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Review

The use of serosurveys following emergency vaccination, to recover the status of "foot-and-mouth disease free where vaccination is not practised"

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ABSTRACT

To eliminate incursions of foot-and-mouth disease (FMD) quickly, a combination of measures, including emergency vaccination, can help block the spread of infection. For the earliest recovery of the FMD-free status for trade, without the slaughter of uninfected vaccinated animals, a serosurvey for antibodies to FMD virus non-structural proteins (NSP) must be used to substantiate absence of occult virus infections. Areas of doubt over requirements for post-vaccination serosurveillance and its feasibility include the required and achievable confidence, the amount of sampling necessary, and the appropriate responses to and consequences of different seropositive findings. This derives largely from uncertainty over the extent of localised pockets of virus infection that may remain within vaccinated populations and the circumstances that permit this. The question therefore remains whether tests are sufficiently sensitive and specific to detect and eliminate infected animals, without excessive culling of uninfected animals, before vaccinated animals mix with non-vaccinated livestock when movement restrictions are lifted. It is recommended to change the rationale for serosurveillance after emergency vaccination. Only when emergency vaccination is used in limited outbreaks is it possible to test and cull comprehensively, an approach compatible with a three-month minimum period to recover the FMD-free status. In other situations, where emergency vaccination is used, such as dealing with large outbreaks in animal-dense regions and where the onset of vaccination has been delayed, post-vaccination serosurveys should be targeted and focus on providing an assurance to detect higher levels of infection, in case of inadequate control measures. As this provides less assurance of absence of infection, the approach would be compatible with a six-month waiting period for free-status recovery and should be complemented by other methods to provide evidence that vaccination and control measures have been effectively implemented, as these are the best guarantee against continuing virus transmission.

1. Introduction

Foot-and-mouth disease (FMD) is of variable severity, dairy cattle and pigs showing obvious signs of illness whilst infection can be mild or sub-clinical, especially in small ruminants and partially immune animals. The causative virus can spread by direct contact with infected animals, or via contaminated animal products, animate and inanimate objects and by atmospheric dispersal. In ruminants, virus may persist beyond 28 days in the oropharynx of so-called "carrier" animals for months to years [1,2]. However, isolation of virus becomes progressively more difficult with time [3,4] and there is little evidence that carrier livestock can transmit FMD virus (FMDV) [5].

Control and eventual elimination of FMD by vaccination has been effective in mainland Europe [6] and South America [7] with vaccine used primarily as a prophylactic tool in cattle, and occasional ring vaccination of sheep and pigs. In many FMD-free countries, disease introductions were controlled by stamping out [8]. After the outbreaks of 2001, the EU Directive on FMD control

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was revised [9]; one aim being to encourage the use of vaccination with retention of vaccinated animals. Outbreak control still requires the killing and destruction of all FMD susceptible animals on farms where known infected animals are present, with vaccination used as a control measure in uninfected farms. However, some EU member states remain reluctant to implement this policy within their contingency plans, whilst other FMD-free regions are still considering their options for FMD control. When FMD caused large outbreaks following introductions to South Korea and Japan in 2010 and 2011 [10,11], vaccination was delayed. This may be partly attributed to continuing uncertainty amongst policy makers and trade partners about the feasibility and reliability with which the FMD-free status can be recovered after using this strategy for FMD control [12,13]. In this paper, we review gaps in knowledge over FMDV persistence and detection in vaccinated populations and address the approaches that can be taken to improve the use and interpretation of serosurveillance methods along with the possible follow-up actions after positive serological findings. We suggest different options for dealing with limited outbreaks compared to epidemics and that more emphasis should be given to complementary approaches to substantiate the effectiveness of emergency vaccination.

2. FMD control in formerly FMD-free countries

FMD is highly contagious, so rapid action is needed to block its spread and eradicate it if introduced into a formerly FMD-free country. This requires surveillance and tracing to diagnose infected farms, and restrictions on movements of infected and potentially infected animals, persons and objects. Farms containing acutely infected animals should be culled,[1] cleansed and disinfected, which may be extended to the preventive culling of potentially infected animals or even to animals that may be at high risk of future infection [14]. Emergency vaccination, in and around affected areas, can supplement, replace or delay preventive culling and the merits and disadvantages of the two approaches have been compared by computational simulation [15–17]. The larger an outbreak becomes, the more unacceptable and unfeasible is control by culling, so factors that predispose to epidemics, favour early adoption of an emergency vaccination policy [9,18]. Countries free of FMD benefit from access to international trade markets for sale of susceptible live animals and their products, especially fresh meat. Loss of this favourable status after FMD introduction can be very costly, so the time to recover the free status affects disease control strategy selection [12].

Once FMD has been controlled, assurance that the infection has been eliminated is required to lift local and national disease control restrictions and to resume trade in livestock and livestock products [19].

3. FMD vaccines and post-vaccination serology to detect infection

FMD vaccines are produced in cell cultures followed by inactivation of infectivity and separation of virus particles from culture medium, debris and viral non-structural proteins (NSP) [20]. If sufficient animals are adequately immunised by vaccination, then within-pen transmission of FMDV will stop [21–24], which will stop between-pen [25] and between-herd transmission [26]. However, infection may spread whilst immunity is developing [27]. Furthermore, if vaccination is inadequate (e.g. poor vaccine quality, non-matching vaccine, or insufficient animals correctly vaccinated), spread may continue [28], especially if other measures, such as movement restrictions, are ineffective [29]. Even well vaccinated animals may become subclinically infected if exposed to a sufficient viral challenge and vaccinated ruminants can develop the FMDV carrier state [30,31]. Such animals shed less virus during the acute stage of infection compared to unvaccinated animals with disease [32–34]. At a population level, emergency vaccination should reduce the overall reproduction ratio below one, but minor outbreaks can continue due to localised failures of control.

The levels of vaccine-induced antibodies directed towards the viral structural proteins (SP) can be measured using serological assays that correlate with the degree of protection [35,36]. Animals infected with replicating FMDV mount an antibody response to both the SP and NSP of the infecting virus and therefore, provided that NSP have been sufficiently removed from FMD vaccines by purification steps during vaccine manufacture, then tests for antibodies to NSP (NSP ELISA) can be used as indicators that infection has occurred, regardless of vaccination status; so-called DIVA tests that differentiate infected from vaccinated animals [13,37]. Following infection, NSP seroconversion takes 7–14 days [38] and antibodies can be detected in serum for months or years [4,39,40]. Different causes of NSP seropositivity are associated with differing risks for FMD transmission and persistence: (1) the animal might have been infected recently, indicating a high risk that FMDV might still be circulating in other animals on the premises or on other epidemiologically linked premises; (2) the animal might have been infected some time ago, with a greater likelihood that transmission of FMDV no longer occurs; (3) the animal might have recovered fully from FMDV infection and no longer harbour virus; (4) the animal might have become a long-term virus carrier; (5) the NSP seroreactivity may be non-specific and the animal in question might not have had any exposure to FMDV. Although virus persists at a low level in carrier animals, virological tests for identifying convalescent animals have a low sensitivity and NSP serology will detect a higher proportion of virus carriers [4].

A workshop to compare NSP tests [41] showed that the former Ceditest (now Prionics PrioCHECK® FMDV NS; [42] combined relatively good sensitivity (Se) and specificity (Sp) with commercial availability, so its performance characteristics are used for "NSP tests" in this review. NSP seroconversion is related to the extent of virus replication, which in turn depends upon levels of host susceptibility, immune status and the nature and severity of exposure [33,34]. Therefore, well-vaccinated animals that become infected may seroconvert weakly and/or transiently, especially in the absence of clinical disease, resulting in wide ranges in Se for detecting different categories of infected animals. Brocchi et al. reported Se of 68–74% for detecting cattle sampled beyond 28 days post infection (>28 dpi) using the Ceditest [41]. Vaccinated animals that progressed to become long-term virus carriers seroconverted more reliably and could be detected with a higher Se (86–89% for cattle at >28 dpi). Conversely, subclinical infection after vaccination was associated with weak NSP seroconversion (Se of 27% at >28 dpi). Although the relationship between likelihood of transmission and degree of subsequent seroconversion in NSP tests remains unquantified, the risk of missing a dangerous occurrence of FMDV transmission is most likely less from failure to detect a transiently NSP seropositive animal than from failure to detect one that has strongly seroconverted.

Modelling has been used to extrapolate outbreak and experimental virus transmission data to predict vaccine-based control in the field. This predicts that if vaccination is optimised and clinical surveillance effectively removes herds with diseased animals, then the number of undisclosed infected herds and animals should be small with few carriers [43–45]. Undetected infected animals would be found mainly in non-vaccinated sheep herds and

[1] Culling is defined as killing followed by exclusion of carcasses from the food chain unless processed to inactivate FMDV.

vaccinated cattle and sheep herds. However, after serosurveillance, carried out according to the EU Directive, vaccination and pre-emptive culling strategies yielded comparable low numbers of undetected infected animals [45]. Schley et al. emphasised that following effective vaccination, the quality of inspection is the principal factor influencing whether or not undisclosed carrier herds occur, supporting the importance of other control measures [44]. Further studies are required to model virus persistence in vaccinated populations through transmission from acutely infected animals, rather than from carrier animals, as the former represent a more significant risk for new FMD outbreaks [12].

NSP serosurveillance of a large number of animals will give rise to many false positive test reactors, since the tests have imperfect specificity (Sp of 98–99.7% for cattle; [41]) and Se/Sp limitations cannot be overcome easily by using a combination of different NSP tests [46]. Furthermore, true positive test results cannot be distinguished readily from false positive ones [47], although a cluster analysis [48] and the use of likelihood ratios to weight the strength of seroconversion might improve the possible discrimination [49]. This makes classification of the infection status of large herds difficult. Arnold et al. concluded that in this situation, the best compromise between maximising the sensitivity for carrier detection, whilst minimising unnecessary culling, will be met by adopting an individual-based testing regime in which all animals in all vaccinated herds are tested and positive animals rather than herds are culled [43]. The remaining risk with this approach is that any carriers that are missed will be free to move to unvaccinated herds on national territory once outbreak restrictions are lifted and those non-vaccinated animals may be traded.

4. Current OIE guidance and EU legislation on post-vaccination measures

Requirements for recovering the FMD-free status where vaccination is not practised are laid out in the OIE Terrestrial Animal Health Code (Supplementary Table 1; [19]) and for EU Member States in the EU FMD Directive [9]. With stamping out (culling) of affected herds and suitable surveillance, the FMD-free status can be regained 3 months after the last case. If emergency vaccination is used, a 3-month recovery period also applies if the vaccinated animals are killed (so-called "vaccination with subsequent slaughter"), but otherwise extends to 6 months (following so-called "vaccination without subsequent slaughter") and also requires additional serosurveillance to substantiate the absence of virus infections. The OIE Code therefore requires that vaccinated animals are tested serologically to show that there is no ongoing virus transmission or "circulation", and, in case of countries wishing to recover the status of "FMD-free where vaccination is not practised", that infected animals are not present. The OIE definition of infection would include carriers, although these are not specifically referred to. In the current FMD Chapter (8.6) of the OIE Code [19], the articles on surveillance (articles 42–47 and article 49) describe the principles that should be followed, but do not specify a sampling frame or design prevalence for detecting virus transmission or infected (including carrier) animals.

The EU Directive on FMD control gives a more detailed account of the post-vaccination surveillance required for EU Member States to recover the status of FMD-free where vaccination is not practiced (Supplementary Table 2, [9]). The requirement in the EU Directive to sample and test all vaccinated animals and their unvaccinated offspring (so-called "census surveillance") arose from the view that NSP serology should be used as a herd test [50] along with the desire to provide a high level of confidence that all carriers are detected and that limited virus transmission within herds is not overlooked by serological surveillance. This would overcome the problem that has led to re-emergence of infection after many years of apparent freedom, and despite targeted annual serosurveillance, in countries continuing with prophylactic mass vaccination after attainment of the status FMD-free where vaccination is practised [7]. This approach also helps to deal with the so-called "small herd problem" in which herd-level freedom cannot be demonstrated with imperfect tests if the expected within-herd prevalence is low, as it allows small herds to be evaluated as an amalgamated stratum rather than at the herd level [51]. The sampling requirements are set out in paragraph 3 of Article 56, although the text appears ambiguous requiring either a sampling protocol suitable for detecting a 5% in-herd prevalence with at least a 95% level of confidence or the sampling and testing of all animals in vaccinated herds. The first option is actually intended to be for non-vaccinated animals within a vaccination zone that are unlikely to show clear clinical signs (e.g. sheep and goats), but this only becomes explicit in the context of the referenced Annex III to that Directive.

Both the OIE Code [19] and the EU Directive [9] require follow-up investigation of all serologically positive findings and a return to the farm to double-check for clinical evidence of FMD and to collect fresh samples from the originally sampled cohort and a number of direct contact animals. Serological testing of the samples should be done to determine whether seroconversion is ongoing, indicative of virus circulation. Samples can also be taken to test for the presence of virus, including oesophagopharyngeal mucus scrapings collected with a probang cup to detect virus carriers. An epidemiological enquiry is also required. At the end of these investigations the herd/flock must be categorised as to whether or not infected animals are present.

The OIE Code clearly describes in Article 8.61 that the occurrence of FMDV infection is confirmed if FMDV is isolated from an animal [19]. The culling strategies for post-outbreak eradication to recover the FMD-free status are summarised in Article 8.6.47 as "the slaughter of all clinically affected and in-contact susceptible animals, but there is no discussion of the requirements to remove subclinically affected animals (that could be cases of recent, historic or carrier infection) if identified only by serology, in the absence of clinically affected companion animals.

The EU Directive requires the stamping out of holdings containing at least one animal where the presence of FMDV is confirmed [9]. As well as depopulation of the susceptible species present, animal products must be treated or disposed of and holdings must be cleansed and disinfected before restocking. Control zones must be established to monitor and regulate animals in surrounding herds. On holdings containing NSP reactors but where further testing confirms the absence of circulating FMDV, the NSP positive animals must be culled. Other test-negative animals in the herd should also be killed but may be slaughtered under controlled conditions and their meat is subject to deboning and maturation (ruminants) or processing into meat products. In case of pork their carcasses can go for consumption (Supplementary Table 2). Cleansing and disinfection of the premises is still required, but no control zones are imposed on neighbouring premises. Thus, the actions required are clearly distinct where acutely infected animals are confirmed (after their detection by virological means or paired serology) compared to other situations where NSP seroreactors are found. However, for both OIE and EU, the presence of a carrier animal (confirmed by virus detection) would invoke the full implications of a new outbreak [9,19]. The requirement to kill the whole herd, including seronegative animals, when FMD infection is confirmed only by serology, could be modified to meet the recommendations of Arnold et al. [43], by selectively removing only the seropositive animals. But the compatibility of this alteration with the requirements of the Directive for cleansing, disinfection and controlled restocking of the herd would also have to be considered.

The declaration of an outbreak has important implications for trade. Supplementary Table 3 provides the definition of an outbreak of FMD according to the EU Directive and these definitions are themselves consistent with those within the OIE Code [19].

5. Feasibility of post-vaccination surveillance

A summary of some of the practical difficulties that arise in using NSP ELISA to help substantiate FMD freedom is provided in Supplementary Table 4.

Three workshops in 2007 examined the design and interpretation of post FMD-vaccination serosurveillance by NSP tests [52]. Their aim was to test the feasibility and consequences of applying the above-described rules after applying emergency vaccination in three plausible scenarios involving different outbreak sizes, affected species and livestock densities. The summary recommendations of the workshops are provided in Supplementary Table 5 and the following key issues are further discussed below: (1) the requirement to sample all vaccinated animals; (2) the follow-up investigation required to establish the significance of seroreactors identified; (3) the criteria for removal of seropositive animals and herds; (4) what can be done with such animals (slaughter for consumption or destruction); (5) the impact of finding seroreactors during the process of surveillance with the objective of regaining the status "FMD free where vaccination is not practised".

6. Discussion

6.1. The requirement to sample all vaccinated animals in all vaccinated herds

Even with tests of suboptimal sensitivity (70–90%), a low prevalence of infection can be detected with high confidence in large groups of animals without sampling and testing every animal. However, in large herds, the animals are often segregated in smaller groups that may be considered as separate epidemiological units and in this case, the number of animals per epidemiological unit would be the denominator for calculation of sample sizes. For NSP serosurveillance, using a test with Sp = 0.995 and Se = 0.7, then detection of seroconversion at 95% confidence, at a prevalence of 2%, in an epidemiological unit of 1000 animals, would require 513 animals to be sampled and the cut-point would be five (i.e. finding five or fewer reactors could still be consistent with absence of true seroconversion, i.e. probability of 2% or more seropositive animals is less than 5%). If it were accepted that only strongly seroconverting animals are likely to (have) spread infection, then the Se figure could be increased to 0.9, in which case 366 samples would need to be tested and the cut-point would become four (FreeCalc; [53]).

Reduction of the numbers sampled in large herds is often relevant for pigs which also do not have risks associated with the development of FMDV carriers. Clinical disease is also rather obvious in pigs so that NSP surveys add less value. Therefore, surveillance in pigs should be targeted towards the identification of disease and virus circulation. Studies on vaccinated pig herds in Hong Kong suggested an all-or-nothing effect, with widespread clinical disease and NSP seroconversion (49–82% seroprevalence) or neither clinical disease nor seroconversion [54]. This all-or nothing effect is also seen in transmission studies in pigs, where exposure to a fixed dose by injection did not lead to infection [21], but exposure to large amounts of virus during contact exposure led to infection and transmission with clear clinical signs [23]. Subclinical infection of vaccinated pigs has been reported, but other vaccinated pen-mates showed disease [33]. Studies on experimentally infected pigs showed that there is a rather short duration of NSP seroreactivity in infected pigs with declining levels of reactors after 9 weeks [40].

7. The follow-up investigation required to establish the significance of seroreactors

If the serosurvey aimed at demonstrating freedom from FMD finds evidence of NSP reactors within herds, then following retesting and use of confirmatory tests, the number and strength of the seroreactors will influence the degree of suspicion that infection occurred [49]. It can be argued that if farm visits for the initial collection of serum samples have already included careful inspection of all the animals without finding any signs of disease and if isolated NSP positive reactors are subsequently found at a level consistent with that expected (from the known specificity of the test used) there should not need to be any follow-up visits for inspection and resampling/testing as prescribed in the OIE Code and the EU Directive [9,19]. Other factors that would mitigate against the need for a follow-up farm visit include the availability of location data for individual animals to rule out clustering of positive cases, samples originating from pigs that do not become long-term virus carriers and only weak positive test reactor findings. Such decisions need to be taken on a case-by-case basis.

If the level of suspicion warrants a follow-up visit, this should check for clinical signs and clustering of positive animals and to examine and resample the initially seropositive animals along with in-contact animals. If clinical or epidemiological evidence for infection or disease were then found, the usual measures for investigating a suspect case would be followed. Past infection would be distinguished from non-specific reactors by presence or absence of clustering and by the number and strength of seroreactors relative to that predicted from the known specificity of the test [55]. Recent infection would be confirmed by clinical checks and/or evidence of seroconversion from the second round of sampling [19,56]. IgM tests could also be helpful in this situation [57]. Oral or nasal swabs could be collected from pigs and oesophagopharyngeal fluids collected from ruminants for virological testing to look for evidence of infection [58]. However, the virological techniques have low sensitivity whilst a false positive test finding could be difficult to identify. Use of an IgA test has been proposed as a proxy for the probang virus test [59,60] as FMDV-specific IgA antibody in mucosal secretions of the upper respiratory tract of cattle is mainly associated with the continued presence of detectable virus in a probang cup sample. However, despite the potential logistic advantages, the IgA test is not yet commercially available.

8. The consequences of detecting seropositive animals and herds

In order to regain the FMD-free status quickly, herds containing infected animals should be culled (stamped out) and their products excluded from the food chain. Evidence of clinical signs and/or virus circulation would clearly justify this action, but the appropriate level of animal removal and of cleansing and disinfection of the holding when only carriers or animals with evidence of past infection are identified, is less straightforward, particularly after the active outbreak phase, and in vaccinated herds, where immunity should prevent virus spread. The least risky category is that of animals that have tested NSP positive, but where there is no evidence for carriers or virus transmission and it is highly likely that the animals are non-specific reactors in NSP tests.

A range of outcomes provides different levels of suspicion and confirmation with regard to detection of infection. First, the prior information, i.e. the degree of suspicion that gave rise to the

sampling and testing in the first place; e.g. the strength of the epidemiological link to other cases that have been confirmed and the degree of clinical suspicion in any sampled animals. Second, the updated prior information after the first test round, i.e. the number and intensity of seropositive reactions and the presence of linkage or clustering between the seropositive animals. Third, the posterior information, i.e. consistency of the results following retesting with the same or alternative tests, combined with the outcome of a second farm visit with further epidemiological and clinical investigations and subsequent sampling and testing results, including evidence of virus circulation provided by detection of additional seropositive animals.

Where unclustered, seropositive animals are detected at a level that is not above the predicted false positive detection rate [53] and epidemiological and clinical suspicions as well as evidence for virus circulation have been ruled out, pig herds could be considered free from infection. In the case of ruminants, the worst-case scenario would be that some of these animals are carriers. To mitigate this risk, the seropositive animals could be sent for slaughter and human consumption so long as the heads of the animals are removed during processing ('Conditional slaughter'; [61]). The remaining herd could be considered uninfected. This is less severe than current EU legislation. Follow-up testing could be used to double-check absence of seroconversion in the same way as sentinels may be tested after depopulated farms are restocked. This is a better approach than virological testing of seropositive ruminants to look for virus carriers due to the low sensitivity of the tests available. For high value individual animals, the cost and effort of virological tests might be justified so as to avoid unnecessary slaughter; multiple sampling and testing being necessary to improve test sensitivity [4].

Where the number, strength and clustering of positive NSP test results are suggestive of infection, then the herd must be revisited and re-examined and either be declared infected and culled in its entirety, or if the evidence of infection is equivocal, only the seropositive animals could be killed initially (conditional slaughter). Other animals on the farm should be closely examined for clinical evidence of infection, possibly sampled virologically via oral or nasal swabs, and rebled for a second round of serological testing to find out if previously seronegative animals have seroconverted. If the culled animals are ruminants, then probang and oral or nasal swabs should be collected at the time of culling for virus isolation. Forwards and backwards tracing should be instigated to find out if there is evidence of infection in other herds that supplied or received animals or had other significant epidemiological contacts (although recent genetic analyses have cast doubt on the predictive value of tracing based on indirect routes of transmission—i.e. not direct animal contacts and movements [62]). If all the follow-up testing and investigation fails to verify infection, then there may or may not have been a localised infection in the past, but the herd can now be considered free from infection and the possibility of past infection should not affect the timing for a declaration of FMD freedom. Further evidence of infection could lead to the conclusion that the herd had probably been infected in the past and/or there was continuing virus circulation. Both scenarios should lead to culling of the entire herd, but the consequences for declaration of FMD freedom could differ. If it were concluded that there was virus circulation, a new outbreak would be declared. However, it might be concluded that only carriers were present and that the disease had been missed at the time of acute infection concurrent to earlier recognised cases of infection. Provided that thorough tracing had not identified later cases of infection, then such findings might not prolong the period for recovery of the FMD-free status.

Fig. 1 provides an overview of the proposed investigative procedure for vaccinated herds.

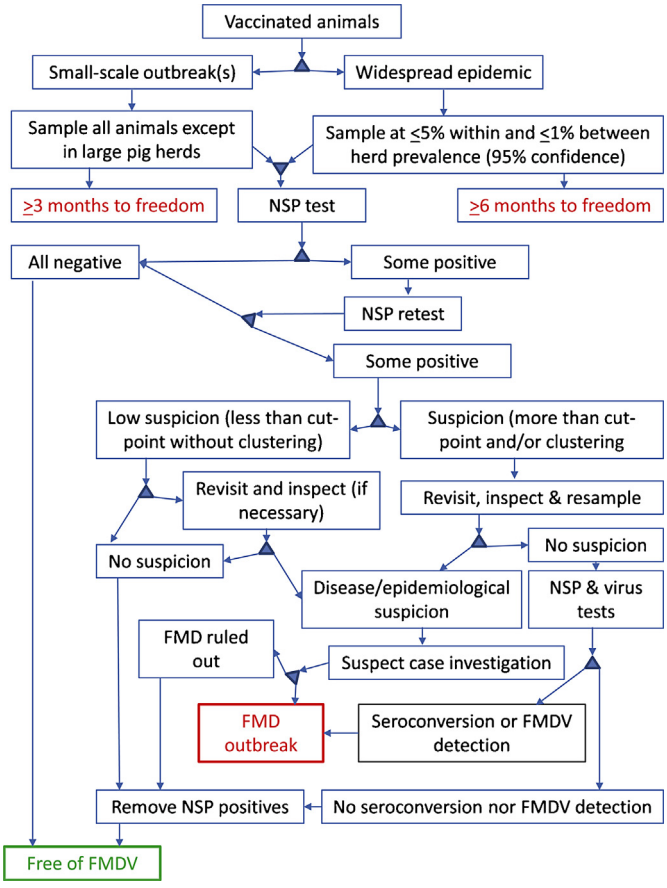

Fig. 1. Decision tree for implementing and interpreting NSP serosurveys.

9. Complementary approaches

Tests of imperfect sensitivity and specificity cannot guarantee the detection and subsequent removal of all infected animals if they are present at a very low prevalence. Instead, NSP serosurveys should supplement other control measures to detect some undisclosed cases and to substantiate that infection is not present at a higher than residual threshold, due to a failure of the FMD control strategy, whether arising from low vaccine effectiveness, or poorly enforced sanitary measures and/or surveillance.

The likelihood of infection continuing to spread despite vaccination may be related to four main factors; the infectiousness of the population immediately prior to vaccination being applied, the quality of surveillance and of control measures, and the effectiveness of the vaccination programme itself. Evidence should be collected on all of these risk factors, for example, evidence in support of the frequency and quality of epidemiological inquiries, tracing operations, veterinary inspection, and enforcement of quarantines and movement controls. Specific measures to demonstrate vaccine effectiveness should include prior knowledge of the potency and match of the vaccine used, accurate numerator and denominator data on the vaccinated population, evidence of an effective storage and distribution network including cold chain maintenance, good records of doses used and of vaccine coverage, and direct demonstration of the quality of immunity induced in vaccinated animals. This information can be collated and analysed to predict its effect in disease spread simulation models to provide a strong baseline to which further evidence from a serosurvey can be added to substantiate freedom from infection.

The procedure for recognition by OIE of the status of FMD-free where vaccination is practised requires applicants to provide

evidence of vaccine effectiveness, including data on population immunity arising from immunisation campaigns. This requirement is absent from applications for recovery of the status of FMD-free where vaccination is not practised following use of "vaccination without subsequent slaughter" [19]. However, random surveys to monitor population immunity are relatively simple to perform in terms of both sample collection and sample testing, since farm visits to inspect vaccinated herds will already be part of the sanitary control measures and because validated tests for SP antibodies are widely available. Another measure would be to undertake a heterologous in vivo vaccine potency test to directly show the level of protection provided by the vaccine used against challenge with the virus causing the outbreaks that are to be controlled. Such potency tests have been considered not worthwhile, as they are too slow to inform a decision on whether or not to proceed with vaccination. However, results could support the downstream application for FMD freedom, as well as assisting the interpretation of serosurvey findings aimed at demonstrating effective vaccine induced population immunity. As a minimum, sera could be obtained from vaccinated animals and tested serologically against the outbreak virus to show the degree of in vitro protection from which in vivo protection could be estimated.

10. Conclusions

In this paper, we review the approaches that can be taken to improve the use and interpretation of serosurveillance using FMDV NSP tests. Even though NSP tests that can differentiate infected from vaccinated animals have become available, countries are reluctant to use emergency vaccination as an additional control measure if FMDV is introduced. One aspect of this problem could be addressed by equalising, at three months, the minimum period needed to reach FMD-free status in the absence of further vaccination, regardless of whether "vaccination without subsequent slaughter" or only stamping out are used; or indeed to make this period risk-based and not a set amount of time [12]. In our paper we mainly evaluate the effect of various surveillance schemes and the risk of missing infected animals. Based on this evaluation, we consider the risk low if all vaccinated ruminants are sampled and a statistical sample on all the farms with vaccinated pigs (to detect 5% prevalence with 95% confidence). In non-vaccinated sheep (or other species where clinical signs are often absent) a sample should be taken to detect 1% of the infected herds with 95% confidence and 5% infected animals on those farms with 95% confidence. In this case a waiting period of 3 months since the last case will be sufficient (N.B. the ambiguity of sampling in Article 56 of the EU Directive should be corrected). If sampling of all vaccinated ruminants is impossible to achieve, then within and between herd design prevalence rates of less than or equal to 5% and 1% should be used for NSP serosurveys. The risk of missing infected animals is then higher, and a waiting period of six months after the last case should be applied. Follow-up of positive NSP reactors should be performed on a case-by-case approach in which laboratory, epidemiological and other information is used in decision-making. Since an effective control programme is the best guarantee that the threat of FMDV infection has been dealt with, more effort should be directed towards demonstrating this, specifically with more emphasis on demonstrating vaccine effectiveness. Countries using emergency vaccination could undertake a heterologous in vivo vaccine potency test to directly show the level of protection provided by the vaccine used against challenge with the virus causing the outbreak and to provide serological correlates of protection to calibrate SP serosurveys of the population immunity achieved by vaccination. Delaying the decision to vaccinate so as to avoid the complications of post-vaccination surveillance will make matters worse if vaccination cannot ultimately be avoided.

Author contributions

DJP drafted the initial manuscript following discussions in the OIE Ad Hoc Group for FMD. All authors reviewed and revised the manuscript and approved the final version as submitted.

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