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Foot-and-mouth disease non-structural protein serology in cattle: Use of a Bayesian framework to estimate diagnostic sensitivity and specificity of six ELISA tests and true prevalence in the field

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Abstract

The diagnostic performance of six foot-and-mouth disease (FMD) assays for detection of antibodies to the non-structural proteins (NSP) of the FMD virus (FMDV) was estimated using a Bayesian analysis on field sera from cattle of unknown infection status originating from post-FMDV outbreak situations in Israel and Zimbabwe. Estimations of the disease prevalence in both populations were also obtained. The diagnostic sensitivity estimates did not differ between both field studies, although overall Bayesian estimates were markedly higher than those previously reported based on sera from comparable experimentally infected (vaccinated) cattle populations. All NSP-based assays demonstrated a lower diagnostic specificity when applied to the Zimbabwean sera compared to both published specificities and similar Bayesian specificity estimates derived for the Israeli dataset. In Israel, the disease prevalence was estimated at 23.9% (95% credibility interval: 19.5–28.8%), whereas 65.4% (59.0–72.5%) was found in Zimbabwe. The need for reliable diagnostic test performance estimates and the benefits of Bayesian analysis in obtaining them are also addressed.

Keywords: Foot-and-mouth disease; Non-structural protein; Bayesian

1. Introduction

Foot-and-mouth disease (FMD) is a highly contagious and devastating disease that affects all species of cloven-hoofed animals, including economically important livestock (cattle, pigs and sheep). The disease is recognised by the World Organisation for Animal Health (OIE) as a major constraint to international trade. In outbreak situations, effective control strategies must, thus, be put in place to stop the spread of the causal virus [1]. Following the 2001 epidemic of FMD in the United Kingdom, Ireland, France and the Netherlands, the European Commission and the Member States revised the legislation by putting greater emphasis on the use of emergency vaccination to control future FMD outbreaks within the Community (Council Directive 2003/85/EC). The adopted "vaccinate-to-live" policy greatly reduces reliance on mass pre-emptive culling of at-risk animals. Nevertheless, to regain the favoured FMD-free status, affected countries need to demonstrate absence of disease and of infection by use of

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clinical and serological surveillance in accordance with OIE requirements. These imply that the serological survey be based on the detection of antibodies to the non-structural proteins (NSP) of the FMD virus (FMDV), which are elicited by infection only [2]. Several such NSP-based assays have been and are being developed.

At a recent workshop held at the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER, Brescia, Italy) in May 2004, six different NSP ELISAs were evaluated for cattle, among which were the OIE-index-test from PANAFTOSA, the IZSLER in-house test and four commercially available assays [3]. The diagnostic specificity (dSp) of each test has been estimated based on test results obtained for 1100 experimental and field sera collected from (vaccinated) non-infected cattle originating in seven different countries. However, the diagnostic sensitivity (dSn) estimates were based solely on test results for sera from experimentally infected (vaccinated) animals, and may not necessarily reflect the performance of the tests in the field. As a result, the true disease prevalence (p) could be wrongly estimated (mainly underestimated) [4].

Ideally, evaluation of the performance of these ELISAs, when used to test field sera derived from animals of unknown (latent) infection status, would involve reference to a "gold standard" test method [5]. Unfortunately, such a gold standard test is lacking for FMD NSP serology. Statistical methods, such as latent class analysis, have been developed to estimate true prevalence, dSn and dSp in the absence of a gold standard. However, without some extraneous constraints, more parameters need to be estimated than the data allows [6], *in casu* 127 parameters to be estimated versus 63 estimable parameters. Therefore, two basic assumptions, known as the Hui–Walter paradigm [7], are regularly made in previously published analyses [8], namely that assay performance characteristics (dSn and dSp) remain constant across populations and that assays are conditionally independent of each other given the true disease status.

However, when diagnostic tests have a similar biological basis, as is the case for all FMDV NSP ELISAs mentioned, the conditional independence assumption is untenable, as shown by the observed covariance of all methods with respect to dSn [3]. Moreover, the dSn estimates varied according to sampling time post infection [3]. Consequently, restrictions on the parameters, other than the Hui–Walter paradigm, need to be imposed to estimate the performance of these NSP-based tests when using field sera collected from animals of unknown infection status.

Recently, Berkvens et al. [4] described a Bayesian approach using probabilistic constraints for the estimation of true disease prevalence and diagnostic test characteristics. The method was validated on data collected for *Cryptosporidium* and porcine cysticercosis using variable numbers of diagnostic tests [4,9]. In this paper, we apply this Bayesian philosophy to six diagnostic NSP ELISAs, using data from two confirmed FMD outbreak situations, in which cattle were subjected to various vaccination regimes.

2. Materials and methods

2.1. Tests

Six different NSP-based ELISAs were compared, these were: NCPanaftosa-screening from PANAFTOSA [10]; 3ABC trapping-ELISA from IZSLER [11]; Ceditest® FMDV-NS (Cedi Diagnostics B.V., Lelystad, The Netherlands) [12,13]; SVANOVIR™ FMDV 3ABC-Ab ELISA (Svanova, Upsala, Sweden) [14]; CHEKIT-FMD-3ABC (Bommeli Diagnostics, Bern, Switzerland) [15,16]; UBI® FMDV NS ELISA (United Biomedical Inc., New York, USA) [17]. For a more detailed description of the tests' specifications and methodologies, refer to Brocchi et al. [3].

2.2. Field specimens

In total, 867 serum samples were collected during two post-outbreak surveillance programmes in 2004. Of these, 465 sera were collected from cattle individually sampled between 30 and 80 days post-FMDV type O infection in four feedlot and/or dairy farms in Israel. All cattle were vaccinated against FMD between May and June 2003 and the actual outbreaks occurred in January 2004 [18]. The remaining 402 sera were derived from a field study conducted in Zimbabwe, in which six herds were sampled; FMDV infection was known to have occurred in five of these herds. The FMD vaccination status was unknown for one of the herds, but otherwise varied from never vaccinated to vaccinated approximately 7 months prior to the occurrence of the FMD outbreak (using a trivalent vaccine to FMDV serotypes SAT1, SAT2 and SAT3). All of the outbreaks were confirmed to be due to infection with either FMDV serotype SAT1 or serotype SAT2 and in each case, specimens were collected between 1 and 5 months after the outbreaks had occurred. The sampling protocol was specifically designed to investigate the dSn of NSP ELISAs for detection of FMDV SAT type carrier cattle amongst vaccinated and subsequently infected animals. Therefore, sampling was targeted towards animals that had either been clinically affected or had been in close contact with infected animals (non-random sampling). Although the sixth herd sample was presumed not to have been infected, some serological evidence, suggesting previous FMDV infection, was nonetheless found [19].

All sera were tested singly and simultaneously in all six ELISAs. The analysis was based on the initial test results and did not include results obtained on retesting of discordant findings. For those ELISAs that allow for an "inconclusive" or "doubtful" interpretation zone between two threshold values, all test results equal to or greater than the lower threshold value were scored as positive. More details on the testing procedure and the specimen database may be found in Brocchi et al. [3]. Table 1 summarises the observed frequencies for all 64 different test outcome combinations based on the test results for the 465 Israeli and 402 Zimbabwean sera.