Veterinary Microbiology 203 (2017) 62–67

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Inter-laboratory validation of foot-and-mouth disease diagnostic capability in Germany

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ARTICLE INFO

Article history:
Received 26 January 2017
Received in revised form 28 February 2017
Accepted 1 March 2017

Keywords:
FMDV
Proficiency test
Ring trial
Serology
Real-time RT-PCR
Quality assurance

ABSTRACT

Germany has been free from foot-and-mouth disease virus (FMDV) without vaccination since 1992, but diagnostic capability at regional laboratories is maintained for FMDV exclusion in suspect cases and as surge capacity for outbreak preparedness. A proficiency test was initiated in 2015 to evaluate the diagnostic performance of 20 regional veterinary laboratories. A panel of two identical samples of FMDV genome for real-time reverse transcription polymerase chain reaction (RT-PCR), four lyophilized bovine sera for antibody detection and eight samples of inactivated vaccine antigen for analysis with a lateral-flow device (LFD) were tested with the systems routinely used at the participating institutions.

With only one exception, all laboratories reliably detected viral RNA with two real-time RT-PCR assays down to a dilution of 10(−4) of the original material. The LFD pen-side test was evaluated at 8 of 20 laboratories, and FMDV antigen was detected by all participants down to a dilution of 1:81. Serological diagnosis was also very consistent at most participating institutions. Very few false-negative results were returned for the diluted positive sera, and testing of a large cohort of negative samples demonstrated a high specificity of over 99% for the two commercial ELISA kits used (PrioCHECK® FMDV NS and ID Screen® FMD NSP). In conclusion, the proficiency test demonstrated the reliable and robust FMDV diagnostic capability of the German regional veterinary diagnostic laboratories. Furthermore, the suitability of the commercially available ELISA systems for the detection of FMDV-specific antibodies was confirmed. Proficiency tests are an important quality assurance measure for transboundary diseases like FMDV where diagnostic capacity in free countries has to be available at the highest possible standard.

1. Introduction

Foot-and-mouth disease (FMD) is a severe vesicular disease affecting cloven-hoofed livestock and wildlife (Alexandersen et al., 2003). It is caused by foot-and-mouth disease virus (FMDV), a non-enveloped, single-stranded, positive-sense RNA virus (*Aphthovirus, Picornaviridae*). Four structural proteins (VP1–VP4) form the viral capsid and give rise to seven immunologically distinct serotypes: O, A, C, Asia 1 and SAT 1, 2 and 3 (Alexandersen et al., 2003; Jamal and Belsham, 2013). The non-structural proteins (NSP), particularly 3A, B, and C, are more conserved and antibodies against these proteins are not serotype-specific. During vaccine preparation, FMDV particles are chemically inactivated and purified. The final vaccine formulation does not contain NSP. Hence, NSP antibody assays can differentiate between infected and vaccinated animals.

Because FMDV is extremely contagious, spreads rapidly among susceptible animals, and requires extreme control measures, it is a major concern for countries with highly developed meat and dairy industries. Preventing the international spread of FMDV is critical because an outbreak in a previously free country inevitably leads to substantial economic losses through culling, movement restrictions and trade embargoes (Thompson et al., 2002).

The early detection of incursions through reliable laboratory diagnosis is essential for FMD control (Ferris et al., 2006), but in Europe, only a small number of high-containment laboratories are certified to work with the virus itself (EuFMD, 2013). Building capacity for low-risk diagnostic testing for FMDV RNA and specific antibodies (by real-time RT-PCR and antibody ELISA) outside of these centers is of utmost importance for two reasons: The early detection of an FMDV incursion is facilitated when regular veterinary diagnostic labs can provide exclusion diagnosis,

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http://dx.doi.org/10.1016/j.vetmic.2017.03.001
0378-1135/

especially in cases that are not suspect of FMD in the legal sense but where FMD is a possible differential diagnosis. Should an outbreak occur, "contingency laboratories" can further assist the national reference laboratory with the large numbers of samples that will need to be tested as quickly as possible (EuFMD, 2013; Knight-Jones et al., 2016). Therefore, it is important to verify that the FMDV diagnostic assays deployed at the German regional veterinary diagnostic laboratories are fit for purpose, and inter-laboratory proficiency tests can be used to detect problems in assay sensitivity and/or specificity.

In 2015, the national reference laboratory for FMD at the Friedrich-Loeffler-Institut organized a proficiency test for 20 regional laboratories in Germany. The objective of this ring trial was to assess the efficacy of the test systems routinely used at each institution for the detection of FMDV RNA by real-time RT-PCR, of FMDV antigen by a commercial available lateral-flow device (LFD), and of specific antibodies to non-structural proteins using commercial ELISA systems. On a voluntary basis, the participants were further asked to test a larger number of negative sera from their routine case load with two different FMDV antibody ELISA systems to confirm their diagnostic specificity and to define which level of background reactivity in the German cattle population has to be expected.

2. Materials and methods

2.1. Overview

Two identical samples of FMDV RNA for RT-PCR, four lyophilized bovine sera for serological analysis and one sample of inactivated vaccine antigen for analysis with a lateral-flow device (LFD) were prepared for the ring trial. Samples were sent without refrigeration, but the participants were advised to freeze the antigen samples upon arrival.

2.2. Samples for RT-PCR

Two FMDV real-time RT-PCR protocols had been provided to the participants earlier: the IRES1 assay by Oem et al. (2005), and the 3D-OIE assay based on a publication by (Callahan et al., 2002). For the PCR part of the ring trial, inactivated vaccine antigen (strain FMDV C_1 Oberbayern, provided by Merial GmbH, Hallbergmoos, Germany) was diluted in serum-free tissue culture media and lysis buffer (RAV1, Macherey-Nagel GmbH & Co. KG, Düren, Germany) before shipping to adjust the nucleic acid content to a quantification cycle (Cq) value of about 20 for the 3D-OIE assay, which corresponded to a Cq value of about 25 for the IRES1 assay. The participants were asked to extract RNA in duplicate with the protocol routinely used at their institution and to prepare a ten-fold dilution series of the eluates from 10^{-1} to 10^{-8}.

2.3. Samples for serology

The serology panel contained one sample of new-born calf serum (NBS; Gibco®, Thermo Fisher Scientific, Waltham, United States) that was negative for antibodies against FMDV (NBS) and three dilutions of a bovine serum containing FMDV-specific antibodies at a dilution of 1:3, 1:5, and 1:7 in NBS. The serum sample had been collected 20 weeks after challenge from an animal vaccinated against and subsequently challenged with FMDV O_1/BFS/1860 that became a persistently infected carrier after challenge. All sera were lyophilized before shipping and reconstituted with deionized water at the receiving institutes. For analysis, two commercial ELISA systems detecting antibodies against FMDV non-structural proteins were used by the participants: PrioCHECK® FMDV NS (Thermo Fisher Scientific) and ID Screen® FMD NSP Competition (ID.vet, Grabels, France). At the time of the trial, only the PrioCHECK® kit was licensed in Germany and was used by all participating laboratories. Parallel testing with the ID Screen® kit was voluntary. To test the association between the results obtained with the two kits, Pearson's product moment correlation coefficient was calculated for all pairwise complete observations and evaluated against a *t*-distribution with n–2degrees of freedom.

Results for the PrioCHECK® assay are reported as percent inhibition (PI) and calculated as follows: PI = 100 – (optical density of the sample/optical density of the negative controls) × 100. Samples with a PI value < 50% are considered negative, and samples with PI ≥ 50% are considered positive. For the ID Screen® assay, a sample/negative quotient (S/N) is calculated as follows: S/N = (optical density of the sample/optical density of the negative control) × 100. Accordingly, samples with a S/N value ≤ 50% are considered positive, and samples with a S/N > 50% are considered negative.

2.4. Samples for LFD rapid tests

A commercially available LFD rapid test for the detection of FMDV antigen (Svanodip® FMDV-Ag, Boehringer Ingelheim Svanova, Uppsala, Sweden) (Ferris et al., 2009) was used by 8 of the 20 laboratories. This is an immunochromatographic test intended for the detection of FMDV antigen in vesicular fluid and epithelium. In the case of a suspected outbreak of FMDV, this test can provide veterinary authorities with a preliminary pen-side diagnosis to support quick decision making.

In the ring trial, the panel for the LFD tests included one sample of inactivated vaccine antigen (FMDV Asia 1, strain Shamir, provided by Intervet Deutschland GmbH, Unterschleißheim, Germany), and one negative sample (NBS). The antigen was diluted 1:10 with NBS before shipping and was then further diluted 1:3 by the participants to obtain dilutions of 1:3, 1:9, 1:27, 1:81, 1:243 and 1:729 in NBS.

2.5. Specificity testing

All participating institutions were asked to test samples from their routine case load with both ELISA systems (PrioCHECK® FMDV NS and ID Screen® FMD NSP Competition), in order to evaluate the diagnostic specificity of the tests in an FMDV-free population. The results were categorized by sample matrix (plasma or serum) and species (cattle, pig, goat, sheep, or other).

2.6. Participating institutions

A total of 20 German regional diagnostic laboratories participated in the ring trial. All of them received the sample panel for PCR analysis. The sample panel for serological analysis was tested in 18 laboratories and the samples provided for rapid testing by LFD were evaluated in 8 laboratories.

2.7. Validation of a shortened protocol for PrioCHECK® FMDV NS

For the ID Screen® FMD NSP kit, the manufacturer offers two protocols, one with an incubation of the diluted sample on the antigen-coated plate at room temperature overnight, and the other with an incubation at 37 °C for 2 h. The recommended incubation time for the PrioCHECK® FMDV NS kit is overnight, but in analogy to the ID Screen® ELISA, shorter incubation times (1 h or 2 h) at higher temperatures (37 °C) and with larger sample volumes (up to 50 µl) were evaluated and compared at the national reference laboratory (NRL). The weak and strong positive controls from the kit, as well as four dilutions of the FMDV-antibody positive reference serum (see Section 2.3) were used for the comparisons.