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Non-capsid proteins to identify foot-and-mouth disease viral circulation in cattle irrespective of vaccination

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Abstract

The ability of foot-and-mouth disease virus (FMDV) to establish subclinical and even persistent infection, the so called carrier state, imposes the need to reliably demonstrate absence of viral circulation, to monitor the progress of control measures, either during eradication programs or after reintroduction of virus in free areas. This demonstration becomes critical in immunized populations, because of the concern that silent viral circulation could be hidden by immunization. This concern originates from the fact that vaccination against foot-and-mouth disease (FMD) protects against clinical disease, but not necessarily against subclinical infection or establishment of the carrier state in cattle.

A novel approach, developed and validated at PANAFTOSA during the 1990s, based on an immunoenzymatic system for detection of antibodies against non-capsid proteins (NCP) has proven valuable for monitoring viral circulation within and between herds, irrespective of the vaccination status. Antibodies against NCP are induced during infection but, in principle, not upon vaccination.

The validation of this system led to its international recognition as the OIE index test. The fitness of this serosurvey tool to assess viral circulation in systematically vaccinated populations was demonstrated through its extensive application in most regions in South America. The experience attained in these regions supported the incorporation of the ''free of FMD with vaccination'' provisions into the OIE code. Likewise, it opened the way to alternatives to the ''stamping out'' policy. The results gave input to an old controversy related to the real epidemiological significance, if any, of carrier animals under the vaccination conditions in South America, and supported the development of recommendations and guidelines that are being implemented for serosurveys that go with control measures in vaccinated populations.

Keywords: Foot-and-mouth disease (FMD); Carriers; Serosurveillance; Non-capsid antibody tests

1. Introduction

Foot-and-mouth disease (FMD) is one of the most important diseases of domestic livestock. It results in a drastic negative impact on the economic progress of animal industry, and generates considerable losses to producers and affected countries. Many trade restrictions are placed upon areas where the infection is present, with imposition of national and international sanitary barriers to movement of both animals and derived products resulting in an important reduction of the availability of animal protein and income.

Since 1987 a Continental Program for eradication of FMD has been implemented in South America based on a resolution of the Inter American Meeting at the Ministerial Level on Animal Health [1]. The objectives of this program included prevention of the disease in free areas and eradication in endemic regions. Approaches to control and eradicate FMD where the disease is prevalent are generally based on quick detection and early response to an outbreak, high vaccine coverage, control of animal movement, slaughter of infected animals or herds and contacts, and a combination of the above. An important characteristic to be considered when control/eradication measures are planned is the ability of the virus to establish

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subclinical infection, which can become persistent, the so called carrier state [2], regardless of whether or not animals were vaccinated before infection [3].

Considering that controversial points of view existed in the international community on the role of carrier animals in the epidemiology of FMD [4] and that over 250 million cattle are systematically vaccinated in South America for the control and eradication of FMD, the availability of methods for identification of asymptomatic viral circulation in a population, regardless of whether they have also been vaccinated, became of great importance. The need for such methods to monitor the progress of the eradication program was particularly important as eradication progressed and, clinical symptoms diminished, as expected. Novel approaches for evaluation of antibodies to FMD non-capsid proteins (NCP), misleadingly referred to as non-structural proteins, were developed and validated at the Pan American Foot-and-Mouth Disease Center (PANAFTOSA), PAHO/WHO. These approaches proved valuable for assessing viral circulation in immunized populations [5–15]. This work highlights the achievements drawn from the wide application of this approach to control FMD in endemic regions, and after emergency situations.

2. Validation of NCP tests: fitness for purpose

During the past decade, PANAFTOSA, PAHO/WHO, as an OIE regional reference center for FMD, has developed and validated an approach to evaluate subclinical FMD viral circulation in animal populations, irrespective of vaccination.

The validation process followed the principles recommended by OIE, which was established based on the concept of fitness for purpose. Validation of methodological development, feasibility, standardization of performance characteristics, and technology transfer and reproducibility was completed by PANAFTOSA in collaboration with the National Laboratory Services of South America and the private sector using a large spectrum of relevant experimental and field model systems. The most important models included: (1) experimental persistently infected cattle, followed over a time period of up to 2 years; (2) animals immunized and re-immunized under various conditions with vaccines from various sources; (3) extensive field samplings representing different epidemiological situations; and (4) follow-up of outbreaks as a function of time and space.

This diagnostic strategy is based on the detection of antibodies against viral NCP that take part in the replication process and that, in principle, are induced only during infection and not upon immunization with conventional vaccines inactivated with binary ethylenimine. Moreover due to the conserved nature of these proteins, infection with any serotype of FMDV can be detected by a single serological assay. This diagnostic approach employs the use of an indirect enzyme-linked immunosorbent assay (I-ELISA) to screen for antibodies against non-capsid polyprotein 3ABC [14,16], followed by confirmation of suspect or reactive samples through a highly specific enzyme-linked immunoelectrotransfer blot test (EITB) using five non-capsid recombinant antigens as serological probes (3A, 3B, 2C, 3D and 3ABC) [5,15,16]. This strategy allows the high level of specificity needed to avoid a distortion in the predictive value in low prevalence regions, where it was mainly going to be applied, without compromising the high sensitivity required to detect low titre sera, which may be frequent at late stages of persistent infection and in vaccinated persistently infected animals with limited viral replication [9].

For the models evaluated, diagnostic specificity values were over 99%, even when multiple vaccinated animals were included in the analysis, provided that samplings are carried out under recommended conditions [5,6,9,10,14]. Specificity values can vary according to the nutritional status of the population, age of animals, vaccine purity, etc.

Regarding sensitivity studies, to date, no false negative results have been detected in the target population analyzed (diagnostic sensitivity ca. 100%), which is mainly composed of animals with established persistent infection. In addition, comparative diagnostic sensitivity of the I-ELISA 3ABC/EITB system with viral isolation from oesophageal pharyngeal fluids (OP) in proven carrier animals indicated 100% sensitivity. Conversely the sensitivity of OP isolation relative to the I-ELISA 3ABC/EITB system is only 46.9% [5,6,9,10,14].

It should be noted, however, that no unique values for sensitivity can be established for the different variables occurring under field situations. Sensitivity values can be lower (97%) when transient subclinically or persistently infected animals are evaluated (manuscript in preparation), as could be the case in animals with high immunization levels, shortly after viral exposure and with rare viral isolation, for which the carrier condition is open to question. Studies should be encouraged to define the pathogenicity of viral infection in animals vaccinated with modern vaccines, to better understand why there is a lack of antibody detection in these cases. This understanding may also lead to a better definition of carriers when referring to immunized animals, which today has no distinction from non-vaccinated populations.

3. Evaluation of viral circulation in herds under systematic vaccination

Since the development of the I-ELISA 3ABC/EITB system, extensive experience in South America has contributed to establishing improved sampling designs, test algorithms, and criteria for interpreting test results. These improvements have enhanced our ability to recognize FMD viral circulation within and between vaccinated herds, as well as to better understand the potential role of carriers in the epidemiology of FMD [7,10,12,13].

As perfect performance of tests is not a realistic assumption, a small number of false negative and false positive results can be expected to occur, depending on the field situation. In addition, positive reactions in previously infected animals can be detected for longer than 12 months after the last positive viral isolation from OP samples [5,9], indicating that positive NCP test results may also reflect previous and not necessarily present FMDV infection. Conversely a negative result for