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Detection of antibodies to non-structural proteins of foot-and-mouth disease virus (review)

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SUMMARY

Foot-and-mouth disease (FMD) is a highly contagious viral disease of cloven-hooved animals that can cause epidemics and great economic losses. The disease remains a huge problem in developing countries and poses a constant threat to developed countries. Detection of antibodies to FMD virus non-structural proteins in the blood of susceptible animals is an important tool for the disease control. This is the only way for reliable differentiation of vaccinated animals from convalescent and virus-carrier animals. Various modifications of solid-phase enzyme immunosorbent assay (ELISA) have been developed for detection of antibodies to FMD virus nonstructural proteins. Recombinant FMD virus non-structural proteins, mostly 3ABC or 3AB, are used as an antigen for such assay. In a short time, recombinant FMD virus antigen-based ELISA has evolved from an in-house laboratory method to commonly available commercial test systems, most of which have high diagnostic specificity and sensitivity. The said method is widely used for FMD surveillance. In the countries and zones free from FMD without vaccination the ELISA for detection of antibodies against FMD virus non-structural proteins is used as a primary method for FMD serological monitoring and retrospective diagnosis. In the countries and zones free from FMD with vaccination this ELISA is used for confirmation of the virus infection absence in vaccinated herds. In South America, ELISA for detection of antibodies against FMD virus non-structural proteins was used for detection of infected animals during the disease eradication. Currently, it is used for monitoring for the virus circulation in still FMD infected Asian and African countries implementing progressive FMD control programme. In Russia having zones where anti-FMD vaccination is carried out this method is a mandatory tool of the disease surveillance. The review is based on the analysis of 65 publications.

Keywords: review, foot-and-mouth disease virus, non-structural proteins, enzyme-linked immunosorbent assay

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Обнаружение антител к неструктурным белкам вируса ящура (обзор)

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РЕЗЮМЕ

Ящур – высококонтагиозное вирусное заболевание парнокопытных животных, способное вызывать эпизоотии и наносить большой экономический ущерб. Болезнь остается огромной проблемой в развивающихся странах и представляет постоянную угрозу для развитых стран. Важным инструментом контроля заболевания является обнаружение антител к неструктурным белкам вируса ящура в крови восприимчивых животных. Это единственный способ, позволяющий достоверно дифференцировать вакцинированных животных от переболевших и вирусоносителей. Для выявления антител к неструктурным протеинам вируса ящура разработаны различные модификации твердофазного иммуноферментного анализа. В качестве антигена в них используются рекомбинантные неструктурные белки вируса ящура, чаще всего 3ABC или 3AB. За короткий срок иммуноферментный анализ с использованием рекомбинантных антигенов вируса ящура прошел путь от внутрилабораторного метода до общедоступных коммерческих тест-систем, большинство из которых обладают высокой диагностической специфичностью и чувствительностью. Данный метод широко применяется в надзоре за ящуром. В странах или зонах, благополучных по ящуру без вакцинации, иммуноферментный анализ, основанный на обнаружении антител к неструктурным белкам вируса ящура, применяется как основной метод серологического мониторинга и ретроспективной диагностики заболевания. В благополучных по ящуру странах и зонах с вакцинацией этот метод используется для доказательства отсутствия вирусной инфекции в вакцинированных стадах. В Южной Америке при эрадикации заболевания иммуноферментный анализ, основанный на обнаружении антител к неструктурным белкам вируса ящура, применялся для выявления инфицированных животных, а в настоящее время этот метод используется для мониторинга циркуляции возбудителя в еще неблагополучных по заболеванию странах Азии и Африки, реализующих программу прогрессивного контроля за ящуром. В России, как стране с зонами вакцинации против ящура, данный метод является обязательным инструментом надзора за заболеванием. Обзор составлен на основе анализа 65 источников.

Ключевые слова: обзор, вирус ящура, неструктурные белки, иммуноферментный анализ

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INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious disease of domestic and wild cloven-hooved animals manifested by vesicular lesions in oral epithelium, limbs and udder [1]. The disease belongs to transboundary diseases capable of crossing the borders between the countries and causing huge economic losses in animal farming industry.

The disease agent is non-enveloped RNA virus of foot-and-mouth disease virus belonging to *Aphthovirus* genus of *Picornaviridae* family [2]. There are seven FMD virus serotypes*: O*, A, C, Asia-1, SAT-1, SAT-2 and SAT-3. Each FMDV serotype is classified into topotypes, genetic lines and sublines [3, 4].

FMD is controlled by preventive vaccination of susceptible livestock animals in enzootic areas and in buffer zones established in some disease-free countries (for example, in Russia) along the borders to the disease-affected countries. Immunization prevents the clinical disease however not always is capable of preventing subclinical infection and virus persistence [5]. Vaccinated animals that have been FMDV infected could potentially become the infection source for other susceptible animals [6]. Therefore, FMD surveillance programme in the countries and zones where vaccination is practiced envisages obligatory proving the virus infection absence in vaccinated herds. Antibodies to FMDV non-structural proteins is the most reliable indicator of the infection in vaccinated animals [7–10].

Four structural proteins (1A, 1B, 1C and 1D) and ten non-structural proteins (L, 2A, 2B, 2C, 3A, $3B_{1-3}$, 3C and 3D) are synthesized in equal amounts during FMDV replication in cells (Fig. 1). Non-structural proteins perform various functions during the virus replication in infected cells: polymerase, helicase, protease functions and etc. Functions of some FMDV non-structural proteins remain unclear [1, 11]. In contrast to structural proteins, non-structural proteins are highly conserved, i.e. they do not differ in different FMDV serotypes when tested with serological methods.

The virus-infected animals develop antibodies to all viral proteins: both to structural and non-structural proteins. Anti-FMD vaccinated animals have a distinct antibody profile: only antibodies against the viral structural proteins are, as a rule, detected in such animals. This is accounted for the fact that modern anti-FMD vaccines are subjected to purification and most of the viral non-structural proteins together with cellular debris are removed from the vaccines during this stage [12, 13]. Thus, detection of FMDV non-structural proteins can serve as evidence that the animal is infected with the virus, regardless of whether it has been vaccinated or not.

DEVELOPMENT OF SEROLOGICAL METHODS FOR DETECTION OF ANTIBODIES AGAINST FMDV NON-STRUCTURAL PROTEINS

Different techniques for detection of antibodies against FMDV non-structural proteins were tested: agar gel immunodiffusion assay [14], immunoblotting [15], etc. However, solid-phase immunosorbent assay (ELISA) has turned out to be the most technologically-advanced method [16].

Development of the ELISA intended for detection of antibodies to FMDV non-structural proteins (NSP-ELISA) requires addressing the antigen preparation challenge. Since preparation of purified FMDV non-structural proteins from infected cell culture is difficult, all researchers used chemically synthesized peptides [17, 18] or, much more often, recombinant proteins [19] as an antigen in NSP-ELISA test-kits. Recombinant proteins were generated by molecular cloning and expression of relevant FMDV genes in *Escherichia coli* (*E. coli*) [19–23] or in baculovirus-insect cells system [24–27].

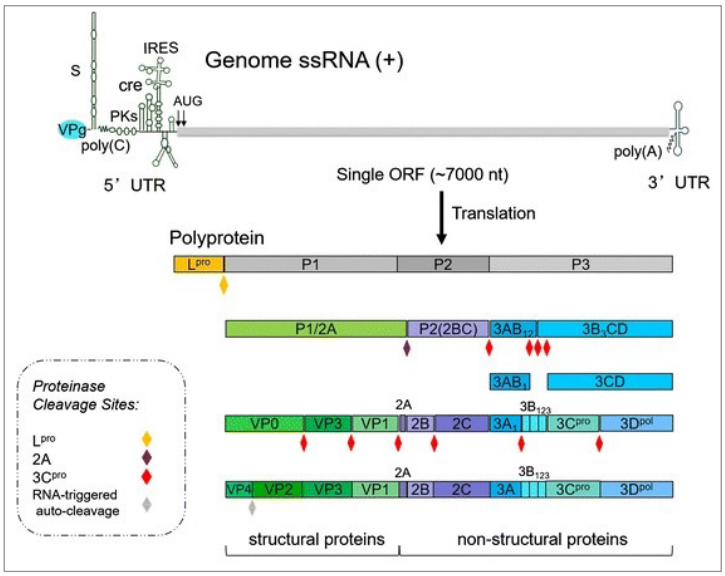

Fig. 1. Schematic diagram of FMD virus genome and processing of viral proteins [11]

Almost all non-structural proteins of FMDV have been tested as antigens for NSP-ELISA test-kit [28–32]. Multiple tests have showed that NSP-ELISA test-kit based on 3AB or 3ABC recombinant proteins provides the best differentiation between post-infection and postvaccinal antibodies [8, 20, 21, 23, 32–37]. Antigenicity of 3 ABC polyprotein appears to be determined by 3A and 3B proteins being its parts, role of 3C protein is significantly lower. Thus, it is shown that 3C protein does not to react with hyperimmune serum when tested by immunoblotting and so does not contain linear epitopes recognized by antibodies [34].

Several solid-phase ELISA variants were developed for detection of antibodies to FMDV non-structural proteins. Anti-species (i.e. species-specific) conjugate was used for indirect ELISA for detection of antibodies bound to FMDV non-structural antigen. Therewith, indirect ELISA in most cases allows for testing sera from cattle, small ruminants as well as from pigs. Enzyme-labeled polyclonal [38] or monoclonal [24] antibodies against FMDV non-structural proteins were used as detection antibodies for competitive ELISA. This ELISA variant allows for testing of sera from animals of all species. In 2015, M. Hosamani et al. proposed to use horseradish peroxidase-conjugated G protein for indirect 3ABC ELISA. This allowed for testing of sera from animals of different species with indirect ELISA [39].

Most described ELISA test-systems based on 3ABC protein or its components demonstrated high specificity and sensitivity. NSP-ELISA allowed detection of post-infection antibodies to FMDV within 12–18 months after infection starting with day 7–10 after infection [21, 23, 28, 40–42]. E. Elnekave et al. [43] have managed to detect antibodies 3 years after infection and W. B. Chung et al. have managed to detect antibodies 3.5 years after infection [25].

Initially, NSP-ELISA was used only in the developer laboratories, then it was commercialized. Currently, several companies (Bionote, IDEXX Laboratories, Inc., ThermoFisher Scientific, Inc. (USA), IDVet (France), IZSLER (Italy), etc.) produce commercial test-kits for detection of antibodies against FMDV non-structural proteins.

There are several publications aimed at comparative assessment of the commercial test-kits [42, 44–46]. E. Brocchi et al. [47] carried out the most extensive comparative study. They compared six diagnostic kits: CHEKIT-FMD-3ABC (Bommeli Diagnostics, Switzerland); UBI® FMDV NS EIA (United Biomedical, Inc., USA); I-ELISA 3ABC/EITB (PANAFTOSA, USA); Ceditest® FMDV-NS ELISA (Cedi Diagnostics B.V., Netherlands); 3ABC trapping-ELISA (IZS-Brescia, Italy); SVANOVIR™ FMDV 3ABC-Ab ELISA (Svanova, Sweden). A total of 3,551 sera from cattle and small ruminants from 9 countries were tested with each test-kit. All tested kits demonstrated high specificity (> 96%), however their sensitivity varied significantly. The following test-kits demonstrated the highest relative sensitivity: 3ABC trapping-ELISA (100%), I-ELISA 3ABC/EITB (99.6%) and Ceditest® FMDV-NS ELISA (99.6%), the sensitivity of the following test-kits was significantly lower: UBI® FMDV NS EIA (88.8%), SVANOVIR™ FMDV 3ABC-Ab ELISA (83.6%) and CHEKIT-FMD-3ABC (81.5%).

Since the diagnostic specificity of all test systems is not absolute, false positive results could be obtained when the said test-kits are used for serological diagnosis of foot-and-mouth disease. It is recommended to use confirmatory test that may be either NSP-ELISA of other manufacturer or immunoblotting, to address this problem [8, 48].

NSP-ELISA FOR FMD SURVEILLANCE

Currently, the World Organization for Animal Health (WOAH) recommends to use NSP-ELISA as an official method for serological diagnosis of FMD [48].

For countries free from FMD without vaccination NSP-ELISA is an ideal tool for FMD seromonitoring and retrospective diagnosis since it allows for detection of antibodies to all seven FMDV serotypes using single test [8, 49].

Detection of antibodies to FMDV non-structural proteins is a key diagnostic tool for FMD surveillance in the regions where this disease is enzootic [50]. NSP-ELISA was used as a screening method for identification of infected animals in vaccinated herds during FMD eradication campaign in South America. FMD-infected countries that have adopted and implement national roadmaps on progressive FMD control in the framework of the Progressive Control Pathway for Food-and-Mouth Disease developed by the Food and Agriculture Organization of the United Nations (FAO) use NSP-ELISA for the virus circulation monitoring [1].

Countries applying for FMD-free status or recovery of the lost FMD-free status should submit a dossier to the WOAH, which, among other things, should contain the results of testing of animals for antibodies to FMDV non-structural proteins. Countries after being recognized as FMD-free should continue serological testing for the free status confirmation.

NSP-ELISA is a mandatory tool used to control transboundary transportation of live animals and animal products. International regulations governing the trade in livestock animals require testing of horned livestock and pigs imported from countries or zones with vaccination for antibodies to the FMDV [51].

The wide use of NSP-ELISA for diagnostic and monitoring tests for foot-and-mouth disease has revealed multiple advantages of the method as well as certain limitations related to its use. The advantages include high diagnostic sensitivity and specificity, throughput capacity, the ability to detect antibodies to all FMDV serotypes using single test.

The limitations related to NSP-ELISA use are accounted for not so much the method characteristics as the peculiarities of the tested samples. Early studies have already shown that NSP-ELISA detects antibodies in some vaccinated animals [7, 52–56]. S. P. Chen et al. using PrioCHECK FMDV NS test-kit (ThermoFisher Scientific, Inc., USA) found that non-vaccinated or once vaccinated pigs were seronegative, but antibodies to non-FMD virus structural proteins were detected in 16.2% of animals vaccinated several times [54]. The study performed by G. K. Sharma et al. showed that the specificity of NSP-ELISA used for tests of non-vaccinated animals was very high and reached 100% [42]. However, antibodies to FMDV non-structural proteins were detected in 33% of animals when samples were collected from animals on day 14 after vaccination and tested with PrioCHECK FMDV NS. Such large number of animals having antibodies to FMDV non-structural proteins can be accounted for the fact that the Indian vaccine was not appropriately purified from the virus non-structural proteins (i.e. not compliant with the requirements for the vaccine purity). However, there are

Fig. 2. FMD-NSP-ELISA test-kit for detection of antibodies against FMD virus non-structural proteins produced by the FGBI "ARRIAH"

other multiple data evidencing that some animals develop antibodies against FMDV non-structural proteins after their vaccination. Thus, in experiments carried out by T. Tekleghiorghis et al. seven out of one hundred cattle had antibodies to FMDV non-structural proteins after single vaccination [57]. It should be noted that the level of such antibodies was low: the maximum percent inhibition was 58% at cut-off of 50% when the sera were tested with PrioCHECK FMDV NS test-kit.

In studies conducted by Chinese experts, the number of vaccinated cattle with antibodies against the FMDV non-structural proteins correlated with the number of vaccinations: there were 2.15% of seropositive animals among the animals vaccinated up to 10 times and already 5.93% of seropositive animals among the animals vaccinated up to 15 times [58].

Vaccine purification appears not to completely remove FMDV non-structural proteins from the vaccine [58, 59]. This was obviously the circumstance that forced the WOAH to amend requirements for anti-FMD vaccines. According to previous rules, the vaccine shall not induce antibodies to FMDV non-structural antibodies after three vaccinations and according current WOAH Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, it is allowed that one of eight animals vaccinated twice has such antibodies [48].

Thus, possible detection of seropositive animals having postvaccinal but not post-infection antibodies to FMDV non-structural proteins should be taken into account when NSP-ELISA is used for diagnostic and monitoring tests carried out in zones where vaccination is practiced.

USE OF NSP-ELISA IN RUSSIA

In the Russian Federations studies aimed at development of ELISA for detection of the antibodies to FMDV non-structural proteins were carried out at the Federal Centre for Animal Health (FGBI "ARRIAH", Vladimir). Recombinant FMDV proteins 3A, 3B and 3AB were produced by expression in *E. coli* [60]. Indirect ELISA for detection of antibodies to FMDV non-structural proteins in cattle sera was developed based on the recombinant antigens [61, 62]. Later, improved 3AB-ELISA variant allowing testing not only cattle sera but also sera from small ruminants was proposed [63]. This method passed validation showing that its diagnostic specificity and sensitivity was 99.8% and 96.6%, respectively [64]. This method is included in the scope of the FGBI "ARRIAH" accreditation.

Since Russia is one of the countries that have zones where anti-FMD vaccination is carried out, NSP-ELISA is a mandatory tool for serological monitoring of the disease. During the serological surveillance the algorithm for diagnostic testing of sera recommended by the WOAH for FMD [51] as well as used in medicine for diagnosis of some highly significant diseases, for example AIDS, is used[1]. Its essence lies in the use of two test-systems: screening and confirmatory. In the FGBI "ARRIAH" initial screening of samples is carried out with 3AB-ELISA developed by A. S. Yakovleva et al. [64], and then all positive samples are tested again with commercial PrioCHECK FMDV NS test-kit. The sample is considered positive when it is tested positive with both tests-systems. Thus, usage of screening and confirmatory tests allows for exclusion of false positive results during diagnostic and monitoring tests [65].

About 250,000 serum samples from cattle and small ruminants submitted from all Russian Federation regions were tested with NSP-ELISA at the FGBI "ARRIAH" within the federal FMD monitoring in 2015–2022. Serological test results were included as evidence in the dossiers

[1] SanPiN 3.3686–21 Sanitary and epidemiological requirements for infectious disease prevention approved by the Ordinance of the Chief Medical Officer of the Russian Federation No. 4 of 28 January 2021. Available at: https://docs.cntd.ru/document/573660140.

submitted to the WOAH. The WOAH based the said dossiers granted the status of the country with FMD free zone without vaccination (the zone includes 52 Subjects of the Russian Federation) and status of the country with three FMD free zones with vaccination (the zones include another 16 Subjects of the Russian Federation) to the Russian Federation[2].

The FGBI "ARRIAH" produces commercial FMD NSP-ELISA test-kit for detection antibodies to FMDV non-structural proteins to provide regional veterinary laboratories of the Russian Federation with up-to-date tools for FMD serological diagnosis (Fig. 2)[3].

CONCLUSION

Detection of antibodies to FMDV non-structural proteins is an important tool for the disease surveillance. Ability of FMDV NSP-tests to detect the infection regardless of the virus serotype has determined their demand in the countries that are FMD free without vaccination and ability of NPS-ELISA tests to detect the infection in vaccinated animals results in their wide use in the countries or zones where vaccination is practiced. However, it should be noted that differentiation between vaccinated and infected animals with FMD NSP-tests becomes possible only if highly purified anti-FMD vaccines are used.

REFERENCES

1. Jamal S. M., Belsham G. J. Foot-and-mouth disease: past, present and future. *Vet. Res*. 2013; 44 (1):116. DOI: 10.1186/1297-9716-44-116.

2. Grubman M. J., Baxt B. Foot-and-mouth disease. *Clin. Microbiol. Rev*. 2004; 17 (2): 465–493. DOI: 10.1128/CMR.17.2.465-493.2004.

3. Samuel A. R., Knowles N. J. Foot-and-mouth disease type O viruses exhibit genetically and geographically distinct evolutionary lineages (topotypes). *J. Gen. Virol*. 2001; 82 (3): 609–621. DOI: 10.1099/0022-1317-82-3-609.

4. Carrillo C., Tulman E. R., Delhon G., Lu Z., Carreno A., Vagnozzi A., et al. Comparative genomics of foot-and-mouth disease virus. *J. Virol*. 2005; 79 (10): 6487–6504. DOI: 10.1128/JVI.79.10.6487-6504.2005.

5. Hutber A. M., Kitching R. P., Conway D. A. Control of foot-and-mouth disease through vaccination and the isolation of infected animals. *Trop. Anim. Health. Prod*. 1998; 30 (4): 217–227. DOI: 10.1023/a:1005071027414.

6. Arzt J., Belsham G. J., Lohse L., Bøtner A., Stenfeldt C. Transmission of foot-and-mouth disease from persistently infected carrier cattle to naive cattle via transfer of oropharyngeal fluid. *mSphere*. 2018; 3 (5):e00365–18. DOI: 10.1128/mSphere.00365-18.

7. Crowther J. R. Review of the developments in the detection of antibodies to non-structural proteins of foot and mouth disease virus. *In: The use of non-structural proteins of foot and mouth disease virus (FMDV) to differentiate between vaccinated and infected animals*. Vienna: IAEA; 2007; 3–46. Available at: https://www-pub.iaea.org/MTCD/publications/PDF/te_1546_web.pdf. (date of access: 18.01.2023).

8. Tewari A., Ambrose H., Parekh K., Inoue T., Guitian J., Nardo A. D., et al. Development and validation of confirmatory foot-and-mouth disease virus antibody ELISAs to identify infected animals in vaccinated populations. *Viruses*. 2021; 13 (5):914. DOI: 10.3390/v13050914.

9. Clavijo A., Wright P., Kitching P. Developments in diagnostic techniques for differentiating infection from vaccination in foot-and-mouth disease. *Vet. J*. 2004; 167 (1): 9–22. DOI: 10.1016/s1090-0233(03)00087-x.

10. Yadin H., Brenner J., Chai D., Oved Z., Hadany Y., Kusak A., Haimovich M. The NSP immune response of vaccinated animals after in-field exposure to FMDV. *Vaccine*. 2007; 25 (49): 8298–8305. DOI: 10.1016/j.vaccine.2007.09.071.

11. Gao Y., Sun S. Q., Guo H. C. Biological function of foot-and-mouth disease virus non-structural proteins and non-coding elements. *Virol. J*. 2016; 13:107. DOI: 10.1186/s12985-016-0561-z.

12. Doel T. R. FMD vaccines. *Virus Res*. 2003; 91 (1): 81–99. DOI: 10.1016/s0168-1702(02)00261-7.

13. Lubroth J., Grubman M. J., Burrage T. G., Newman J. F., Brown F. Absence of protein 2C from clarified foot-and-mouth disease virus vaccines provides the basis for distinguishing convalescent from vaccinated animals. *Vaccine*. 1996; 14 (5): 419–427. DOI: 10.1016/0264-410x(95)00172-w.

14. McVicar J. W., Sutmoller P. Foot-and-mouth disease: the agar gel diffusion precipitin test for antibody to virus-infection-associated (via) antigen as a tool for epizootiologic surveys. *Am. J. Epidemiol*. 1970; 92 (4): 273–278. DOI: 10.1093/oxfordjournals.aje.a121207.

15. Bergmann I. E., de Mello P. A., Neitzert E., Beck E., Gomes I. Diagnosis of persistent aphthovirus infection and its differentiation from vaccination response in cattle by use of enzyme-linked immunoelectrotransfer blot analysis with bioengineered non-structural viral antigens. *Am. J. Vet. Res*. 1993; 54 (6): 825–831. PMID: 8391765.

16. Ma L. N., Zhang J., Chen H. T., Zhou J. H., Ding Y. Z., Liu Y. S. An overview on ELISA techniques for FMD. *Virol. J*. 2011; 8:419. DOI: 10.1186/1743-422X-8-419.

17. Shen F., Chen P. D., Walfield A. M., Ye J., House J., Brown F., Wang C. Y. Differentiation of convalescent animals from those vaccinated against foot-and-mouth disease by a peptide ELISA. *Vaccine*. 1999; 17 (23–24): 3039–3049. DOI: 10.1016/s0264-410x(99)00148-6.

18. Oem J. K., Kye S. J., Lee K. N., Park J. H., Kim Y. J., Song H. J., Yeh M. Development of synthetic peptide ELISA based on non-structural protein 2C of foot and mouth disease virus. *J. Vet. Sci*. 2005; 6 (4): 317–325. PMID: 16293996.

19. Crowther J. R. The use of non-structural (NS) antigens of FMD virus to assess antibodies in vaccinanted and infected livestock. *In: Foot-and-mouth disease: control strategies. Symposium Proceedings (2–5 June 2002)*. Lyons, France: 2002; 377.

20. Rodríguez A., Dopazo J., Sáiz J. C., Sobrino F. Immunogenicity of non-structural proteins of foot-and-mouth disease virus: differences between infected and vaccinated swine. *Arch. Virol*. 1994; 136 (1–2): 123–131. DOI: 10.1007/BF01538822.

21. De Diego M., Brocchi E., Mackay D., De Simone F. The non-structural polyprotein 3ABC of foot-and-mouth disease virus as a diagnostic antigen in ELISA to differentiate infected from vaccinated cattle. *Arch. Virol*. 1997; 142 (10): 2021–2033. DOI: 10.1007/s007050050219.

22. Foster M., Cook A., Cedillo L., Parkhouse R. M. Serological and cellular immune responses to non-structural proteins in animals infected with FMDV. *Vet. Q*. 1998; 20 (Suppl. 2): S28–30. DOI: 10.1080/01652176.1998.9694960.

23. Mackay D. K., Forsyth M. A., Davies P. R., Berlinzani A., Belsham G. J., Flint M., Ryan M. D. Differentiating infection from vaccination in foot-and-mouth disease using a panel of recombinant, non-structural proteins in ELISA. *Vaccine*. 1998; 16 (5): 446–459. DOI: 10.1016/s0264-410x(97)00227-2.

24. Sørensen K. J., de Stricker K., Dyrting K. C., Grazioli S., Haas B. Differentiation of foot-and-mouth disease virus infected animals from vaccinated animals using a blocking ELISA based on baculovirus expressed FMDV 3ABC antigen and a 3ABC monoclonal antibody. *Arch. Virol*. 2005; 150 (4): 805–814. DOI: 10.1007/s00705-004-0455-z.

[2] Available at: https://www.woah.org/en/disease/foot-and-mouth-disease/#ui-id-2.

[3] Available at: https://shop.arriah.ru/catalog/diagnostikum/nabor-dlya-obnaruzheniya-antitel-k-nestrukturnym-belkam-virusa-yashchura-immunofermentnym-metodom-v-.

25. Chung W. B., Sorensen K. J., Liao P. C., Yang P. C., Jong M. H. Differentiation of foot-and-mouth disease virus-infected from vaccinated pigs by enzyme-linked immunosorbent assay using nonstructural protein 3AB as the antigen and application to an eradication program. *J. Clin. Microbiol*. 2002; 40 (8): 2843–2848. DOI: 10.1128/JCM.40.8.2843-2848.2002.

26. Kweon C. H., Ko Y. J., Kim W. I., Lee S. Y., Nah J. J., Lee K. N., et al. Development of a foot-and-mouth disease NSP ELISA and its comparison with differential diagnostic methods. *Vaccine*. 2003; 21 (13–14): 1409–1414. DOI: 10.1016/s0264-410x(02)00684-9.

27. Meyer R. F., Babcock G. D., Newman J. F., Burrage T. G., Toohey K., Lubroth J., Brown F. Baculovirus expressed 2C of foot-and-mouth disease virus has the potential for differentiating convalescent from vaccinated animals. *J. Virol. Methods*. 1997; 65 (1): 33–43. DOI: 10.1016/s0166-0934(96)02165-9.

28. Biswal J. K., Jena S., Mohapatra J. K., Bisht P., Pattnaik B. Detection of antibodies specific for foot-and-mouth disease virus infection using indirect ELISA based on recombinant nonstructural protein 2B. *Arch. Virol*. 2014; 159 (7): 1641–1650. DOI: 10.1007/s00705-013-1973-3.

29. Biswal J. K., Ranjan R., Pattnaik B. Diagnostic application of recombinant non-structural protein 3A to detect antibodies induced by foot-and-mouth disease virus infection. *Biologicals*. 2016; 44 (3): 157–162. DOI: 10.1016/j.biologicals.2016.02.004.

30. Mahajan S., Mohapatra J. K., Pandey L. K., Sharma G. K., Pattnaik B. Truncated recombinant non-structural protein 2C-based indirect ELISA for FMD sero-surveillance. *J. Virol. Methods*. 2013; 193 (2): 405–414. DOI: 10.1016/j.jviromet.2013.07.003.

31. Mohapatra A. K., Mohapatra J. K., Pandey L. K., Sanyal A., Pattnaik B. Diagnostic potential of recombinant nonstructural protein 3B to detect antibodies induced by foot-and-mouth disease virus infection in bovines. *Arch. Virol*. 2014; 159 (9): 2359–2369. DOI: 10.1007/s00705-014-2089-0.

32. He C., Wang H., Wei H., Yan Y., Zhao T., Hu X., et al. A recombinant truncated FMDV 3AB protein used to better distinguish between infected and vaccinated cattle. *Vaccine*. 2010; 28 (19): 3435–3439. DOI: 10.1016/j.vaccine.2010.02.072.

33. Bergmann I. E., Malirat V., Neitzert E., Beck E., Panizzutti N., Sánchez C., Falczuk A. Improvement of a serodiagnostic strategy for foot-and-mouth disease virus surveillance in cattle under systematic vaccination: a combined system of an indirect ELISA-3ABC with an enzyme-linked immunoelectrotransfer blot assay. *Arch. Virol*. 2000; 145 (3): 473–489. DOI: 10.1007/s007050050040.

34. Sun T., Lu P., Wang X. Localization of infection-related epitopes on the non-structural protein 3ABC of foot-and-mouth disease virus and the application of tandem epitopes. *J. Virol. Methods*. 2004; 119 (2): 79–86. DOI: 10.1016/j.jviromet.2004.02.016.

35. Lu Z., Cao Y., Guo J., Qi S., Li D., Zhang Q., et al. Development and validation of a 3ABC indirect ELISA for differentiation of foot-and-mouth disease virus infected from vaccinated animals. *Vet. Microbiol*. 2007; 125 (1–2): 157–169. DOI: 10.1016/j.vetmic.2007.05.017.

36. Zia M. A., Dobson S. J., Rowlands D. J., Stonehouse N. J., Shah M. S., Habib M. Development of an ELISA to distinguish between foot-and-mouth disease virus infected and vaccinated animals utilising the viral non-structural protein 3ABC. *J. Med. Microbiol*. 2022; 71 (4). DOI: 10.1099/jmm.0.001516.

37. Brocchi E., De Diego M. I., Berlinzani A., Gamba D., De Simone F. Diagnostic potential of Mab-based ELISAs for antibodies to non-structural proteins of foot-and-mouth disease virus to differentiate infection from vaccination. *Vet. Q*. 1998; 20 (Suppl. 2): S20–24. PMID: 9652058.

38. Sørensen K. J., Madsen K. G., Madsen E. S., Salt J. S., Nqindi J., Mackay D. K. Differentiation of infection from vaccination in foot-and-mouth disease by the detection of antibodies to the non-structural proteins 3D, 3AB and 3ABC in ELISA using antigens expressed in baculovirus. *Arch. Virol*. 1998; 143 (8): 1461–1476. DOI: 10.1007/s007050050390.

39. Hosamani M., Basagoudanavar S. H., Tamil Selvan R. P., Das V., Ngangom P., Sreenivasa B. P., et al. A multi-species indirect ELISA for detection of non-structural protein 3ABC specific antibodies to foot-and-mouth disease virus. *Arch. Virol*. 2015; 160 (4): 937–944. DOI: 10.1007/s00705-015-2339-9.

40. Nanni M., Alegre M., Compaired D., Taboga O., Fondevila N. Novel purification method for recombinant 3AB1 nonstructural protein of foot-and-mouth disease virus for use in differentiation between infected and vaccinated animals. *J. Vet. Diagn. Invest*. 2005; 17 (3): 248–251. DOI: 10.1177/104063870501700306.

41. Silberstein E., Kaplan G., Taboga O., Duffy S., Palma E. Foot-and-mouth disease virus-infected but not vaccinated cattle develop antibodies against recombinant 3AB1 nonstructural protein. *Arch. Virol*. 1997; 142 (4): 795–805. DOI: 10.1007/s007050050119.

42. Sharma G. K., Mohapatra J. K., Mahajan S., Matura R., Subramaniam S., Pattnaik B. Comparative evaluation of non-structural protein-antibody detecting ELISAs for foot-and-mouth disease sero-surveillance under intensive vaccination. *J. Virol. Methods*. 2014; 207: 22–28. DOI: 10.1016/j.jviromet.2014.06.022.

43. Elnekave E., Shilo H., Gelman B., Klement E. The longevity of anti NSP antibodies and the sensitivity of a 3ABC ELISA – a 3 years follow up of repeatedly vaccinated dairy cattle infected by foot and mouth disease virus. *Vet. Microbiol*. 2015; 178 (1–2): 14–18. DOI: 10.1016/j.vetmic.2015.04.003.

44. Bronsvoort B. M., Sørensen K. J., Anderson J., Corteyn A., Tanya V. N., Kitching R. P., Morgan K. L. Comparison of two 3ABC enzyme-linked immunosorbent assays for diagnosis of multiple-serotype foot-and-mouth disease in a cattle population in an area of endemicity. *J. Clin. Microbiol*. 2004; 42 (5): 2108–2114. DOI: 10.1128/JCM.42.5.2108-2114.2004.

45. Moonen P., van der Linde E., Chénard G., Dekker A. Comparable sensitivity and specificity in three commercially available ELISAs to differentiate between cattle infected with or vaccinated against foot-and-mouth disease virus. *Vet. Microbiol*. 2004; 99 (2): 93–101. DOI: 10.1016/j.vetmic.2003.12.003.

46. Lee F., Lin Y. L., Jong M. H. Comparison of ELISA for the detection of porcine serum antibodies to non-structural proteins of foot-and-mouth disease virus. *J. Virol. Methods*. 2004; 116 (2): 155–159. DOI: 10.1016/j.jviromet.2003.11.004.

47. Brocchi E., Bergmann I. E., Dekker A., Paton D. J., Sammin D. J., Greiner M., et al. Comparative evaluation of six ELISAs for the detection of antibodies to the non-structural proteins of foot-and-mouth disease virus. *Vaccine*. 2006; 24 (47–48): 6966–6979. DOI: 10.1016/j.vaccine.2006.04.050.

48. Foot and mouth disease (infection with foot and mouth disease virus). *In: WOAH. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chapter 3.1.8*. Available at: https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.01.08_FMD.pdf.

49. Paton D. J., Ferris N. P., Hutchings G. H., Li Y., Swabey K., Keel P., et al. Investigations into the cause of foot-and-mouth disease virus seropositive small ruminants in Cyprus during 2007. *Transbound. Emerg. Dis*. 2009; 56 (8): 321–328. DOI: 10.1111/j.1865-1682.2009.01088.x.

50. Chitray M., Grazioli S., Willems T., Tshabalala T., De Vleeschauwer A., Esterhuysen J. J., et al. Development and validation of a foot-and-mouth disease virus SAT serotype-specific 3ABC assay to differentiate infected from vaccinated animals. *J. Virol. Methods*. 2018; 255: 44–51. DOI: 10.1016/j.jviromet.2018.02.006.

51. WOAH. Terrestrial Animal Health Code. Available at: https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access.

52. Armstrong R. M., Cox S. J., Aggarwal N., Mackay D. J., Davies P. R., Hamblin P. A., et al. Detection of antibody to the foot-and-mouth disease virus (FMDV) non-structural polyprotein 3ABC in sheep by ELISA. *J. Virol. Methods*. 2005; 125 (2): 153–163. DOI: 10.1016/j.jviromet.2005.01.012.

53. Robiolo B., Seki C., Fondevilla N., Grigera P., Scodeller E., Periolo O., et al. Analysis of the immune response to FMDV

structural and non-structural proteins in cattle in Argentina by the combined use of liquid phase and 3ABC-ELISA tests. *Vaccine*. 2006; 24 (7): 997–1008. DOI: 10.1016/j.vaccine.2005.08.071.

54. Chen S. P., Ellis T. M., Lee M. C., Cheng I. C., Yang P. C., Lin Y. L., et al. Comparison of sensitivity and specificity in three commercial foot-and-mouth disease virus non-structural protein ELISA kits with swine sera in Taiwan. *Vet. Microbiol*. 2007; 119 (2–4): 164–172. DOI: 10.1016/j.vetmic.2006.09.013.

55. Mohapatra J. K., Pandey L. K., Sanyal A., Pattnaik B. Recombinant non-structural polyprotein 3AB-based serodiagnostic strategy for FMD surveillance in bovines irrespective of vaccination. *J. Virol. Methods*. 2011; 177 (2): 184–192. DOI: 10.1016/j.jviromet.2011.08.006.

56. Lee F., Jong M. H., Yang D. W. Presence of antibodies to non-structural proteins of foot-and-mouth disease virus in repeatedly vaccinated cattle. *Vet. Microbiol*. 2006; 115 (1–3): 14–20. DOI: 10.1016/j.vetmic.2005.12.017.

57. Tekleghiorghis T., Weerdmeester K., van Hemert-Kluitenberg F., Moormann R. J., Dekker A. Comparison of test methodologies for foot-and-mouth disease virus serotype A vaccine matching. *Clin. Vaccine. Immunol*. 2014; 21 (5): 674–683. DOI: 10.1128/CVI.00034-14.

58. Liu Z. Z., Zhao F. R., Gao S. D., Shao J. J., Zhang Y. G., Chang H. Y. Development of a chemiluminescence immunoassay using recombinant non-structural epitope-based proteins to accurately differentiate foot-and-mouth disease virus-infected and vaccinated bovines. *Transbound. Emerg. Dis*. 2018; 65 (2): 338–344. DOI: 10.1111/tbed.12811.

59. Barnett P. V., Geale D. W., Clarke G., Davis J., Kasari T. R. A review of OIE country status recovery using vaccinate-to-live versus vaccinate-to-die foot-and-mouth disease response policies I: benefits of higher potency vaccines and associated NSP DIVA test systems in post-outbreak surveillance. *Transbound. Emerg. Dis*. 2015; 62 (4): 367–387. DOI: 10.1111/tbed.12166.

60. Yakovleva A. S., Kanshina A. V., Scherbakov A. V. Expression of recombinant nonstructural proteins 3A, 3B and 3AB of foot-and-mouth disease virus in *E. coli*. *Proceedings of the Federal Centre for Animal Health*. 2005; 3: 105–114. EDN: UNWYYB. (in Russ.)

61. Yakovleva A. S., Shcherbakov A. V., Kanshina A. V., Mudrak N. S., Fomina T. A. Recombinant non-structural 3A, 3B and 3AB proteins of foot-and-mouth disease virus: use in indirect ELISA for differentiation of vaccinated and infected cattle. *Mol. Biol*. 2006; 40 (1): 146–151. DOI: 10.1134/S0026893306010195.

62. Yakovleva A. S., Kanshina A. V., Scherbakov A. V., Mudrak N. S., Fomina T. A. Development of an indirect ELISA based on *E. coli* expressed 3A, 3B and 3AB nonstructural proteins of FMDV to differentiate between infected and vaccinated cattle. *Proceedings of the Federal Centre for Animal Health*. 2005; 3: 115–126. EDN: UNWYYL. (in Russ.)

63. Yakovleva A., Kanshina A., Scherbakov A. Development of indirect ELISA for detection of antibodies to foot-and-mouth disease virus nonstructural proteins. *In: The Global Control of FMD – Tool, Ideas and Ideals: Book and Abstr. Europ. Commiss. Control FMD*. FAO; 2008; 80.

64. Yakovleva A. S., Kanshina A. V., Scherbakov A. V., Orlova Ye. S. Development and validation of 3AB-ELISA test-system for detection of antibodies to FMD virus nonstructural proteins in blood sera from cattle and small ruminants. *Veterinary Science Today*. 2015; (4): 36–42. EDN: VODNXP. (in Russ.)

65. Kanshina A. V., Yakovleva A. S., Orlova Y. S., Scherbakov A. V. Seromonitoring yashchura = Seromonitoring for foot-and-mouth disease. *Molekulyarnaya diagnostika: sbornik nauchnykh trudov X Yubileinoi mezhdunarodnoi nauchno-prakticheskoi konferentsii (Moskva, 9–11 noyabrya 2021 g.) = Molecular diagnosis: Proceedings of the X Anniversary International Scientific and Practical Conference (Moscow, November 9–11, 2021)*. Tambov: OOO firma "Yulis"; 2021; 2: 349–350. EDN: FEDYZQ.

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