Effectiveness of systematic foot and mouth disease mass vaccination campaigns in Argentina

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Summary

The objective of this paper is to evaluate the effectiveness of systematic mass vaccination campaigns against foot and mouth disease in Argentina. The analysis was based on an estimation of the proportion of protected animals and protected farms in vaccinated populations, as reflected by levels of antibodies measured in liquid-phase enzyme-linked immunosorbent assay. The analysis was carried out in 42 animal health districts in Buenos Aires province, using data collected from four cross-sectional studies, in 2004, 2007, 2008 and 2011. Cattle were assigned to one of two categories on the basis of correlation between serological titres and expected percentage protection: non-adequately protected (expected protection <75%) and adequately protected (expected protection ≥75%). The proportions of adequately protected cattle and significantly non-adequately protected farms were estimated and compared among sampled locations. Protection was variable among the districts; cattle aged one to two years showed higher levels of protection than cattle six to 12 months old, and the proportion of protected cattle was higher in the more recent studies. The results of the analysis will allow the national animal health service to investigate in depth those districts where protection was lower than the regional background protection. The authors propose that this methodology could be used to evaluate the effectiveness of vaccination campaigns in other countries or zones where systematic foot and mouth disease mass vaccination campaigns are undertaken.

Keywords

Adequately protected animal – Evaluation of vaccination campaign – Foot and mouth disease – Liquid-phase blocking ELISA – Mass

vaccination campaign – Sampling design – Significantly non-adequately protected herd.

Introduction

Foot and mouth disease (FMD) is arguably one of the most contagious infectious diseases of mammals and has great potential for causing severe economic loss in susceptible cloven-hoofed animals. There are seven serotypes of FMD virus (FMDV); namely, O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1, incorporating a large and indeterminate spectrum of subtypes. Typical cases of FMD are characterised by a vesicular condition of the feet, buccal mucosa and, in females, the mammary glands; clinical signs may vary from mild to severe and fatalities may occur, especially in young animals (1). Infection or vaccination with one FMDV serotype does not confer immunity against another serotype, and the protection conferred by one subtype against another subtype of the same serotype is variable, ranging from none to complete (2).

A number of South American countries are exporters of animals and animal products derived from FMD-susceptible species; such products have the potential to carry the virus. Importing countries implement strict health barriers in this international trade and it is a priority for exporter countries to preserve their FMD-free status. The virus persists in some parts of the South American continent (3, 4) and outbreaks have been recently reported in Ecuador (2006 to 2011) and Venezuela (2007 and 2011) (5). In addition, during recent years clinical cases have been sporadically detected in FMD-free countries and zones such as Argentina (2006), Brazil (2006), Bolivia (2007), Colombia (2008 and 2009) and Paraguay (2011 and 2012) (6). Consequently, most South American animal health services have designed and implemented compulsory systematic FMDV mass vaccination programmes for cattle at the whole-country level or at zone level. The ultimate objective of such programmes is to raise herd immunity to the level that prevents the introduction of FMDV or mitigates its spread within the susceptible population.

The FMDV vaccine currently used in Argentina is oil-adjuvanted and tetravalent, comprising strains A24/Cruzeiro, A/Argentina/2001, O1/Campos and C3/Indaial (7). To be approved for general use, each batch of vaccine is subject to tests for safety and potency by the national animal health service (*Servicio Nacional de Sanidad y Calidad Agroalimentaria* [SENASA]) (8). The vaccination programme is administrated by SENASA for the entire territory, with the exception of Patagonia, which is recognised by the World Organisation for Animal Health (OIE) as an FMD-free zone where vaccination is not practised. Vaccination takes place twice per year, although other strategies such as annual vaccination are used in very limited and isolated areas. Only cattle are vaccinated and each campaign lasts approximately two months. In any given year, all cattle (independently of age) are vaccinated in the first campaign, and then cattle under two years of age are vaccinated in the second campaign. The vaccination area contains more than 95% of the country's cattle population.

The operational aspects of the vaccination campaign, including its design and the purchase, storage and delivery of the vaccines, are coordinated by SENASA-audited local authorities in each animal health district. Each district is administered by an elected group of producers, has a defined geographical area under its mandate and has a veterinarian as technical director. Vaccination is administered by contracted personnel. Throughout Argentina there are 305 animal health districts with a total of approximately 220,000 cattle producers and 50 million cattle.

The effectiveness of a vaccination programme depends on a number of factors (9, 10). First, the vaccine should be of adequate potency and safety, should contain strains of FMDV that match field strains, and should be subjected to a modern quality assurance system, according to the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, Chapter 2.1.5. (1). In addition, the vaccine should be stored and distributed under proper conditions, using a cold chain, and the shelf life of the product should not be exceeded. Secondly, the interval between vaccination events on a single farm, the age of the calves at

first vaccination and the duration of the vaccination campaign should be consistent with the expected pattern of natural and artificial immunity. The level of vaccination coverage that is required depends on several factors and it is impossible to be prescriptive; however, the aim should be to achieve at least 80% herd immunity (11). Lastly, the vaccine should be used according to the procedure prescribed by the manufacturer. Failure to achieve and control compliance with these factors contributes to the maintenance of FMDV circulation, even in countries where the main control strategy has been massive vaccination over a number of years (12).

Post-vaccination monitoring is necessary for estimating FMD protection at the level of both the individual animal and the farm, and for identifying possible campaign failures (13). The objective of this study was to design and apply an analytical method for evaluation of the effectiveness of systematic FMDV mass vaccination campaigns. The method was used to evaluate the effectiveness of a mass vaccination programme in Argentina.

Materials and methods

Sampling frame

The province of Buenos Aires covers approximately 300,000 km^2. Within the province there are 105 animal health districts that manage the vaccination of approximately 20 million cattle distributed on approximately 60,000 livestock farms. Approximately 40% of Argentina's cattle population is located within the province of Buenos Aires.

Sampling design

Four independent cross-sectional studies were carried out in 2004, 2007, 2008 and 2011. In each of them, the animal health districts were enrolled on a voluntary basis. A two-stage, random-sampling design was used to estimate the proportion of cattle protected against FMD per farm in each district. In the first stage, farms (primary sampling units) were selected with a probability of selection proportional to the

number of animals on the farm. In the second stage, cattle (secondary sampling units) were systematically selected from each farm.

The number of farms to be selected per district (*n*) was calculated using the following formula (14):

$$n = \frac{p \times (1-p) \times z^2 \times [ROH \times (b-1)+1]}{e^2 \times b}$$

where *p* is the expected proportion of animals with serological levels of antibodies compatible with protection, *z* is the level of confidence, *ROH* is the rate of homogeneity, *b* is the number of animals selected per farm (may be variable: the fewer individuals per farm, the greater the number of farms to be selected) and *e* is the acceptable absolute error.

For cattle aged six to 12 months (category 1), the parameters were defined as *p*: 65%, based on previous studies (15, 16); level of confidence 95%; *ROH* low (0.11), based on previous studies (17); *b*: 10 (adequate value for comparison of results between farms); error: 6.5%.

For cattle one to two years old (category 2), the parameters were *p*: 86% (15, 16); level of confidence 95%; *ROH*: 0.5 (17); *b*: 3 (value adjusted to obtain the same number of farms in both calculations); error 8.6%.

For both age categories the result was that 42 farms were needed. Thus, 42 farms per district were randomly selected, with the probability of selection proportional to the total number of cattle per farm. Lastly, 10 cattle from category 1 and three cattle from category 2 were sampled on each farm. This procedure was used in each cross-sectional study.

Sample collection

Blood samples were collected during the first vaccination campaign of the year (February to March, i.e. early autumn in the southern hemisphere). It was expected that all animals more than six months

old on a given farm would have been vaccinated on average six months earlier than the date of sampling. Cattle in category 1 had received at least their first FMDV vaccine dose; cattle in category 2 had already received two or three doses. It was expected that immunity to FMDV had reached its lowest level in early autumn, immediately before the vaccination round.

Diagnostic testing

Serum samples were analysed in a single dilution liquid-phase competitive blocking enzyme-linked immunosorbent assay (slpELISA) to determine antibody titres against FMDV strain O1/Campos, using the protocol described previously (7). Briefly, a 1:64 dilution of each serum was tested in 96-well plates. Each plate allowed the testing of 68 samples, six positive-control sera of known titres (high, medium, low) and one negative-control serum. Six wells were used for control of antigen concentration (100% reactivity) and two wells were used as reaction blanks.

The absorbance value at a dilution of 1:64 of each sample was interpolated in a standard curve generated for each slpELISA plate, using the absorbance values of the six positive control sera at 1:64 dilutions versus the reference titres of the same controls, determined by end-point dilution. A straight line was obtained by linear regression analysis in the titre range of 1.40 to 2.40.

Calculations and validation of each plate were as described for liquid-phase competitive blocking ELISA (lpELISA) (8).

Case definition

Each animal was classified as either non-adequately protected (expected percentage protection [EPP] <75%) or adequately protected (EPP ≥75%) according to the slpELISA titres. The EPP is a measure of the association between ELISA titres and protection (8, 18, 19, 20). For potency testing, vaccinated cattle were challenged by intradermal injection of FMDV into the tongue and then classified as protected or not protected on the basis of post-inoculation clinical signs or lesions.

The association between the probability of protection (i.e. the EPP) and slpELISA titres was quantified using logistic regression. For vaccine approval, Argentinean legislation requires slpELISA titres associated with an EPP ≥75%, measured 60 days post-vaccination (21). Accordingly, the ELISA titre for which an EPP ≥75% is verified is considered the cut-off value for case definition (protected, unprotected). Currently, the cut-off value for FMDV strain O1/Campos is 2.10, meaning that cattle with an slpELISA titre ≥2.1 are predicted to have ≥75% probability of protection against infection (i.e. EPP ≥75%).

Data analysis

The proportion of animals adequately protected was estimated, grouping animals according to age category, farm and district. A 95% confidence interval was calculated for the estimated proportion of adequately protected animals, taking into account the two-stage procedure used for sampling selection (14).

A significantly non-adequately protected herd (SNAPH) was defined as a herd in which the proportion of adequately protected category 1 animals was significantly <65% at a 95% level of confidence. As stated above, ten category 1 bovines were sampled from each farm. Assuming that test results followed a binomial distribution of the form $n = 10$, $p = 0.65$ (sample size 10, expected proportion of adequately protected category 1 animals 65%), the cumulative probability of a farm having ≤3 adequately protected samples was <0.05. A farm was therefore classified as a SNAPH where ≤3 category 1 animals were adequately protected. The proportion of SNAPHs per district was subsequently estimated.

Chi-squared tests were used to estimate whether:

a) the district-specific proportion of adequately protected animals was significantly different from that expected, which (as described above) was defined as 65% for category 1 animals and 86% for category 2

b) a district had a proportion of SNAPHs significantly different from that expected, defined for each survey as the mean proportion of SNAPHs in the survey

c) the proportion of adequately protected animals and SNAPHs varied between and among surveys.

The association between the number of adequately protected animals and SNAPHs was quantified using linear regression.

Results

Samples (n = 42,547) were collected from 3,309 farms in 49 animal health districts during the four survey periods: 24, 9, 13 and three districts participated in one, two, three and four of the cross-sectional studies, respectively (Table I).

The proportion of adequately protected animals and the 95% confidence interval for category 1 and category 2 animals are shown for the four studies in Figures 1 to 4. As expected, the proportion of adequately protected animals was consistently higher in category 2 animals than in category 1.

For each of the four studies, the percentage of districts with a proportion of adequately protected cattle greater than expected was 33%, 22%, 18% and 76% (category 1) and 92%, 61%, 64% and 81% (category 2), respectively.

The percentage of adequately protected category 1 animals was significantly ($p < 0.05$) higher in 2011 than in previous years. However, the range of values (the difference between the maximum and minimum percentage of adequately protected category 1 animals per district) was smaller in 2011 than in previous years (i.e. 47, 41, 35 and 26 for the 2004, 2007, 2008 and 2011 studies, respectively). The proportion of adequately protected category 2 cattle was significantly ($p < 0.05$) greater in 2011 than in 2007, but the differences with the other studies were not significant.

The distribution of SNAPHs by district for the four surveys is shown in Figure 5. The percentage of SNAPHs per survey was 18%, 25%, 27% and 11%, respectively. The number and percentage of districts with SNAPHs significantly lower than average for each survey was 5 (13%), 2 (11%), 1 (9%) and 3 (14%), respectively.

For category 1 cattle in the 2011 study, the proportion of adequately protected animals and the proportion of SNAPHs by district were significantly associated (r^2: 0.63; $p < 0.01$) (Fig. 6).

Discussion and conclusions

Regular evaluation of systematic and mass vaccination campaigns is of great importance in order to quantify their effectiveness, detect problems and monitor the effect of interventions designed to correct identified problem areas. The method proposed in this paper has already been implemented in four situations and the results have supported the decision-making process in animal health districts and at SENASA.

The observed proportion of adequately protected animals per district was consistently lower for category 1 cattle than for category 2, and results were more variable in the younger animals. Although this was expected, because older cattle have had the opportunity to receive more vaccinations than younger ones, the results provide reassurance that the sampling protocol used in each of the cross-sectional studies was appropriate.

The higher proportion of adequately protected category 1 cattle and the lower proportion of SNAPHs observed in 2011 were probably associated with adjustments made to the vaccination campaign protocols, mainly to improve vaccination coverage and the timing of vaccination on each farm. It is important to have a high proportion of adequately protected category 1 cattle, because they have the lowest level of immunity in the population, and at weaning they are frequently moved to other premises where they are mixed with cattle of the same category from different origins. For these reasons they are the most likely age group to be infected with and spread FMDV.

Although the proportion of adequately protected cattle and the proportion of SNAPHs in each district were significantly associated, it should be noted (Fig. 6) that two districts may have a similar proportion of adequately protected cattle but a different proportion of SNAPHs, and vice versa. For example, in the districts where the proportion of adequately protected cattle was close to 70%, the percentage of SNAPHs ranged from 3% to 20% (districts no. 17 and no. 39), whereas among districts with about 5% SNAPHs, the percentage of adequately protected cattle ranged from 68% to 83% (districts no. 46 and no. 16). Both the proportion of adequately protected cattle and the proportion of SNAPHs are important indicators when analysing the effectiveness of vaccination campaigns. An appropriate level of protection in a vaccinated animal population requires not only a high proportion of adequately protected animals but also a lack of clusters of non-adequately protected animals. An even distribution of a given proportion of non-adequately protected animals in a population may have little effect on the risk of FMDV diffusion, but clusters of highly susceptible animals on a small number of farms may pose a serious risk. The frequency and distribution of non-adequately protected animals are closely related to the design, implementation and supervision of vaccination campaigns.

Although the vaccine used in Argentina at the time of the four cross-sectional studies was tetravalent, only strain O1/Campos was analysed in the present study. However, a high level of concordance between the four vaccine strains was observed in an earlier study where the levels of antibodies were determined in samples from 279 category 1 cattle and 76 category 2 cattle (B. Robiolo, personal communication).

In order to simplify the analyses, the authors classified cattle into two categories: adequately and non-adequately protected. The criteria used for classification were based on the results of potency testing for the purpose of vaccine approval. In this process, animals are challenged with an intradermal FMDV inoculation of 1×10^4 infective dose 50% into the tongue. The cut-off point for a given serotype is the dose that protects 75% of challenged animals. The inoculation of 1×10^4 infective dose 50% is probably a greater challenge than animals might

experience under field conditions. Two considerations should be therefore kept in mind: *i*) the defined criterion for classification of an animal as adequately protected was very strict, and *ii*) animals that were classified as not adequately protected are not completely unprotected, and certainly many of them could resist infection or not show clinical signs when given an infective dose.

In Argentina, the distribution of the cattle population according to age category varies across zones, seasons and productive systems. In autumn, on average, about 25% of cattle are less than one year old, 35% are between one and two years and 40% are older than two years. Herd immunity should be the weighted average of adequately protected animals in all age categories. The objective of this study was to evaluate the efficacy of the vaccination campaign and including cattle >2 years old in the analysis would bias the results, because of the effect of multiple vaccinations. For that reason, the results presented here are an underestimate of the true level of herd immunity.

Moreover, blood samples were collected from cattle when vaccine-induced antibodies were at one of their lowest possible levels, just before revaccination. This may not have had an important effect in cattle that had been vaccinated several times, but it is likely to be important in young cattle (category 1) that had received only one vaccination four to six months earlier. This issue is particularly important if cattle were first vaccinated at a time when they still had a relatively high level of colostrum antibodies. The frequency of these events needs to be considered when interpreting the results of studies of this type.

Another possible reason for the low proportion of adequately protected category 1 cattle is the timing of vaccination relative to the time of the calving period in seasonally managed herds. In areas where calving is concentrated in a three- or four-month period, vaccination campaigns frequently take place either before or immediately after the end of the calving period. Thus, some calves are young, still having high levels of colostrum antibodies at the time of

first vaccination, and passive immunity may affect the response to vaccination in these animals. This factor is crucial and must be considered when planning and analysing systematic vaccination campaigns, particularly in cattle production systems that are managed on a seasonal basis. The requirement to revaccinate category 1 cattle before moving them to other premises helps to address these problems.

The described method for evaluation of the effectiveness of a systematic FMDV vaccination programme has been used in Argentina on several occasions, and it was useful to estimate protection against FMDV at the level of both the individual animal and the farm, and then to implement and monitor corrective measures. The authors propose that this methodology could be applied in other countries or zones where vaccination is systematically used, provided that the correlation between serum antibody level and protection has been determined. In cases where this has not been determined or vaccination is not systematically used, evaluation of the effectiveness of vaccination campaigns may need some adjustments.

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Table I

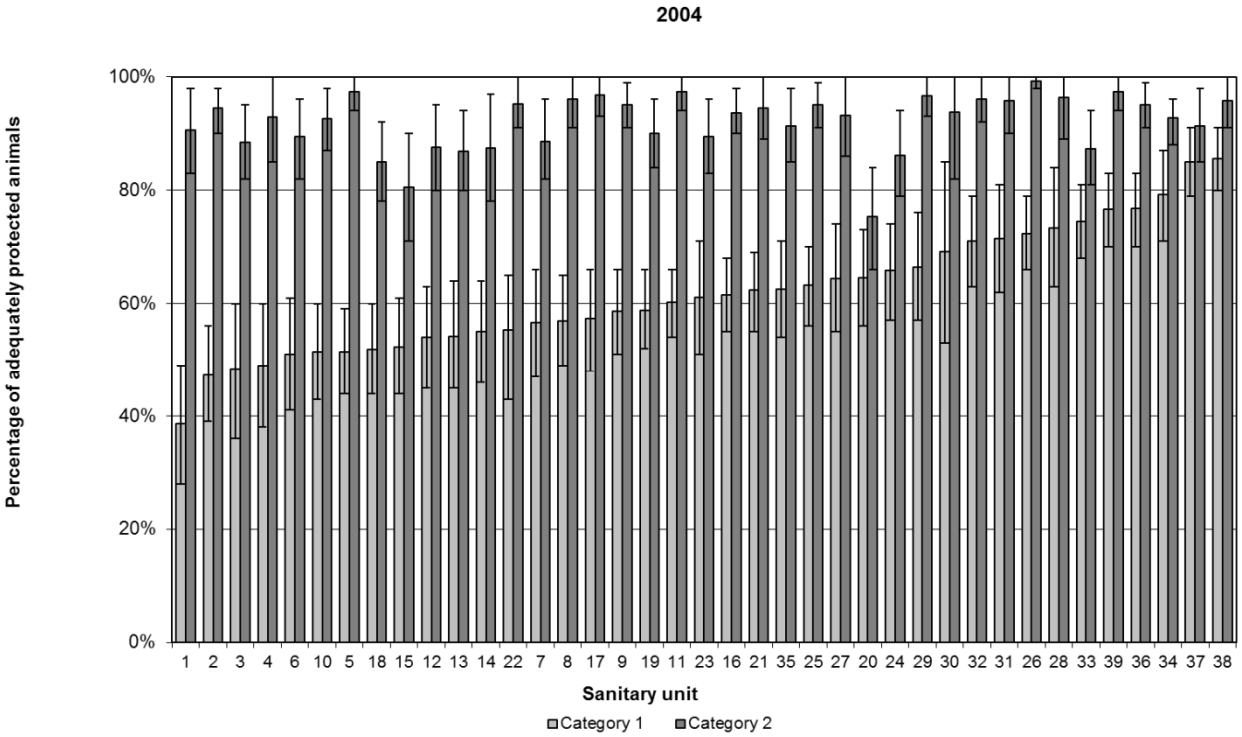
Evaluation of systematic mass vaccination campaigns against foot and mouth disease in Argentina, 2004 to 2011

Number of animal health districts participating in the study, number of selected farms and number of cattle in each age category, stratified by study

Study	Districts	Farms	Animals	
			Category 1 [(a)]	Category 2 [(b)]
2004	39	1,387	13,832	4,118
2007	18	660	6,569	1,881
2008	11	433	4,331	1,144
2011	21	829	8,239	2,433
Total	89	3,309	32,971	9,576

a) Cattle 6 to 12 months of age

b) Cattle 1 to 2 years of age



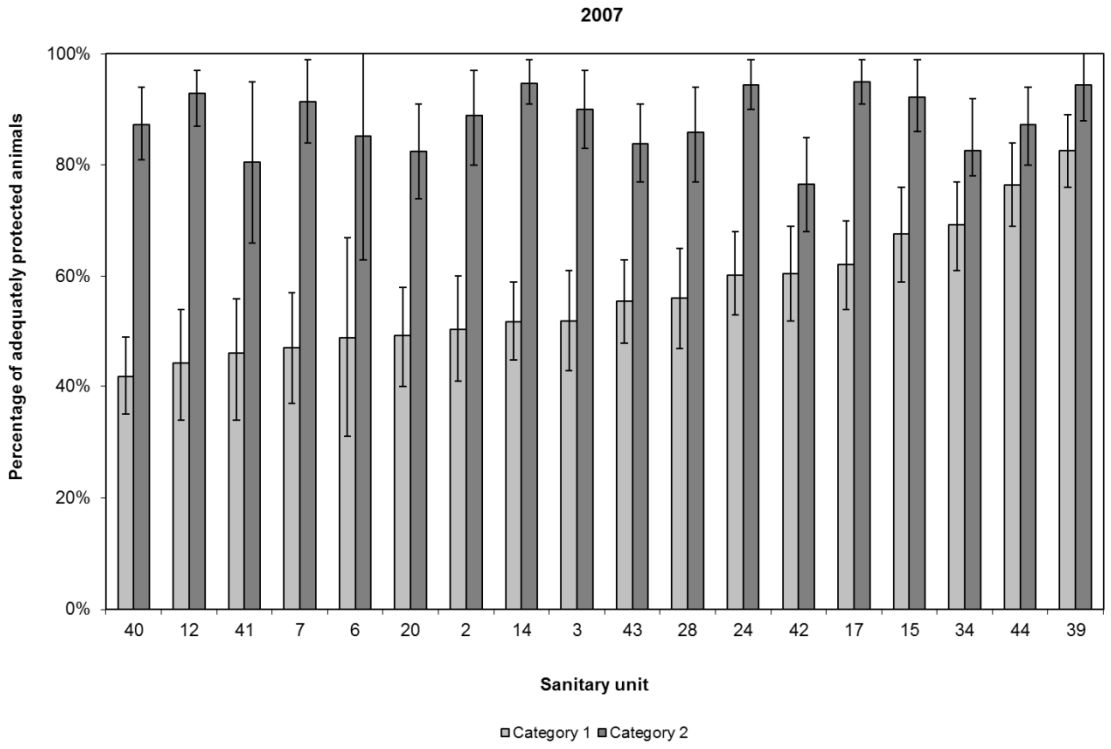
Category 1: cattle 6 to 12 months of age

Category 2: cattle 1 to 2 years of age

Fig. 1

Ranked bar plot showing the percentage and 95% confidence interval of adequately protected animals per animal health district and age category, 2004 study

Foot and mouth disease virus strain O1/Campos



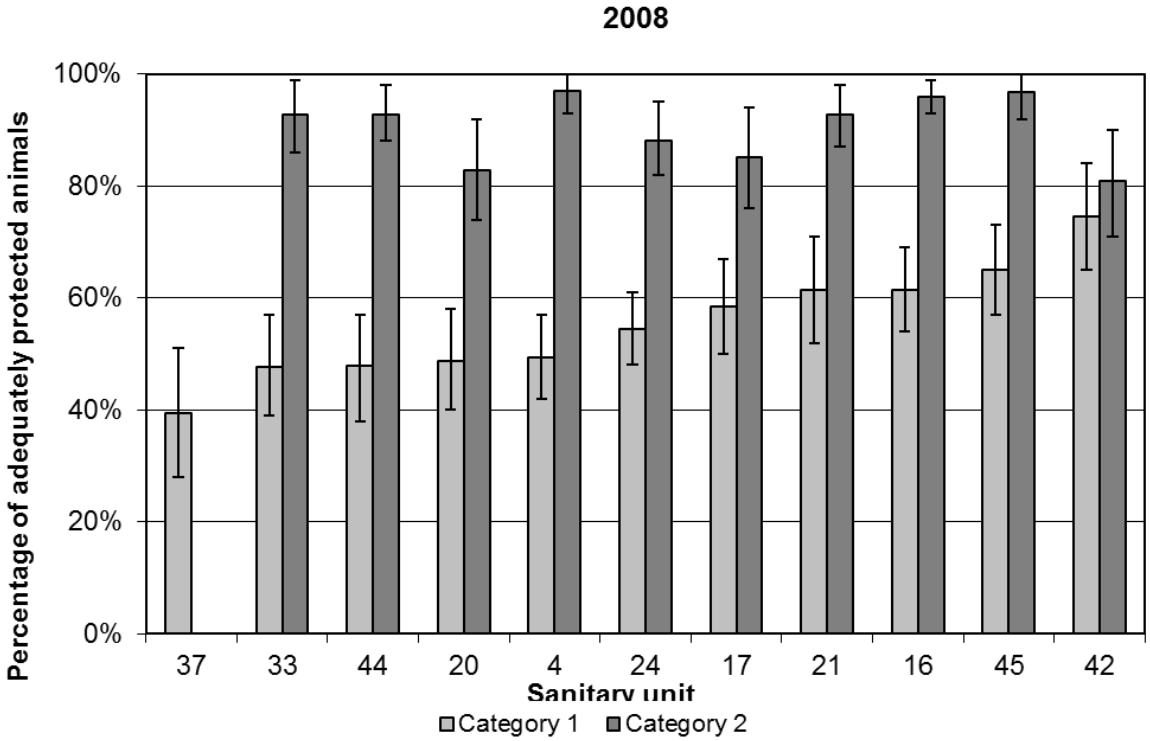
Category 1: cattle 6 to 12 months of age

Category 2: cattle 1 to 2 years of age

Fig. 2

Ranked bar plot showing the percentage and 95% confidence interval of adequately protected animals per animal health district and age category, 2007 study

Foot and mouth disease virus strain O1/Campos



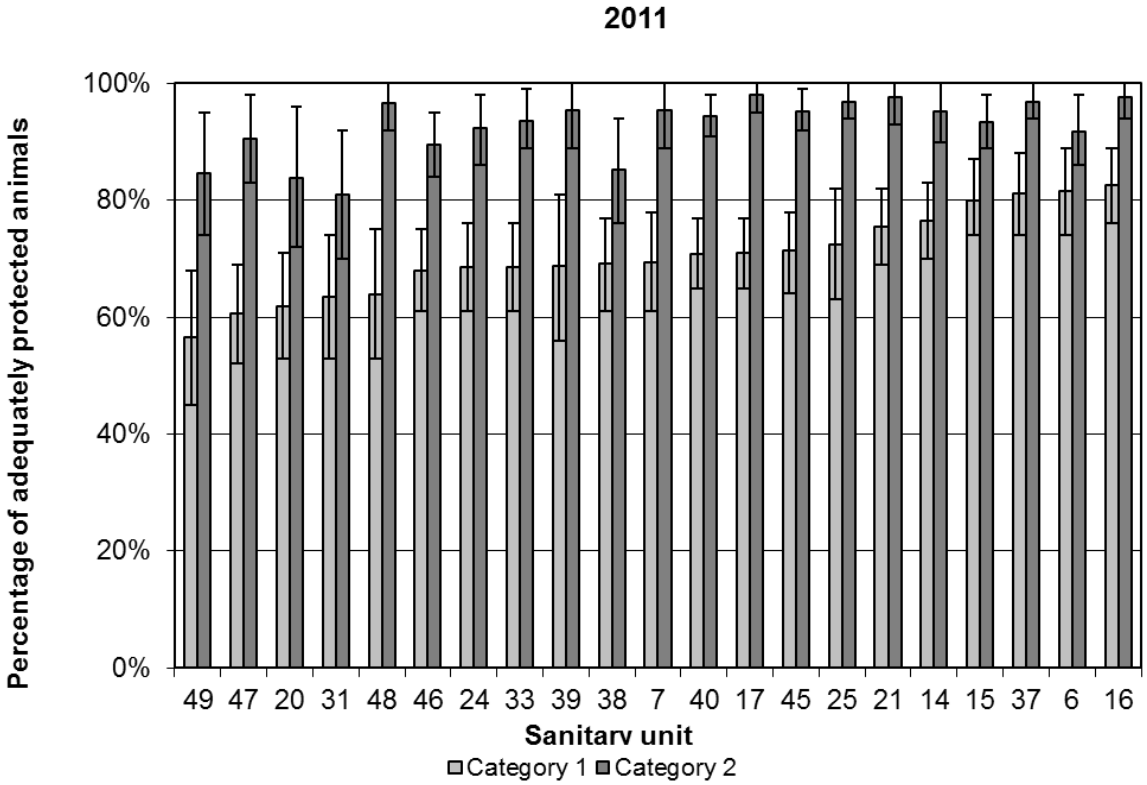
Category 1: cattle 6 to 12 months of age

Category 2: cattle 1 to 2 years of age

Fig. 3

Ranked bar plot showing the percentage and 95% confidence interval of adequately protected animals per animal health district and age category, 2008 study

Foot and mouth disease virus strain O1/Campos



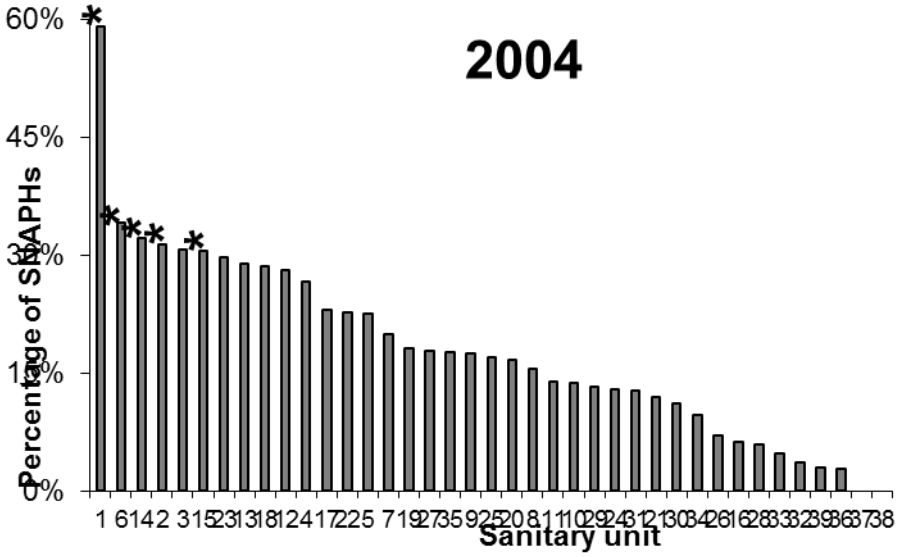
Category 1: cattle 6 to 12 months of age

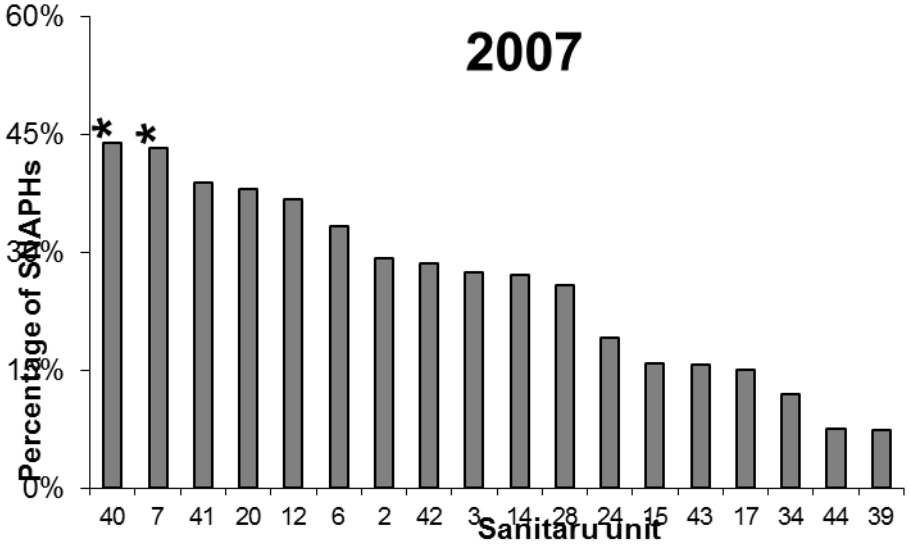
Category 2: cattle 1 to 2 years of age

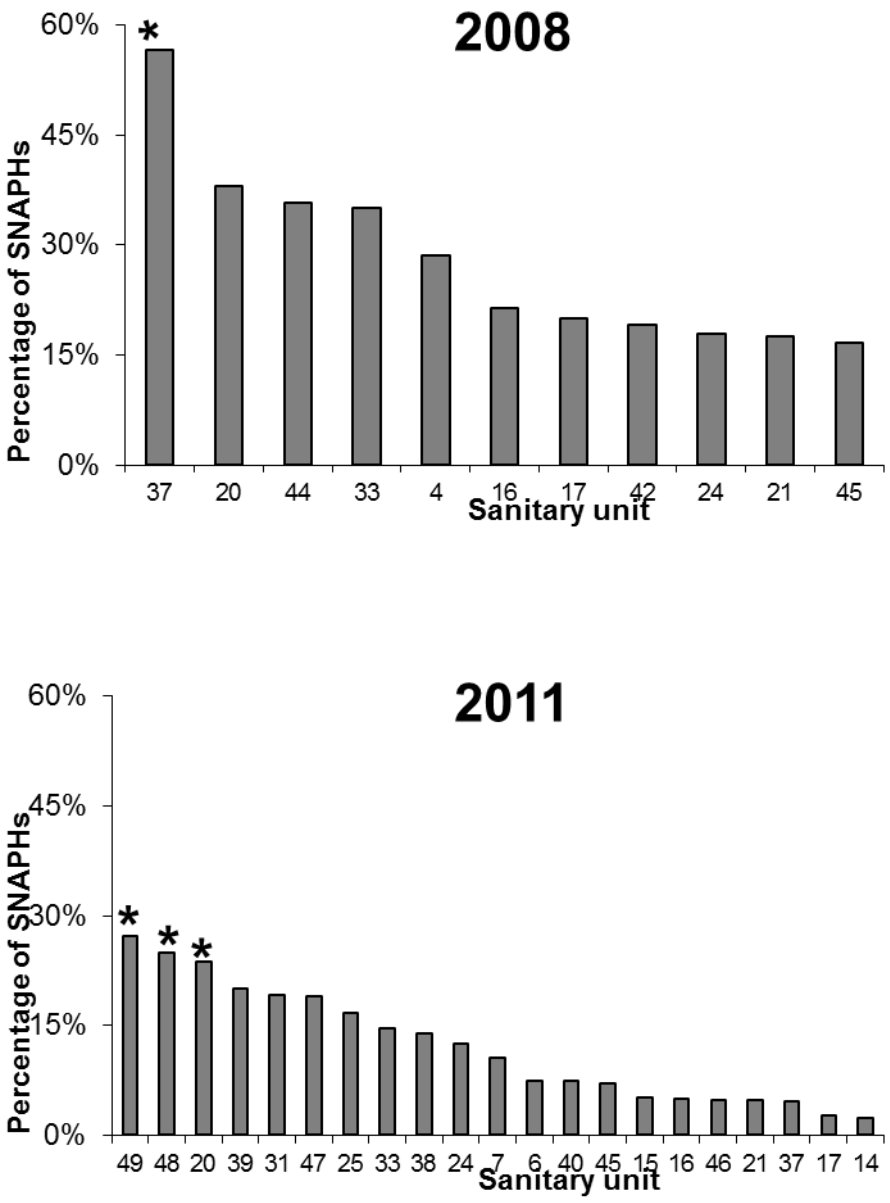
Fig. 4

Ranked bar plot showing the percentage and 95% confidence interval of adequately protected animals per animal health district and age category, 2011 study

Foot and mouth disease virus strain O1/Campos


2004
60%
45%
30%
15%
0%
Percentage of SNAPHs
Sanitary unit


2007
60%
45%
30%
15%
0%
Percentage of SNAPHs
40 7 41 20 12 6 2 42 3 14 28 24 15 43 17 34 44 39
Sanitary unit

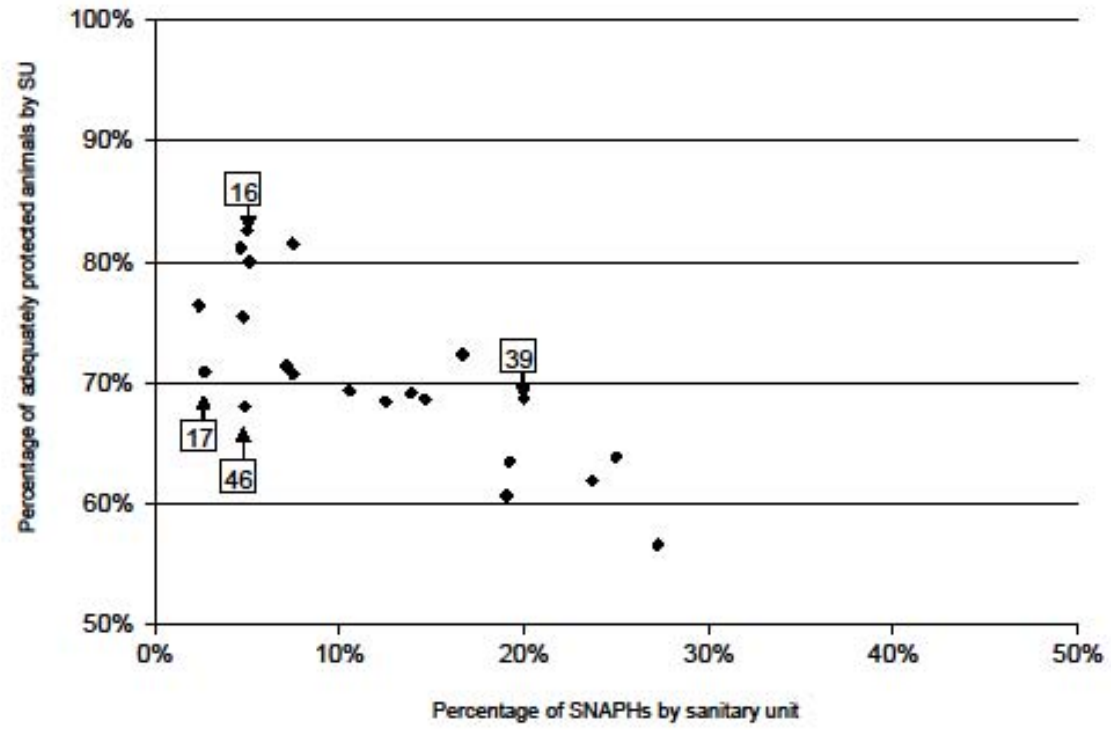


SNAPHs: significantly non-adequately protected herds

* Significant difference (p <0.05) from average proportion of SNAPHs per district

Fig. 5

Ranked bar plots showing the percentage of significantly non-adequately protected herds per animal health district for each study



SNAPHs: significantly non-adequately protected herds

Fig. 6

Scatter plot showing the percentage of adequately protected animals per animal health district as a function of the percentage of significantly non-adequately protected herds per district

Data from 2011 study for category 1 animals and FMDV strain O1/Campos. Diamonds represent districts, labels identify particular districts