

Importance of foot and mouth disease vaccine purity in interpreting serological surveys

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Summary

The aim of this study was to determine whether the degree of purity achieved in conventional vaccines against the foot and mouth disease virus in Argentina interferes with the interpretation of seroepidemiological surveys for confirming the absence of viral activity, which are performed to support the recognition of free zones practising vaccination.

The evaluation of 168 vaccine series due to be marketed in Argentina (2006–2012) and subjected to official control testing in cattle, as well as repeated vaccination of cattle and other species using vaccines with high antigen concentrations, demonstrated that they did not induce antibodies to non-structural proteins (NSPs).

The results show clearly that vaccines with satisfactory potency do not induce a response to NSPs, even by forcing the immune response through more concentrated doses with multiple valences and revaccination protocols at shorter intervals than in vaccination campaigns. These results confirm that the vaccines used in routine vaccination plans have a degree of antigen purification consistent with the needs observed on the basis of sampling for serological surveillance. Moreover, serological surveys conducted in 2006–2011 by Argentina's official Veterinary Service – the national health and agrifood quality service (SENASA) – on more than 23,000 sera per year from cattle included in the vaccination plan, in order to confirm the absence of virus circulation, revealed an average 0.05% of reactive results, consistent with the specificity of the tests.

In conclusion, the vaccines produced by conventional methods and with proven potency that are available in Argentina are sufficiently purified to ensure that they do not interfere with the interpretation of sampling for serological surveillance performed to support the recognition of FMD-free zones practising vaccination.

Keywords

Animal disease status – Argentina – DIVA vaccine – Foot and mouth disease – Non-structural protein – Purified vaccine – Serological survey – Vaccine – Viral activity.

Introduction

Foot and mouth disease (FMD) is an acute, highly contagious disease of cloven-hoofed animals. It is the most economically important disease of livestock because of its impact on international trade in animals and animal products.

In its standards, the World Organisation for Animal Health (OIE) stipulates the use of control measures to enable countries or zones to obtain recognition of their FMD-freedom with or without vaccination, which is essential for gaining access to markets for the products and by-products of FMD-susceptible species. To obtain or regain disease-free status following outbreaks, every country's Veterinary Services must demonstrate the effectiveness of control and/or eradication programmes. They do this by means of clinical, virological and serological surveillance to ensure the absence of clinical cases and cases of infection or asymptomatic virus circulation, the latter particularly when vaccination is administered.

In FMD monitoring, control and eradication campaigns implemented in zones or countries practising vaccination where no clinical cases have occurred, serological methods for detecting antibodies to viral structural proteins are not useful because they detect the antibodies produced in animals as a result of both infection and vaccination. For over a decade, serological indicators have been available for detecting antibodies to viral non-structural proteins (NSPs) (1, 2, 3, 4) and it is widely documented that the presence of NSP antibodies is associated with viral replication in the population, whether or not the animals have been vaccinated. Tests to determine the presence of antibodies to NSPs have therefore been very useful in identifying FMD-free zones (5) and hence in supporting international recognition of free zones practising vaccination. It is essential to ensure that the vaccines used in vaccination campaigns or emergency vaccination meet the purity requirement with regard to their NSP content and that they do not induce antibodies to NSPs in vaccinated animals.

The use of vaccines for routine vaccination of cattle and emergency vaccination of all susceptible species has proved to be an effective tool in controlling the disease (6, 7) and has reduced virus circulation significantly to undetectable levels. In Argentina, the FMD control and eradication programme of Argentina's official Veterinary Service, the national health and agrifood quality service (SENASA), provides for the routine vaccination of cattle in the free zone practising vaccination: every six months for cattle under two years of age and

every year for those over two years of age. These intervals may be modified in accordance with the epidemiological situation and risk. The programme does not include the vaccination of pigs, sheep or other species, except in risk situations determined by SENASA.

In the production of viral antigens and inactivated FMD vaccines, there have been significant improvements in the substrates used for replicating the virus and in inactivating agents, antigen purification procedures (8, 9) and the quality of adjuvants employed (10). As a result, vaccines whose production process includes purification stages have been found not to induce NSP antibodies, even after several vaccinations and/or using a double dose with a high antigen concentration (2, 9, 11, 12).

This makes these vaccines a product of choice for use in routine vaccination or as a high-potency emergency vaccine, not only because they have met the purity, safety, potency and efficacy requirements laid down in the regulations of the country where they are marketed but also because they have been administered extensively to the susceptible animal population.

This study presents data on the potency of inactivated vaccines marketed in South America and on the guarantees of their purity in terms of NSP content. The strategy used was NSP antibody determination under different vaccination and revaccination schedules of different hosts under controlled conditions. The study also presents the results of potency control tests and of NSP antibody determination obtained in official control testing of the vaccines and data from serological surveys of cattle included in Argentina's routine vaccination plan. It shows that the conventional vaccines with satisfactory potency used in Argentina do not interfere with serological surveys to determine viral activity, which are performed to support recognition/maintenance of the disease status of FMD-free zones practising vaccination.

Materials and methods

Vaccines

The vaccines were produced by the Biogénesis Bagó laboratory (Buenos Aires, Argentina) in accordance with good manufacturing practice. The antigens (O1 Campos, A24 Cruzeiro, A Argentina 2001 [A2001], A Argentina 2000 [A2000] and C3 Indaial) were grown in baby hamster kidney (BHK-21) cells in suspension, inactivated with binary ethyleneimine, then concentrated and purified using polyethylene glycol. The vaccines were formulated as simple water-in-oil emulsion with antigen concentrations greater than 30 micrograms (μg) per dose in the case of polyvalent vaccines and greater than 15 μg /dose in the case of monovalent vaccines. These concentrations are considered high compared with those documented by European manufacturers (13). Trivalent vaccines were used at 5 millilitres (ml) per dose, and quadrivalent or pentavalent vaccines (Table I) and monovalent vaccines (O1 Campos) were used at 2 ml/dose. The commercial vaccines submitted for official control testing between 2006 and 2012 were quadrivalent vaccines (O1 Campos, A24 Cruzeiro, A2001 and C3 Indaial). They were tested by the production laboratory before being submitted to SENASA for official control testing to determine their safety, purity and potency, and the vaccines met the official control testing requirements satisfactorily under current Argentinian regulations (in the case of the 2 ml/dose vaccines) (14, 15) and under current Brazilian regulations (in the case of the 5 ml/dose vaccines).

The vaccines for experiments 2, 3 and 4 were in commercial use, while the vaccine for experiment 1 was experimental. A commercial monovalent vaccine for export was used for pigs.

Vaccines from a number of manufacturers have been used in vaccination campaigns in Argentina. During the 2006–2011 period, more than 88% of the vaccines administered came from the Biogénesis Bagó laboratory.

Repeated vaccination testing in cattle

Repeated vaccination tests were carried out under controlled conditions on cattle in the SENASA experimental field in Argentina. The animals used ($n = 87$) were of the Hereford breed, aged between 18 and 24 months and free from FMD antibodies; they came from the Patagonia region of Argentina (a zone not practising FMD vaccination, 42nd parallel south), had been wormed and were in good health. In experiment 1a, a single dose (2 ml) was administered three times at 30-day intervals, and in experiment 1b, a double dose (4 ml) was administered following the same schedule. In experiment 2, four single doses of vaccine were administered at intervals ranging from 30 to 90 days, and in experiments 3 and 4, three single doses were administered at intervals of 30 to 90 days. Table I describes the design of each vaccination experiment using repeat doses, and specifies the number of cattle used in each test, the strains included in each vaccine and the volume per dose, the number of vaccinations administered and the timing of vaccinations. The blood for obtaining serum was drawn on day 0 of the test and between 30 and 60 days following each vaccination. In each test, two unvaccinated cattle were used as a control and blood samples were taken from them at the same times as from the vaccinated cattle.

Repeated vaccination testing in pigs and sheep

The pigs ($n = 15$) used for testing were of the Duroc Jersey breed. They were 2 months old, had never been vaccinated against FMD and had been wormed. The sheep used ($n = 23$) were of the Merino breed, aged between 12 and 48 months, had never been vaccinated against FMD and had been wormed. The repeated vaccination tests were conducted under controlled conditions in establishments in the Province of Buenos Aires (Argentina).

The pigs and sheep were given two vaccinations (using the monovalent vaccine O1 Campos in the case of pigs, and the quadrivalent vaccine O1 Campos, A2001, A24 Cruzeiro and A2000 in the case of sheep) at 30-day intervals, and blood samples were taken every 30 days up to 60 days post primary vaccination (dpv).

Determination of antibodies to structural proteins

Liquid-phase blocking enzyme-linked immunosorbent assay (LPB-ELISA) was used to detect specific antibodies to vaccine virus strains of the above-mentioned vaccines. The titres obtained by LPB-ELISA were converted into expected percentage of protection (EPP) values for each of the vaccine strains, in accordance with tables of correlation between LPB-ELISA titres and protection against the challenge virus (16). The official potency control testing performed on the vaccine series consists of using this technique to determine antibodies in the sera of a group of 17 cattle 60 dpv (SENASA [Argentina], 14, 15). For each batch to be approved, the EPP value must be equal to or greater than 75%. The sera obtained 28–30 dpv with 5 ml/dose trivalent vaccines were also evaluated using LPB-ELISA supplied by the Pan American Foot and Mouth Disease Center (PANAFTOSA), and the results were extrapolated to the corresponding EPP curve (17).

Detection of antibodies to non-structural proteins

NSP antibodies were determined in all the cattle samples using a screening test that consists of an ELISA kit for detecting the non-capsid protein 3ABC of the FMD virus (indirect I-ELISA 3ABC), called the NCPanaftosa screening test, followed by a confirmatory test performed using the NCPanaftosa confirmatory kit (with which an enzyme-linked immunoelectrotransfer blot [EITB] assay is performed) (4, 18). The two tests combined are known as the I-ELISA 3ABC Panaftosa/EITB system. The results were expressed as test to control (T/C), which is the ratio between the absorbance value obtained with test serum (T) and the absorbance value obtained with control serum (C). Non-reactive sera were considered to be those yielding T/C values of <0.8 , borderline sera, those yielding T/C values of between 0.8 and <1 , and reactive sera, those yielding T/C values of ≥ 1 . Sera with borderline or reactive results were confirmed by EITB.

The sera from repeated vaccination testing in cattle were also analysed using the PrioCHECK FMDV NS method (Prionics), which is a blocking ELISA for the detection of antibodies in any animal species. The results were expressed as a percentage of inhibition (PI). Non-

reactive sera were considered to be those yielding a PI value of <50% and reactive sera, those yielding a PI value of $\geq 50\%$. In addition, the sera for experiment 1 were examined using the 3ABC-CEVAN method of Argentina's Animal Virology Centre (CEVAN) (19), in which the results were expressed as percent positivity (PP). Non-reactive sera were considered to be those yielding a PP value of <15%, borderline sera, those yielding PP values of 15–20%, and reactive sera, those yielding PI values of $\geq 20\%$.

The samples from sheep and pigs were analysed using only the PrioCHECK FMDV NS method (Prionics).

In all cases the manufacturer's instructions were followed.

To check the purity of each batch of vaccines with regard to NSP content, Argentina's official body uses the I-ELISA 3ABC Panaftosa/EITB system to determine the presence of reactions in sera obtained 60 dpv and in prevaccination sera, while simultaneously checking the potency and safety of all vaccine series. This study includes the official results using I-ELISA 3ABC Panaftosa/EITB for more than 2,800 sera relating to 168 series of quadrivalent vaccines (O1 Campos, A24 Cruzeiro, A2001, C3 Indaial) used in the years 2006 ($n = 49$), 2007 ($n = 42$), 2008 ($n = 23$), 2009 ($n = 22$), 2010 ($n = 10$), 2011 ($n = 9$) and 2012 ($n = 13$) – representing a total of over 730 million doses produced by the Biogénesis Bagó laboratory that underwent control testing.

Seroepidemiological surveillance

For more than a decade, SENASA has conducted random serological surveys to assess the control measures implemented and to obtain OIE recognition and annual reconfirmation of the animal disease status of free zones with and without vaccination. Sampling was carried out in accordance with the OIE *Terrestrial Animal Health Code (Terrestrial Code)* (20). The sampling used was that required to detect an event, in two stages. To calculate the sample size, the assumptions were: 1% prevalence of infected herds; 10% prevalence of infected animals; and 95% confidence interval in each of the zones into which the country

was divided. This study reports the results of NSP antibody determination using I-ELISA 3ABC Panaftosa and of the confirmation of reactions using EITB in cattle samples taken during the first vaccination campaign, in 2006 to 2011, prior to administering the next vaccine dose. Farms with reactor animals were then subjected to a virological and epidemiological investigation that confirmed the absence of virus circulation (not shown). These data were included in the documentation submitted by SENASA to the OIE every year to support the continued animal disease status of FMD-free zones.

Statistical analysis

The antibody titres determined by LPB-ELISA following the first two vaccinations of repeated vaccination testing in cattle were compared using analysis of variance (ANOVA) and the post-hoc test used was Tukey-Kramer; the significance threshold was set at 5% ($p = 0.05$).

Results

Repeated vaccination testing in cattle: determination of antibodies to non-structural proteins

The use of repeated vaccination tests forces an immune response, thus making it possible to detect NSP antibody responses that might not be detected with a primary vaccination or with conventional single-dose vaccines. These tests are performed to demonstrate the efficiency of the purification process and are used as support in the product authorisation or licensing process. This makes the interpretation of sampling reliable by evaluating viral activity in zones included in the vaccination plan or during animal health emergencies, ruling out possible interference of vaccines with the tests used. As described in the 'Materials and methods' section, vaccines with 3, 4 and 5 valences were used in a single dose (2 ml or 5 ml) or in a double dose (4 ml), administered either 3 or 4 times at intervals of 30–90 days.

Figures 1a, 1b and 1c show, at each post-vaccination stage, how many cattle sera were classified into each category of T/C results after being analysed by the I-ELISA 3ABC Panaftosa method in experiments 1a

and 1b (Fig. 1a), experiment 2 (Fig. 1b) and experiments 3 and 4 (Fig. 1c).

As can be seen, the reactivity profile found in each test is highly consistent. No reactions were induced even after forcing the immune response with 1, 2 or 3 revaccinations or by using double-dose vaccines in schedules of up to two revaccinations. No positive reactions were detected in cattle sera following 1, 2, 3 or 4 vaccinations in any of the tests. A point of note is that the I-ELISA 3ABC Panaftosa/EITB system entails determination by I-ELISA 3ABC Panaftosa, after which all suspect sera (T/C values of 0.8–1) or positive sera (T/C values of ≥ 1) are subjected to the EITB confirmatory test. Of all the sera studied ($n = 360$), only one reacted to the I-ELISA test (0.28%) but, when it was analysed by EITB, it was found not to react. The serum had reacted only following revaccination, as a transient reaction, but proved to be non-reactive at the following blood sample (120 dpv, Fig. 1a).

The sera for all the experiments were analysed using the PrioCHECK FMDV NS method and those for experiment 1 were analysed using the ELISA 3ABC CEVAN method, with no reactions being detected at any post-vaccination stage (data not shown).

According to the methods used, the unvaccinated cattle included in each of the tests remained non-reactive throughout the study.

Repeated vaccination testing in cattle: determination of antibodies to structural proteins

The determination of specific antibodies (to structural proteins) by LPB-ELISA ensured that the vaccines used had induced adequate seroconversion for every strain included in each vaccine (Fig. 2).

Average levels of antibodies per group to each of the vaccine strains at 60 dpv was greater than $\log_{10} = 2.3$, exceeding the values established by current Argentinian regulations for the approval of commercial 2 ml/dose quadrivalent vaccines (14, 15). In the case of commercial 5 ml/dose trivalent vaccines, the EPP values (17) exceeded the

required level of antibodies at 28–30 dpv for each of the vaccine strains. The average antibody titres following one or two vaccinations increased significantly ($p < 0.01$) in each experiment.

Repeated vaccination testing in pigs and sheep

In Argentina, FMD vaccination in sheep and pigs is administered in animal health emergencies or in cases of FMD infection risk. This makes it necessary to ensure that FMD vaccination and revaccination of these species does not induce NSP antibodies; this indicator can therefore be used to detect infected animals following an outbreak. Neither of the two vaccines studied induced NSP antibodies in either pigs or sheep, after either one or two vaccinations (Table II). The specific antibody levels to O1 Campos evaluated using LPB-ELISA (average/group) following the first vaccination (at 30 dpv) were $\log_{10} = 2.10$ in pigs and $\log_{10} = 2.91$ in sheep. At that stage, antibody levels to A2001 were $\log_{10} = 3.07$ in sheep. The second vaccination induced an increase in the titres of specific antibodies to O1 Campos detected at 60 dpv in both sheep ($\log_{10} = 4.2$) and pigs ($\log_{10} = 3.3$).

Official (batch by batch) control testing of vaccine potency

To ensure the potency of products for use in campaigns, animal health authorities in South America require potency control testing in cattle of every batch of FMD vaccine to be marketed.

Figure 3 shows the potency results, expressed as EPP values in cattle, corresponding to vaccine series destined for Argentina in the period 2010–2012 and evaluated by SENASA. The EPP values of all the vaccines exceeded the established cut-off value ($\geq 75\%$).

Official control testing of vaccines for induction of antibodies to non-structural proteins

SENASA checks all batches of vaccine for NSP lack of reactivity in the final product. This evaluation gives greater assurance of purity in terms of NSP content, as a supplement to the information provided in the product registration dossier.

Table III shows the number of vaccine series for local use that are submitted for official control testing in Argentina every year (2 ml/dose quadrivalent vaccine), as well as the final results from the I-ELISA 3ABC Panaftosa/EITB system of all the sera evaluated in a production laboratory in the period 2006–2012. No reactions were detected in the sera of vaccinated cattle.

Seroepidemiological surveillance

One of the keys to the success of vaccination campaigns is the quality of vaccines used. The results of seroepidemiological surveillance of the zones included in the vaccination plan therefore represent an indirect measure of quality of this tool. In Argentina, SENASA performs annual seroepidemiological surveys in order to demonstrate the absence of virus circulation or infection in zones with or without vaccination of cattle and other FMD-susceptible species. This study takes into consideration the results of NSP antibody determination in cattle from the zone included in the vaccination plan, using samples from age category 1 (6–12 months) and age category 2 (12–24 months). The percentage of positives to NSP antibodies ranged from 0.01% to 0.07% in category 1 and from 0.05% to 0.13% in category 2, using the I-ELISA 3ABC Panaftosa/EITB system (Table IV), with an average 0.05% of reactive results – values consistent with the specificity of the test (4). The epidemiological investigation conducted into additional sampling and into the distribution of these reactive results (data not shown) indicated the absence of virus circulation in the zone included in the vaccination plan and, hence, non-interference of the vaccines with these determinations.

Discussion

It is generally accepted that the most efficient and cost-effective method of preventing and controlling infectious diseases such as FMD is to use vaccines. The OIE *Terrestrial Code* provides guidelines on the animal health surveillance needed to support submissions for recognition of freedom from infection or absence of virus circulation, a status that allows access or continued access to markets for meat and

by-products. Countries practising vaccination that wish to obtain OIE recognition for FMD freedom with vaccination must demonstrate sufficient herd immunity and absence of virus circulation by means of serological surveys and NSP antibody determination using OIE-validated and recognised methodologies (20).

Countries with routine vaccination campaigns, as well as FMD-free countries not practising vaccination, should have available, for their campaigns or for possible emergency vaccination, vaccines of proven efficacy that do not interfere with methods for detecting infected animals.

The chapter on purity (18) in the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* recommends that, as part of the registration process, the production laboratory should provide information on the non-induction of NSP antibodies following three double-dose vaccinations in calves. Argentinian regulations for the registration of FMD vaccines require that evidence of potency, safety and purity be provided. To obtain evidence of the latter, the product to be registered must be used in revaccination tests and demonstrate absence of NSP seroconversion. After registration has been approved, purity control testing of vaccine series consists of determining the presence of NSP antibodies in the sera of vaccinated animals, which is carried out in parallel with efficacy control testing (14, 15).

NSP lack of reactivity in repeated vaccination tests of cattle, sheep and pigs in this study supports the conclusion that the purification method is satisfactory and that it has been verified using different methodologies and vaccination schedules. Earlier reports of unpurified vaccines not authorised in Argentina had found an increase in the T/C value following each vaccination (21) and a majority of reactor cattle.

Specific humoral response in the repeated vaccination experiments indicates antibody levels above the cut-off values required by current regulations and significant increases following the first two vaccinations. This confirms an enhancing effect on specific humoral response and hence significant antigen concentration. Following the third vaccination, antibody titres remained high, with EPP values of

over 99% with no significant differences from previous titres. According to Black *et al.* (22), high antibody levels could limit the response to subsequent vaccinations owing to neutralisation of vaccine antigen or a regulatory effect on antibody production.

Furthermore, no reactions were detected in the batch-by-batch control testing of industrial series using the I-ELISA 3ABC Panaftosa/EITB system. This guarantees that vaccine series for marketing are sufficiently purified. A point of note is that all the series were of appropriate potency and that more than 75% showed EPP values of over 90%.

Serological surveys to determine the prevalence of NSP antibodies in the various zones with vaccination programmes are used to assess the degree of virus circulation. Should vaccines with residual NSP be used, positive reactions to NSP could be detected, which would require further analysis to make sure that the reactions stem from post-vaccine reaction rather than from virus circulation, as the latter would affect the status granted by the OIE to that country or zone. The low percentage of reactive results detected, consistent with the specificity of the test (4), and the subsequent virological and epidemiological investigation indicated the absence not only of virus circulation but also of interference from vaccines with the tests to determine NSP antibodies.

This is the first study to demonstrate that potent vaccines produced by conventional methods achieve a degree of purity, in terms of NSP content, that allows for an accurate interpretation of the results of seroepidemiological surveillance; this accuracy is important, as the detection of NSP antibodies during surveillance provides supporting evidence to confirm the animal disease status of FMD-free zones. This conclusion is based on the use of vaccines with a high antigen concentration and multiple valences, after forcing the immune response by increasing doses and revaccinating, and by vaccinating at shorter intervals than in vaccination campaigns. Furthermore, these data were supplemented by SENASA batch-by-batch control testing

of vaccines prior to their release, in which more than 5,500 sera were analysed.

In conclusion, the inactivated vaccines described in this study, which are on sale and are strictly controlled by the animal health authorities, provide an essential tool for eradication campaigns to support recognition of freedom from viral activity in zones where vaccination is practised or following one or more emergency vaccinations.

Conclusions

In repeated vaccination tests in cattle, sheep and pigs, vaccines subjected to purification processes with a high antigen concentration and multiple valences do not induce NSP antibodies.

SENASA batch-by-batch control testing on vaccines in cattle prior to their release ensures satisfactory potency and absence of NSP seroconversion in the vaccines released.

Potent vaccines produced by conventional methods where the production process includes antigen purification stages allow for an accurate interpretation of the results of seroepidemiological surveillance of which the NSP antibody determination is used to support the animal disease status of FMD-free zones.

References

1. Neitzert E., Beck E., Augé de Mello P., Gomes I. & Bergmann I.E. (1991). – Expression of the aphthovirus RNA polymerase gene in *Escherichia coli* and its use together with other bioengineered nonstructural antigens in detection of late persistent infections. *Virology*, **184** (2), 799–804.

2. Bergmann I.E., de Mello P.A., Neitzert E., Beck E. & Gomes I. (1993). – Diagnosis of persistent aphthovirus infection and its differentiation from vaccination response in cattle by use of enzyme-linked immunoelectrotransfer blot analysis with bioengineered nonstructural viral antigens. *Am. J. Vet. Res.*, **54** (6), 825–831.

3. Fondevila N., O'Donnell V., Duffy S., León E., Smitsaart E. & Schudel A.A. (1997). – Sero-epidemiological indicators for evaluating foot and mouth disease control campaigns [in Spanish]. *Rev. Sci. Tech. Off. Int. Epiz.*, **16** (3), 784–792.

4. Bergmann I.E., Malirat V., Neitzert E., Beck E., Panizzuti N., Sanchez C. & Falczuk A. (2000). – Improvement of a serodiagnostic strategy for foot-and-mouth disease virus surveillance in cattle under systematic vaccination: a combined system of an indirect ELISA-3ABC with an enzyme-linked immunoelectrotransfer blot assay. *Arch. Virol.*, **145** (3), 473–489.

5. Bergmann I.E., Malirat V., Dias L.E. & Dilandro R. (1996). – Identification of foot-and-mouth disease virus-free regions by use of a standardized enzyme-linked immunoelectrotransfer blot assay. *Am. J. Vet. Res.*, **57** (2), 972–974.

6. Mattion N., König G., Seki C., Smitsaart E., Maradei E., Robiolo B., Duffy S., León E., Piccone M., Sadir A., Bottini R., Cosentino B., Falczuk A., Maresca R., Periolo O., Bellinzoni R., Espinoza A., Torre J.L. & Palma E.L. (2004). – Reintroduction of foot-and-mouth disease in Argentina: characterisation of the isolates and development of tools for the control and eradication of the disease. *Vaccine*, **22** (31–32), 4149–4162.

7. Pluimers F.H., Akkerman A.M., van der Wal P., Dekker A. & Bianchi A (2002). – Lessons from the foot and mouth disease outbreak in the Netherlands in 2001. *Rev. Sci. Tech. Off. Int. Epiz.*, **21** (3), 711–721. Available at: web.oie.int/boutique/extrait/3.8.Pluimers.pdf (accessed on 1 April 2015).

8. Barteling S. (2004). – Modern inactivated foot-and-mouth disease (FMD) vaccines: historical background and key elements in production and use. *In* Foot and mouth disease current perspectives (F. Sobrino & E. Domingo, eds). CRC Press, United Kingdom, 305–333

9. Espinoza A.M., Maradei E., Mattion N., Cadenazzi G., Maddonni G., Robiolo B., La Torre J., Bellinzoni R. & Smitsaart E. (2004). – Foot-and-mouth disease polyvalent oil vaccines inoculated repeatedly in cattle do not induce detectable antibodies to non-structural proteins when evaluated by various assays. *Vaccine*, **23** (1), 69–77.

10. Aucouturier J., Dupuis L. & Ganne V. (2001). – Adjuvants designed for veterinary and human vaccines. *Vaccine*, **19** (17–19), 2666–2672.

11. Bergmann I.E., Neitzert E., Malirat V., Ortiz S., Colling A., Sánchez C. & Correa Melo E. (2003). – Rapid serological profiling by enzyme-linked immunosorbent assay and its use as an epidemiological indicator of foot-and-mouth disease viral activity. *Arch. Virol.*, **148** (5), 891–901.

12. Bergmann I.E., Neitzert E., Malirat V., de Mendonça Campos R., Pulga M., Muratovik R., Quintino D., Morgados J.C., Oliveira M. & de Lucca Neto D. (2006). – Development of an inhibition ELISA test for the detection of non-capsid polyprotein 3ABC in viral suspensions destined for inactivated foot-and-mouth disease vaccines. *Dev. Biol. (Basel)*, **126**, 241–50, Discussion 327.

13. Goris N., Merkelbach-Peters P., Diev V.I., Verloo D., Zakharov V.M., Kraft H.P. & de Clercq K. (2007). – European Pharmacopoeia foot-and-mouth disease vaccine potency testing in cattle: between test variability and its consequences. *Vaccine*, **25** (17), 3373–3379. doi:10.1016/j.vaccine.2006.12.049.

14. Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA) (Argentina) (2006). – Resolution 351/2006. Boletín Oficial N° 30.940, 5 July 2006. Available at: infoleg.mecon.gov.ar/infolegInternet/anexos/115000-119999/117636/norma.htm (accessed on 1 April 2015).

15. Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA) (Argentina) (2010). – Resolution 111/2010. Available at:

infoleg.mecon.gov.ar/infolegInternet/anexos/160000-164999/164766/norma.htm (accessed on 1 April 2015).

16. Maradei E., La Torre J.L., Robiolo B., Esteves J., Seki C., Pedemonte A., Iglesias M., D'Aloia R. & Mattion N. (2008). – Updating of the correlation between IpELISA titers and protection from virus challenge for the assessment of the potency of polyvalent aphotavirus vaccines in Argentina. *Vaccine*, **26** (51), 6577–6586. doi:10.1016/j.vaccine.2008.09.033.

17. Ministério da Agricultura, Pecuária e Abastecimento: SISLEGIS (2008). – Instrução Normativa Nº 50, Regulamento técnico para a produção, controle da qualidade, comercialização e emprego de vacinas contra a febre aftosa [Normative Instruction No. 50, Technical Regulation for the production, quality control, marketing and use of vaccines against FMD]. Available at: sistemasweb.agricultura.gov.br/sislegis/action/detalhaAto.do?method=abreLegislacaoFederal&chave=50674&tipoLegis=A (accessed on 1 April 2015).

18. World Organisation for Animal Health (OIE) (2012). – Chapter 2.1.5. Foot and mouth disease. *In* Manual of diagnostic tests and vaccines for terrestrial animals. OIE, Paris. Available at: www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.05_FMD.pdf (accessed on 26 November 2015).

19. Robiolo B., Seki C., Fondevilla N., Grigera P., Scodeller E., Periolo O., La Torre J. & Mattion N. (2006). – Analysis of the immune response to FMDV structural and non-structural proteins in cattle in Argentina by the combined use of liquid phase and 3ABC-ELISA tests. *Vaccine*, **24** (7), 997–1008.

20. World Organisation for Animal Health (OIE) (2014). – Chapter 8.8. Infection with foot and mouth disease virus. *In* Terrestrial animal health code. OIE, Paris. Available at: www.oie.int/fileadmin/Home/eng/Health_standards/tahc/2010/chapitre_fmd.pdf (accessed on 1 de abril 2015).

21. Maradei E. (2007). – Use of non-structural proteins of FMDV to differentiate vaccinated and infected animals in Argentina. *In* The use of non-structural proteins of foot-and-mouth disease virus (FMDV) to differentiate between vaccinated and infected animals. RCA Project. Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, IAEA-TECDOC-1546. International Atomic Energy Agency, Vienna, 197–204. Available at: www-pub.iaea.org/MTCD/publications/PDF/te_1546_web.pdf (accessed on 1 April 2015).

22. Black L., Rweyemamu M. & Boge A. (1984). – Revaccination response of cattle as a function of the 140S foot and mouth disease antigen dose. *J. Comp. Pathol.*, **94** (3), 417–424.

Table I
Design of repeated vaccination tests in cattle

Experiment no.	No. of cattle	Strains included in the vaccine	Doses/volume	Timing of vaccinations (dpv)
1a	17	O1 Campos, A24 Cruzeiro, A2001, A2000	1 dose/2 ml	0, 30 and 60
1b	17	O1 Campos, A24 Cruzeiro, A2001, A2000	2 doses/4 ml	0, 30 and 60
2	16	O1 Campos, A24 Cruzeiro, A2001, A2000, C3 Indaial	1 dose/2 ml	0, 90, 120 and 150
3	17	O1 Campos, A24 Cruzeiro, C3 Indaial	1 dose/5 ml	0, 60 and 90
4	18	O1 Campos, A24 Cruzeiro, C3 Indaial	1 dose/5 ml	0, 42 and 90

dpv: days post primary vaccination

Table II
Antibody response to structural proteins (determined using liquid-phase blocking enzyme-linked immunosorbent assay [LPB-ELISA]) and antibody response to non-structural proteins (determined using the PrioCHECK FMDV NS method) in sheep and pigs following repeated vaccinations

Species	Vaccine	Titres of LPB-ELISA O1 Campos		Titres of LPB-ELISA A2001		Reactions to PrioCHECK FMDV NS	
		Post 1st vac ^(a)	Post 2nd vac ^(b)	Post 1st vac	Post 2nd vac	Post 1st vac	Post 2nd vac
		Pigs	Monovalent O1 Campos	2.10 ^(c) (0.19) ^(d)	3.3 (0.27)	ND	ND
Sheep	Polyvalent	2.91 (0.52)	4.20 (0.45)	3.07 (0.58)	4.14 (0.49)	0/23	0/23

a) After first vaccination (30 days post primary vaccination)

b) After second vaccination (60 days post primary vaccination)

c) Average antibody titres per group according to liquid-phase blocking enzyme-linked immunosorbent assay (LPB-ELISA), expressed as inverse of log₁₀

d) In brackets: standard deviation

e) Reactive sera as a ratio of total sera analysed

ND: not determined

ELISA: enzyme-linked immunosorbent assay

Table III
Official control testing in Argentina of vaccines produced by the
Biogénesis Bagó laboratory with respect to the induction of
antibodies to non-structural proteins

Year	No. of vaccine series	Millions of doses	Sera reactive to ELISA 3ABC/EITB ^(a) as a ratio of total sera evaluated	Series approved as a ratio of total series evaluated
2006	49	132	0/1,650	49/49
2007	42	119	0/1,428	42/42
2008	23	127	0/782	23/23
2009	22	122	0/748	22/22
2010	10	74	0/340	10/10
2011	9	67.4	0/306	9/9
2012	13	94.7	0/442	13/13
Total	168	736.1	0/5,696	168/168

a) The sera (pre-vaccination and 60 days post primary vaccination) of each bovine vaccinated with each of the vaccine series were determined using the ELISA kit for detecting the non-capsid protein 3ABC of the FMD virus (indirect I-ELISA 3ABC Panaftosa), after which sera with a test to control (T/C) value of ≥ 0.8 (suspect and positives) were analysed by enzyme-linked immunoelectrotransfer blot (EITB) assay

Table IV

Results from sampling (2006-2011) cattle in age category 1 (6-12 months) and age category 2 (12-24 months) in zones included in the vaccination plan drawn up by Argentina's official Veterinary Service, the national health and agrifood quality service (SENASA)

Year	Age category 1		Age category 2	
	No. of cattle	Percentage of positives*	No. of cattle	Percentage of positives
2006	16,200	0.02	10,413	0.07
2007	16,464	0.02	10,121	0.10
2008	20,632	0.04	13,034	0.07
2009	14,762	0.07	9,749	0.11
2010	15,969	0.01	1,0382	0.13
2011	15,646	0.01	10,281	0.05

*Percentage of reactive results using the I-ELISA 3ABC Panaftosa/EITB system

ELISA: enzyme-linked immunosorbent assay

Fig. 1

Non-structural protein antibody determination in cattle following several vaccinations

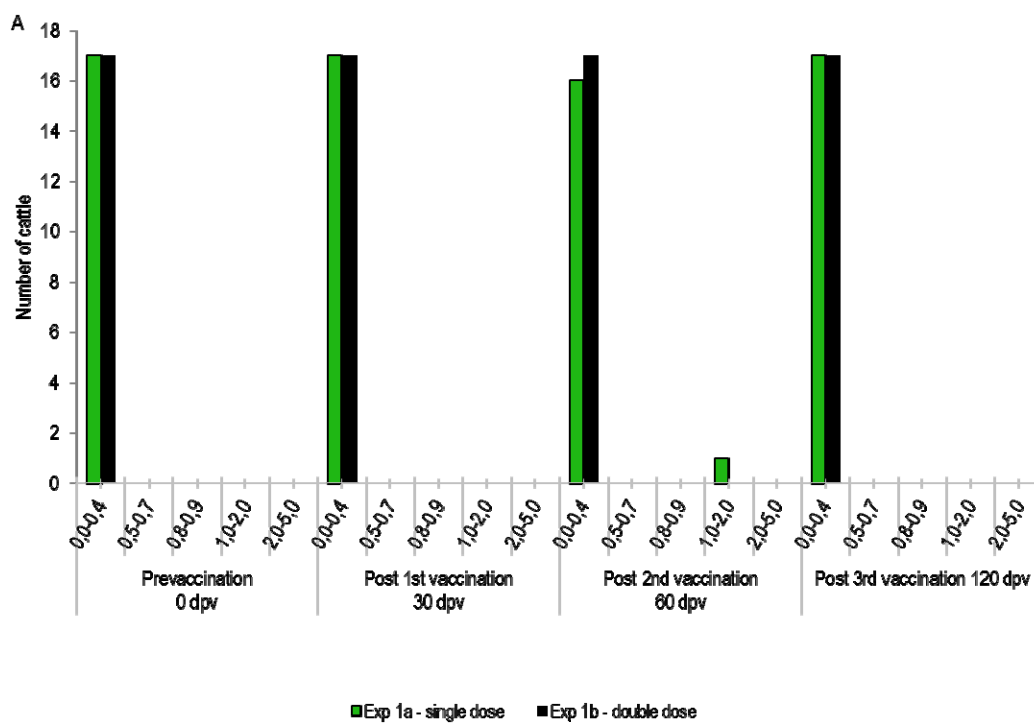
The bars indicate how many cattle sera were classified into each category of test to control (T/C) results after being analysed by the I-ELISA 3ABC Panaftosa method

The y-axis denotes the number of cattle, while the x-axis denotes the T/C values

Panel A: experiments 1a and 1b

Panel B: experiment 2

Panel C: experiments 3 and 4



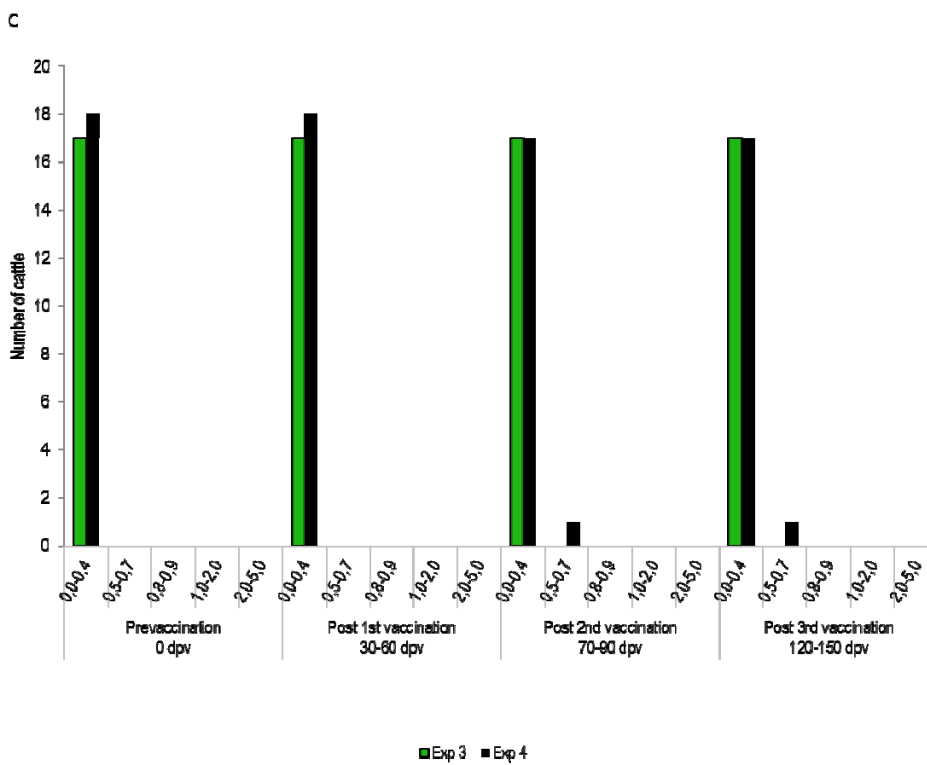
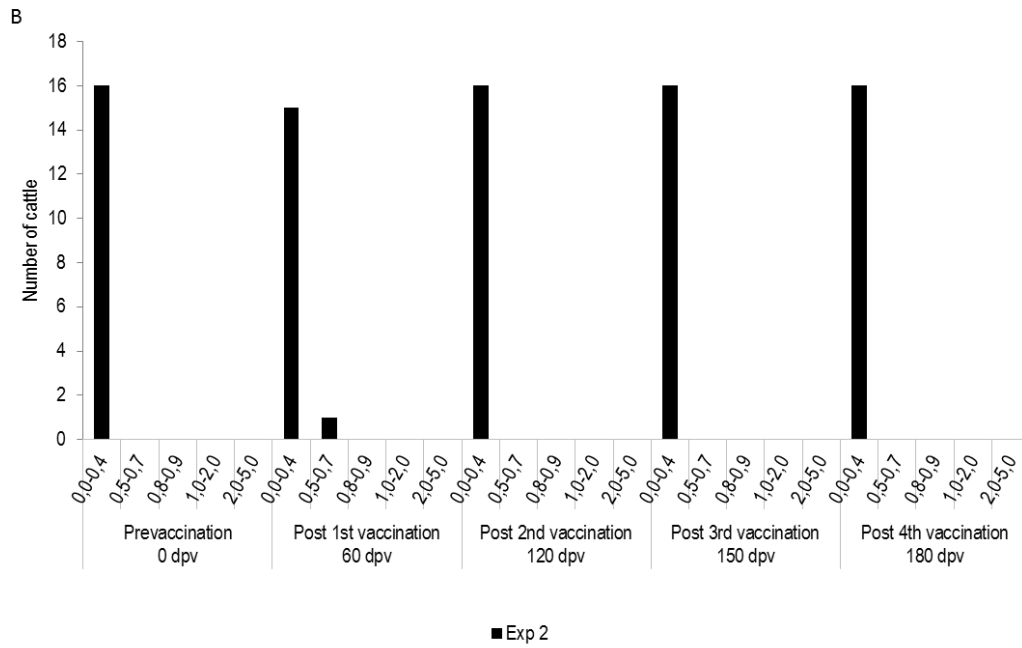


Fig. 2

Average antibody titres per group to O1 Campos, determined using liquid-phase blocking enzyme-linked immunosorbent assay (LPB-ELISA) (\log_{10}), before and after each cattle vaccination, corresponding to experiments 1a, 1b, 2, 3 and 4

The vertical lines indicate standard deviation

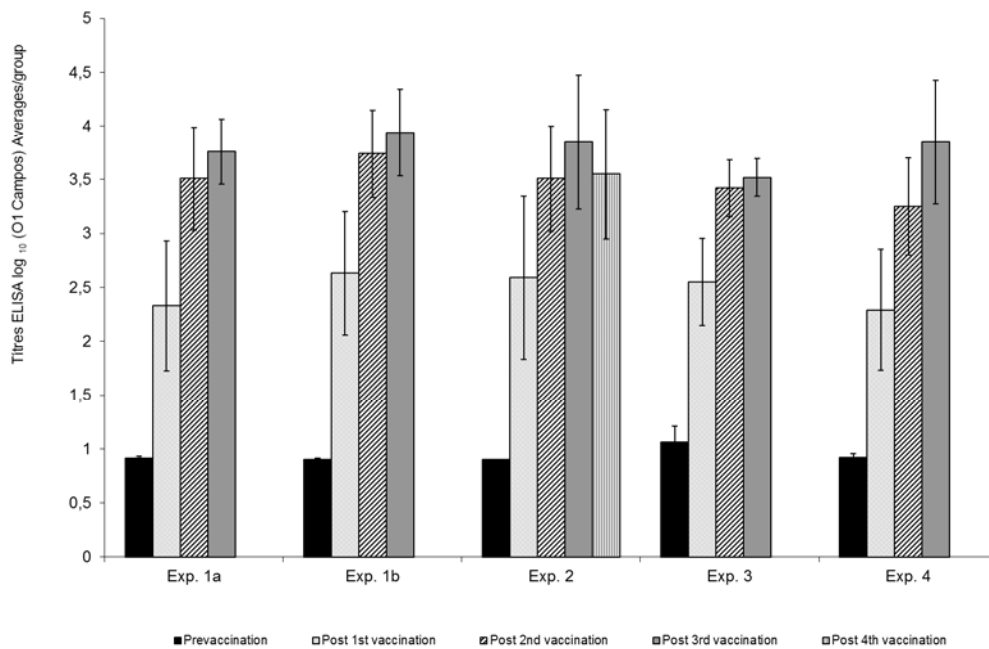


Fig. 3

Results of the potency control tests, expressed as expected percentage of protection, performed on series of foot and mouth disease vaccine submitted in the period 2010-2012 to Argentina's official Veterinary Service, the national health and agrifood quality service (SENASA)

The bars indicate the expected percentage of protection

