (Fig.2).

In 2003 – **5754** serum samples have been tested (Table 3) using the NSP FMDV ELISA CHEKIT Bommeli. The FMDV surveillance program started again from 01.04.2003 and finished at 30.11.2003. The same 35 sentinel villages were located in a 10-th kilometre border strip zone in Bourgas, Yambol, Haskovo and Kardjali districts of Bulgaria as shown in Map 1.

5694 samples of them were derived from the sentinel animals and in a difference from previous 2002 year they had mainly ovine and caprine origin. All 5754 tested serum samples have been determined as negative for presence of NSP FMDV antibodies.
The CHEKIT-FMD-3ABC ELISA OD % value results are shown on Fig 3. The only 5 samples that have a OD% value more than 30% are result of testing additional positive control sera for different purposes and not have the origin from suspected or FMDV positive samples or animals.

Discussion and Conclusions
The virus neutralization test (VNT) and monotype LPHBL ELISA are currently the only prescribed and recommended tests by the O.I.E. for the trade and surveillance. However, these tests require each serum sample to be tested separately for presence of FMDV specific-type antibodies and can not differentiate vaccinated from infected animals. The work with live virus for performance the VNT requires cell culture and virus containment facilities and takes 2-3 days minimum to provide the results.
The evaluation of the specificity of CHEKI-FMD-3ABC ELISA on the basis of distribution of % OD value correlated with the NSP serum samples found positive. Its well shown that only in May 2002, when the suspected animals for the presence of FMDV NSP antibodies have been found and tested in our laboratory the number of samples demonstrated with % OD value as positive or non-conclusive with CHEKI-FMD-3ABC ELISA are grater. Therefore for Bulgaria, which has not enough financial recourses and laboratory staff capacity, and meanwhile is free from the disease for a several years it's easier and chipper to use only one test for FMDV serum-surveillance purposes from the economically point of view.
On the basis of these results and from the experience derived from testing more than 10 000 serum samples from sentinel animas for presence of NSP FMDV antibodies during 2002-2003 we can conclude that CHEKIT-FMD-3ABC ELISA Bommeli can be use with the success as a tool in FMDV serum-surveillance programs in countries free from FMD, using non vaccination practice for the control of FMDV and bordering with regions where FMDV still circulate or still endemic. The test is easy to perform, quicker with reproducible results and with high specificity. Therefore the non-conclusive serum samples for presence of NSP FMDV antibodies have to be retested using the same or the second confirmation test up to the their full determination.

Acknowledgements
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Map1. FMDV sentinel villages 2002-2003

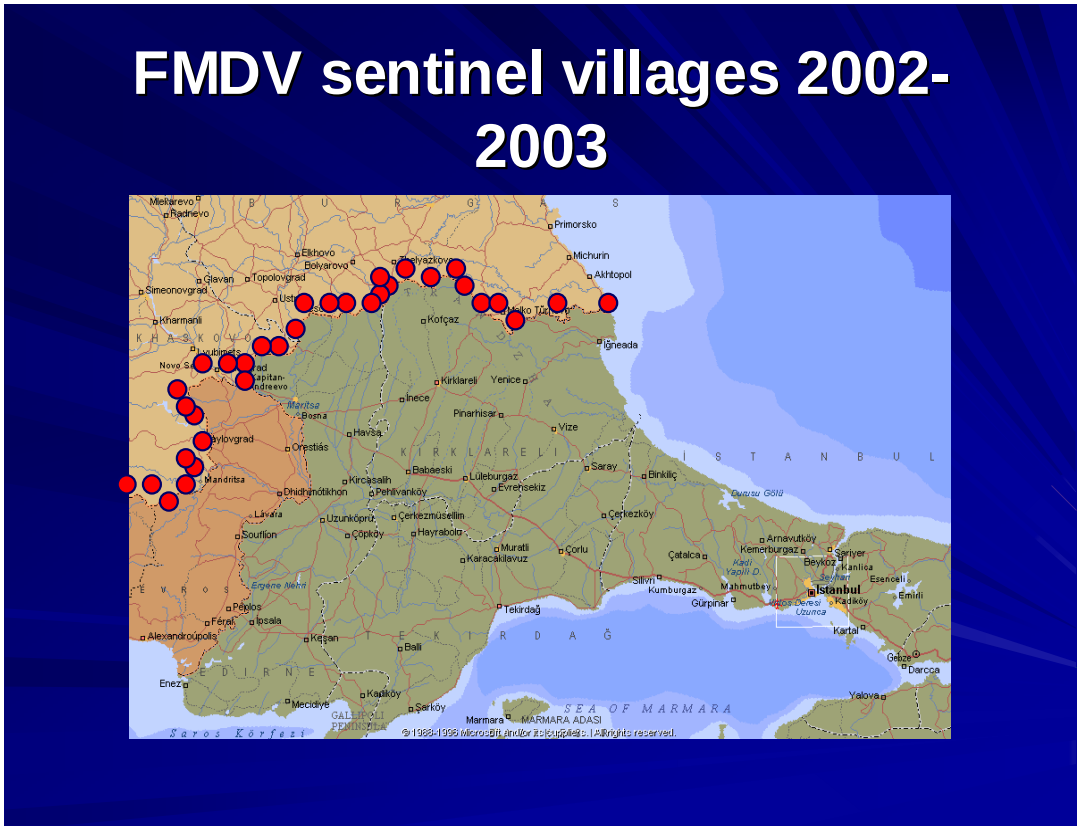


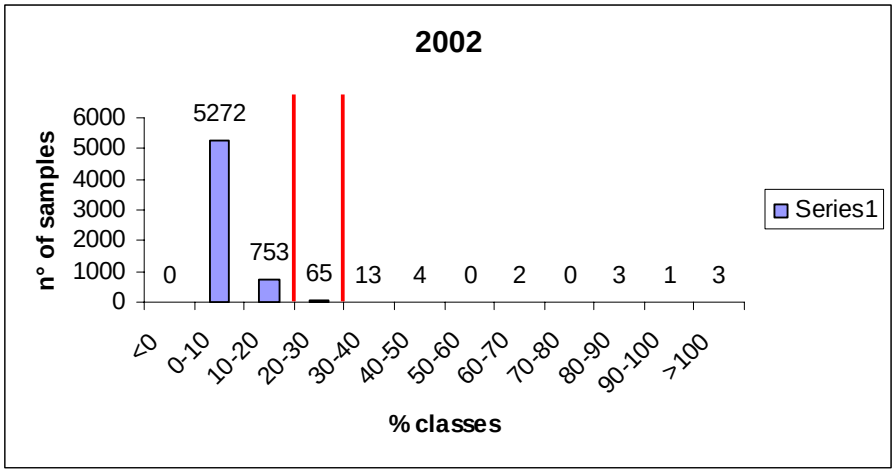
Table 1. Distribution of samples investigated for FMDV antibodies according to the National serum-surveillance programme during 2002

District	Species				NSP FMDV CHEKIT ELISA Result
	Sheep	Goat	Calf	Cattle	
Bourgas	950	175	20	-	NEG
Yambol	1174	40	24	2	NEG
Kardjali	1439	-	-	-	NEG
Haskovo	730	165	20	79	NEG
Total	**4293**	**380**	**64**	**81**	**NEG**

Table 2. Results from further investigations of serum samples from cattle positive by CHEKIT-FMD-3ABC Bommeli

Cattle ear tag number	Result from CHEKIT Bommeli	WRL-IAH Pirbright NSP FMDV ELISA	IZSP Brescia NSP FMDV ELISA	CISA Valdeolmos NSP FMDV ELISA	WRL-IAH Pirbright LPBE O (O-1 Manisa)	WRL-IAH Pirbright LPBE A (A-5.A-22, A-24)	WRL-IAH Pirbright LPBE Asia 1
042888	POS	POS	POS	POS	NEG	NEG	NEG
042504	POS	POS	POS	POS	NEG	NEG	NEG
042505	POS	POS	POS	POS	NEG	NEG	NEG
042526	POS	POS	POS	POS	NEG	NEG	NEG

Fig. 1: Distribution of % OD value for 2002 serum samples investigated with CHEKIT-FMD-3ABC ELISA, calculating by the ELISA results from 6115 plate wells.



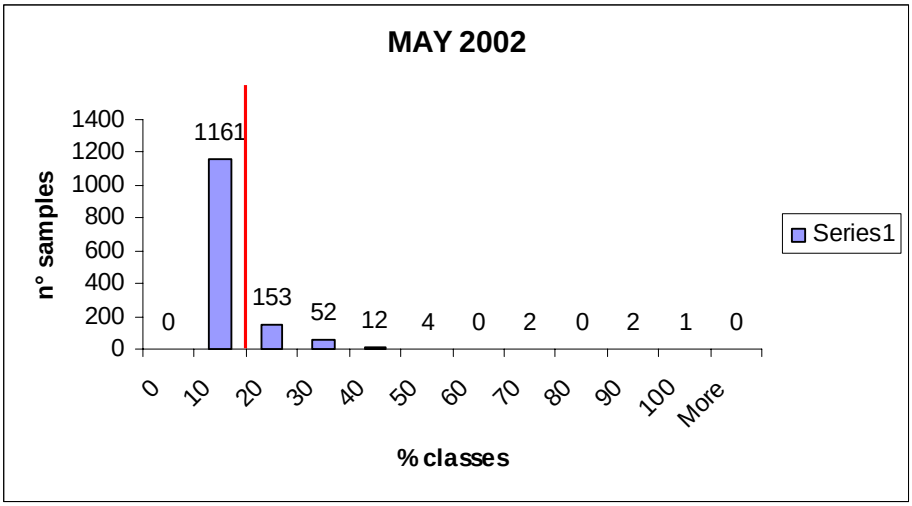


Fig. 2 Distribution of % OD value for May 2002 serum samples investigated with CHEKIT-FMD-3ABC ELISA

Table 3. Distribution of samples investigated for FMDV antibodies according to the National serum-surveillance programme during 2003 and others

District	Species				NSP FMDV CHEKIT ELISA Result
	Goat	Sheep	Wild and circus animals	Trans-border	
Bourgas	96	607	2	12	NEG
Yambol	-	1062	-	2	NEG
Kardjali	170	1174	-	-	NEG
Haskovo	-	2585	-	-	NEG
Blagoevgrad	-	-	19	18	NEG
Sofia	-	7	-	-	NEG
Total	**266**	**5435**	**21**	**32**	**NEG**

Fig. 3 Distribution of % OD value for 2003 serum samples investigated with CHEKIT-FMD-3ABC ELISA, calculating by the ELISA results from 9116 plate wells