Ministry of Agriculture, Livestock and Food Supply
Secretariat of Animal and Plant Health and Inspection

Serological Monitoring to assess efficiency of vaccination against foot-and-mouth disease in the free zone

Final Report

Department of Animal Health
Brasília, November, 2011

Serological Monitoring to assess efficiency of vaccination against foot-and-mouth disease in the free zone - Final Report - 2010/2011

1. Introduction

As of 2005, under DSA/SDA/MAPA coordination and with support of the PANAFTOSA, State Veterinary Services (SVEs) have been carrying out serum-epidemiological studies with aim at monitoring effectiveness of vaccination campaigns against the foot-and-mouth disease (FMD) in the Units of the Federation (UFs) that make up the disease's free zone. These studies are part of a set of management activities implemented by the National Program for the Eradication and Prevention of Foot-and-mouth Disease (PNEFA) and; in addition, they seek to satisfy health certification commitments agreed with countries that import beef cattle. Similar to the first survey carried out in 2005, this study included all UFs that are part of the FMD free zone with vaccination.

2. Goal

To estimate immunity coverage of bovine population between 6 and 24 months old of the UFs located in the foot-and-mouth disease free zone with vaccination.

3. Related documents

- *Evaluation of population immunity resulting from vaccination campaigns against the foot-and-mouth disease. Final report, August 2007.*
  http://www.agricultura.gov.br/arq_editor/file/Aniamal/programa%20nacional%20sanidade%20aftosa/Assessment%20of%20the%20population%20immunity.pdf
- *Epidemiological study to evaluate efficiency of vaccination against the foot-and-mouth disease in the free zone – Guide for Standardization of Activities / August, 2010.*

4. Time-frame

Planning, sample collection and laboratory analysis activities were conducted between August, 2010 and September, 2011. Sample collection, specifically, was concentrated in September and October, 2010 (96% of total samples), before the November vaccination stage and distant as much as possible from the previous vaccination stage, which took place in May, 2010, as depicted in Figure 01. Thus, the samples were collected in third final period between vaccination stages, allowing us to assess the time period of lesser expectation regarding immunity coverage of the bovine population.

In all of the UFs, immunity coverage was assessed for the three types of viruses that are used in the vaccines traded in the country (virus O, A and C). The work involved with preparing and delivering samples to LANAGRO/MG was carried out between October and December 2010, and the laboratory results for virus O and A were made available in April 2011 and for virus C, in the beginning of September of 2011.

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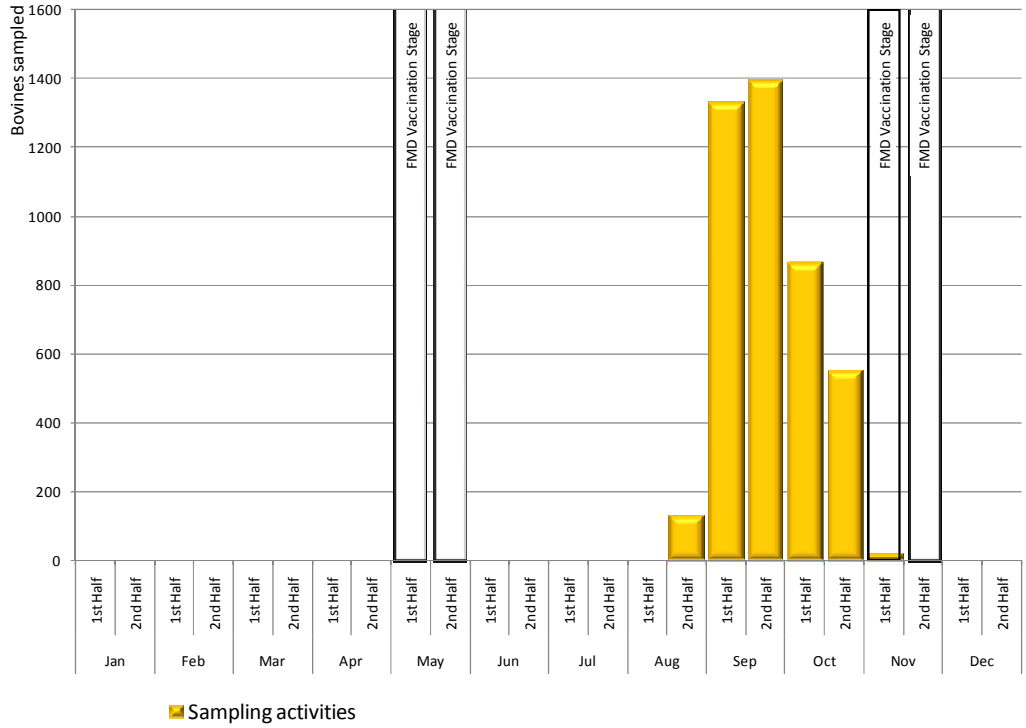

Figure 01. Time-frame of sample collection, with emphasis on vaccination months, 2010

5. Methods

Independent studies were carried out for each UF of the FMD free zone with vaccination. Specifically in UFs located on international border regions, two independent studies were carried out: one on the region made up by municipalities located on the border, and another on the region encompassing other municipalities in the state.

The DSA/SDA/MAPA, with PANAFTOSA´s support, was responsible for coming up with the sample model and choosing the rural properties. These activities were carried out based on the databases of farms provided by the SVEs involved, which were responsible for all field activities, including sample collection and gathering information such as the age of the animals, estimated number of vaccinations of each sampled bovine, brand of the vaccine used in the last two vaccination stages, size of the herd, among others.

For each UF and international border region, a stratified sample was determined according to age (6 to 12 months and 13 to 24 months) and herd size (up to 50 animals and more than 50 animals), in order to estimate the amount of protected animals within each population of the survey. The chart below shows a summary of the parameters used to determine the sample size. The total expected amount of samples for each subpopulation (UF) involved is presented in Table 1.

Stratum		Expected percentage of protected animals
Age group	**Herd Size**	
6 to 12 months old	Up to 50 bovines	70%
	More than 50 bovines	75%
13 to 24 months old	Up to 50 bovines	80%
	More than 50 bovines	85%

Parameters used to determine sample size
Confidence Level =95%
Lab test parameters: Sensitivity and Specificity = 0.8333
Sampling error: 0.085

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Table 01. Estimates of samples for each subpopulation involved in the FMD vaccination efficiency study

State	Region	Category	Sample			
			Holdings	6-to-12 m. Bovines	13 to 24 m. bovines	Total Bovines
Acre	Border	Up to 50 bovines	11	14	8	22
		50+ bovines	17	103	68	171
		Total	**28**	**117**	**76**	**193**
	Non-border	Up to 50 bovines	9	11	7	18
		50+ bovines	17	102	72	174
		Total	**26**	**113**	**79**	**192**
		AC Total	**54**	**230**	**155**	**385**
Bahia		Up to 50 bovines	27	30	24	54
		50+ bovines	14	73	67	140
		Total	**41**	**103**	**91**	**194**
Distrito Federal		Up to 50 bovines	40	48	32	80
		50+ bovines	12	72	48	120
		Total	**52**	**120**	**80**	**200**
Espírito Santo		Up to 50 bovines	18	21	15	36
		50+ bovines	16	92	68	160
		Total	**34**	**113**	**83**	**196**
Goiás		Up to 50 bovines	7	9	5	14
		50+ bovines	17	93	81	174
		Total	**24**	**102**	**86**	**188**
Minas Gerais		Up to 50 bovines	24	30	18	48
		50+ bovines	15	89	61	150
		Total	**39**	**119**	**79**	**198**
Mato Grosso do Sul	Border	Up to 50 bovines	11	14	8	22
		50+ bovines	17	100	70	170
		Total	**28**	**114**	**78**	**192**
	Non-border	Up to 50 bovines	3	4	2	6
		50+ bovines	18	110	74	184
		Total	**21**	**114**	**76**	**190**
		MS Total	**49**	**228**	**154**	**382**
Mato Grosso	Border	Up to 50 bovines	4	6	2	8
		50+ bovines	19	117	73	190
		Total	**23**	**123**	**75**	**198**
	Non-border	Up to 50 bovines	5	7	3	10
		50+ bovines	18	115	67	182
		Total	**23**	**122**	**70**	**192**
		MT Total	**46**	**245**	**145**	**390**
Pará		Up to 50 bovines	6	7	5	12
		50+ bovines	18	108	72	180
		Total	**24**	**115**	**77**	**192**
Paraná	Border	Up to 50 bovines	63	77	49	126
		50+ bovines	9	56	34	90
		Total	**72**	**133**	**83**	**216**
	Non-border	Up to 50 bovines	30	35	25	60
		50+ bovines	14	78	62	140
		Total	**44**	**113**	**87**	**200**
		PR Total	**116**	**246**	**170**	**416**
Rio de Janeiro		Up to 50 bovines	22	25	19	44
		50+ bovines	15	84	66	150
		Total	**37**	**109**	**85**	**194**
Rondônia	Border	Up to 50 bovines	10	14	6	20
		50+ bovines	17	108	66	174
		Total	**27**	**122**	**72**	**194**
	Non-border	Up to 50 bovines	18	25	10	35
		50+ bovines	16	105	58	163
		Total	**34**	**130**	**68**	**198**
		RO Total	**61**	**252**	**140**	**392**
Rio Grande do Sul	Border	Up to 50 bovines	19	24	14	38
		50+ bovines	16	89	71	160
		Total	**35**	**113**	**85**	**198**
	Non-border	Up to 50 bovines	39	50	28	78
		50+ bovines	12	71	52	123
		Total	**51**	**121**	**80**	**201**
		RS Total	**86**	**234**	**165**	**399**
Sergipe		Up to 50 bovines	48	54	42	96
		50+ bovines	11	58	52	110
		Total	**59**	**112**	**94**	**206**
São Paulo		Up to 50 bovines	21	27	15	42
		50+ bovines	16	106	54	160
		Total	**37**	**133**	**69**	**202**
Tocantins		Up to 50 bovines	9	12	6	18
		50+ bovines	18	111	69	180
		Total	**27**	**123**	**75**	**198**
Total			**786**	**2584**	**1748**	**4332**

The analytical method used to assess the immunity level of the bovine population was the enzyme-linked immunosorbent assay (ELISA-LPB) standardized by PANAFTOSA to detect specific antibodies against proteins of the virus capsid (structural or capsid proteins). Information on such assay is presented in the final report published by DSA/SDA/MAPA in 2007. In addition, Figure 2 shows a logistic regression function resulting from the statistical analysis between the indirect (level of antibodies) and direct response (outcome of the PGP - podal generalization test), revealing the existence of significant co-relationship between circulating antibody titers and PGP protection.

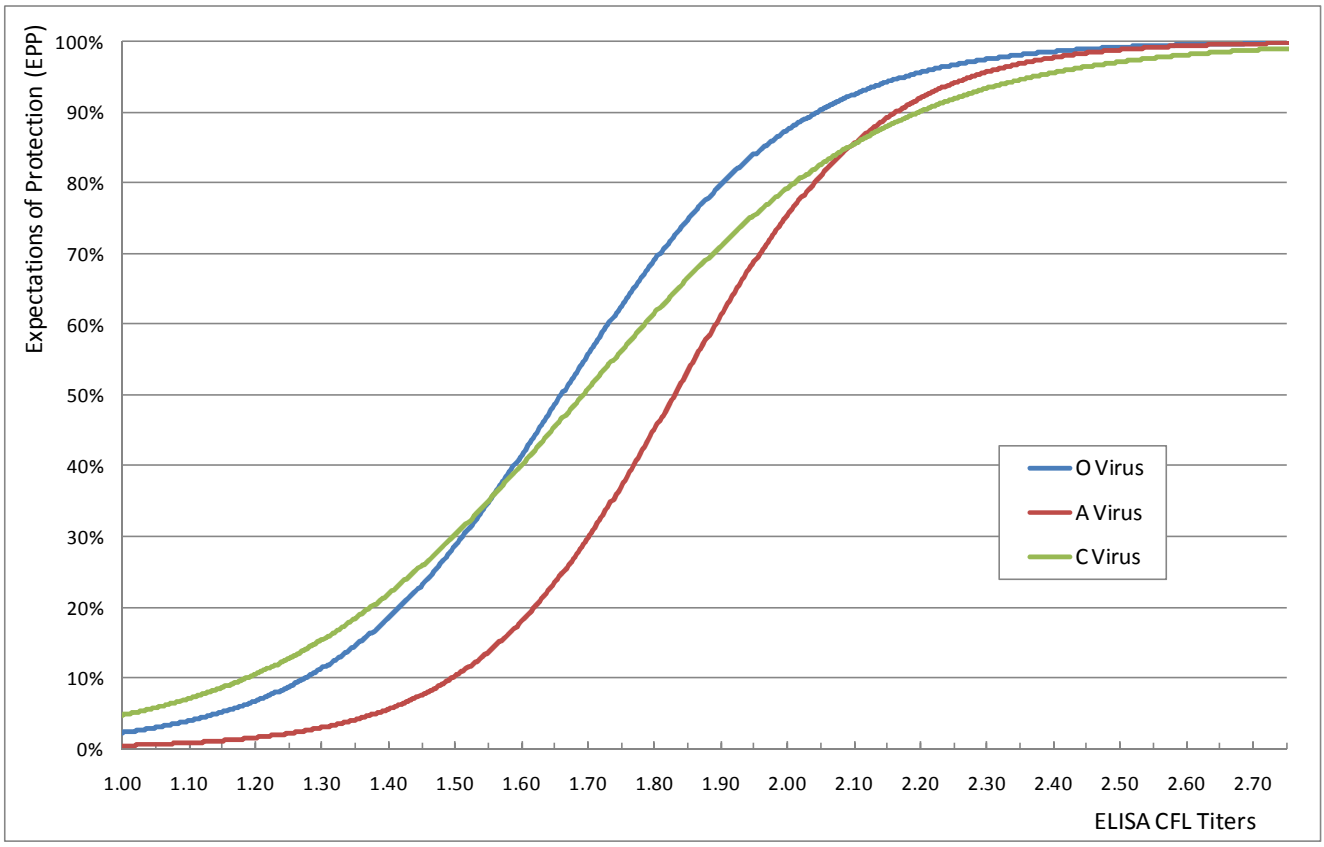

Figure 02. Expectation of protection – ELISA LPB (polyclonal detector), according to FMD types of viruses (O, A and C)
Source: PANAFTOSA

The model may be used both as a regression function and a discriminating function. In the first situation, we may estimate the probability of being protected based on knowing its antibody titers, and, in the second situation, by first establishing a cut-off value (or discriminating) and then classifying, based on knowing a bovine's antibody titers, if such animal belongs to the population of protected or not protected bovine whenever exposed to 10.000 bovine infectious doses (Bid 50%) of the FMD virus.

The sensitivity and specificity of the diagnostic test used to assess the immunity level of the bovine population are determined by the cut-off point that is going to be used in order to classify, based on antibody titers measured by ELISA-LPB, if the bovine belongs to the population of protected or not protected . Bovine which

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serum reveal titer below the cut-off or discriminating value are classified as not protected, whereas those with titer equal or above the cut-off or discriminating value are classified as protected. The sample size determined for the investigation also depends on the sensitivity and specificity of the diagnostic method.

During the planning stage of this study we considered the country's experience accumulated from studies conducted since 2005. Initially, we decided to consider, in determining the size of bovine sample, figures for sensitivity and specificity of the diagnostic method as equal to 0.8333 for evaluation of any of the valences present in the vaccines against the FMD disease that are manufactured and used in Brazil. At first, the intention was to quickly define the information on the samples size to begin field collection activities and, within laboratories, proceed with determining the cut-off point to be used to evaluate each valence, corresponding to the sensitivity and specificity considered to determine the size of the sample.

Following that, handling three different dilutions with bovine serums, determined by using different cut-off point for each valence of vaccine strains, would affect the laboratory's performance with regards to how fast it could analyze the samples collected. In this context, we decided to use titer 2.1 as the cut-off point for ELISA-LPB, for any of the vaccine strains. Respectively corresponding to that cut-off value, we have the strains: O1 Campos, A24 Cruzeiro and C3 Indaial, expectations of protection 0.9272; 0.8599 and 0.8577. Based on such protection expectations, and using the regression logistic function, we established sensitivity and specificity values for each one of the vaccine strains, considering the cut-off titer as ≥ 2.1 with ELISA-LPB, as shown in the chart below:

Sensitivity and specificity values and 95% confidence limits for the three types of viruses.
Titer with ELISA-LPB (Cut off point ≥ 2.1)

Vaccine strain	Sensitivity (%)			Specificity (%)		
	Value	Lowest limit	Highest limit	Value	Lowest limit	Highest limit
O1 Campos	81.67	74.74	88.59	85.71	78.23	93.20
A24 Cruzeiro	70.80	66.27	75.33	91.49	83.51	99.47
C3 Indaial	65.64	58.98	72.31	69.44	54.40	84.49

Source: PANAFTOSA

Once independent tests have been carried out for each virus strain in each of the subpopulations, the method to classify the bovine as truly protected or not protected may be through individual, series or parallel tests. For individual interpretation, we use the sensitivity and specificity values presented in the previous chart to calculate the real prevalence. The adoption of tests in series or in parallel imply in sensitivity and specificity values resulting from the combination of the tests, according to the follow possibilities:

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	Sensitivity	Specificity
• Evaluation of the three tests in parallel	98.2%	54.5%
• Evaluation of the three tests in series	38.0%	99.6%
• Evaluation of tests O and A in parallel:	94.6%	78.4%
• Evaluation of tests O and C in parallel:	93.7%	59.5%
• Evaluation of tests A and C in parallel:	90.0%	63.5%
• Evaluation of tests O and A in series:	57.8%	98.8%
• Evaluation of tests O and C in series:	53.6%	95.6%
• Evaluation of tests A and C in series:	46.5%	97.4%

Out of the figures found, we may observe that the best balance of sensitivity and specificity value is obtained by using two tests, two by two, in parallel; thus choosing this method for interpretation of the outcomes of the laboratory tests employed. The use of three tests in parallel or in series produces expressive losses in specificity and sensitivity values, respectively. On the other hand, using tests two by two and in series leads to losses in sensitivity values.

In order to determine confidence limits for the real proportion of protected animals we used the method proposed by Lew and Levy (1989)[1], "Bayesian" estimator, as an estimator of the real proportion of protected animals, instead of the traditional maximum likelihood estimator of the true prevalence and its variance in the evaluation of screening studies. To calculate the corrected prevalences (CP) the following programs were used: X(PLORE)[2] and EpiTools epidemiological calculators[3], selection "Bayesian estimation of true prevalence from survey testing with one or two tests".

6. Results and Discussions

Table 2 provides information on the samples collected from each subpopulation. There was good correspondence between the total expected number of samples and the total number collected (comparing with Table 01). Generally speaking, small differences between the expected and the total amount of bovine samples collected amidst the age groups being addressed were found in Bahia (four samples), Federal District (four samples), Mato Grosso do Sul (two samples), Mato Grosso (one sample), Paraná (two samples) and Rio Grande do Sul (two samples). The average time-frame between collection and the last vaccination varied from 3.3 months on the Mato Grosso do Sul border to 5.2 months on the Rio Grande do Sul border. The average time frame for the entire foot-and-mouth disease free zone was of 4.3 months.

Figures 3 and 4 allow evaluating the geographic distribution of the municipalities where the farms, from which samples were collected, are located; and show their space distribution according to the herd size (up to 50 bovines and more than 50 bovines).

[1] Lew, R. A. & Levy, P. S. (1989) Estimation of prevalence on the basis of screening tests. Statistics in Medicine, v. 8, 1225-1230.
[2] David Meredith. (2001) Department of Mathematics, San Francisco State University San Francisco. CA 94132
[3] Sergeant, ESG, 2009. Epitools epidemiological calculators. AusVet Animal Health Services and Australian Biosecurity Cooperative Research Centre for Emerging Infectious Disease. Available at: http://epitools.ausvet.com.au.

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Table 02. Total amount of collected samples, per herd size and age groups, and average time-lapse between collection and the last vaccination against foot-and-mouth disease on that farms

State	Region	Category	Sample				Average time between vaccination and sampling (days)
			Holdings	6-to-12 m. Bovines	13 to 24 m. bovines	Total Bovines	
Acre	Border	Up to 50 bovines	11	14	8	22	141
		50+ bovines	17	103	68	171	135
		Total	**28**	**117**	**76**	**193**	**137**
	Non-border	Up to 50 bovines	9	11	7	18	131
		50+ bovines	17	102	72	174	126
		Total	**26**	**113**	**79**	**192**	**128**
		AC Total	**54**	**230**	**155**	**385**	**133**
Bahia		Up to 50 bovines	26	34	26	60	138
		50+ bovines	15	65	69	134	132
		Total	**41**	**99**	**95**	**194**	**136**
Distrito Federal		Up to 50 bovines	40	48	30	78	148
		50+ bovines	12	70	48	118	151
		Total	**52**	**118**	**78**	**196**	**149**
Espírito Santo		Up to 50 bovines	18	21	15	36	106
		50+ bovines	16	92	68	160	117
		Total	**34**	**113**	**83**	**196**	**111**
Goiás		Up to 50 bovines	7	9	5	14	112
		50+ bovines	17	93	81	174	114
		Total	**24**	**102**	**86**	**188**	**114**
Minas Gerais		Up to 50 bovines	24	30	18	48	110
		50+ bovines	15	89	61	150	114
		Total	**39**	**119**	**79**	**198**	**112**
Mato Grosso do Sul	Border	Up to 50 bovines	10	13	7	20	88
		50+ bovines	18	102	70	172	106
		Total	**28**	**115**	**77**	**192**	**100**
	Non-border	Up to 50 bovines	3	4	2	6	113
		50+ bovines	18	111	73	184	112
		Total	**21**	**115**	**75**	**190**	**112**
		MS Total	**49**	**230**	**152**	**382**	**105**
Mato Grosso	Border	Up to 50 bovines	4	6	2	8	154
		50+ bovines	19	117	73	190	154
		Total	**23**	**123**	**75**	**198**	**154**
	Non-border	Up to 50 bovines	5	7	3	10	151
		50+ bovines	18	114	68	182	151
		Total	**23**	**121**	**71**	**192**	**151**
		MT Total	**46**	**244**	**146**	**390**	**152**
Pará		Up to 50 bovines	6	7	5	12	129
		50+ bovines	18	108	72	180	128
		Total	**24**	**115**	**77**	**192**	**128**
Paraná	Border	Up to 50 bovines	63	77	49	126	124
		50+ bovines	9	53	35	88	128
		Total	**72**	**130**	**84**	**214**	**125**
	Non-border	Up to 50 bovines	30	35	25	60	129
		50+ bovines	14	78	62	140	131
		Total	**44**	**113**	**87**	**200**	**130**
		PR Total	**116**	**243**	**171**	**414**	**127**
Rio de Janeiro		Up to 50 bovines	20	24	16	40	138
		50+ bovines	17	85	69	154	136
		Total	**37**	**109**	**85**	**194**	**137**
Rondônia	Border	Up to 50 bovines	10	14	6	20	140
		50+ bovines	17	108	66	174	144
		Total	**27**	**122**	**72**	**194**	**143**
	Non-border	Up to 50 bovines	18	25	10	35	143
		50+ bovines	16	105	58	163	132
		Total	**34**	**130**	**68**	**198**	**138**
		RO Total	**61**	**252**	**140**	**392**	**140**
Rio Grande do Sul	Border	Até 50 bovinos	17	21	13	34	151
		Mais de 50 bovinos	18	93	71	164	160
		Total	**35**	**114**	**84**	**198**	**155**
	Non-border	Up to 50 bovines	39	51	27	78	144
		50+ bovines	12	67	54	121	156
		Total	**51**	**118**	**81**	**199**	**146**
		RS Total	**86**	**232**	**165**	**397**	**150**
Sergipe		Up to 50 bovines	44	56	40	96	121
		50+ bovines	15	60	50	110	122
		Total	**59**	**116**	**90**	**206**	**121**
São Paulo		Up to 50 bovines	21	27	15	42	104
		50+ bovines	16	106	54	160	115
		Total	**37**	**133**	**69**	**202**	**109**
Tocantins		Up to 50 bovines	9	12	6	18	129
		50+ bovines	18	117	63	180	124
		Total	**27**	**129**	**69**	**198**	**126**
Total			**786**	**2584**	**1740**	**4324**	**130**

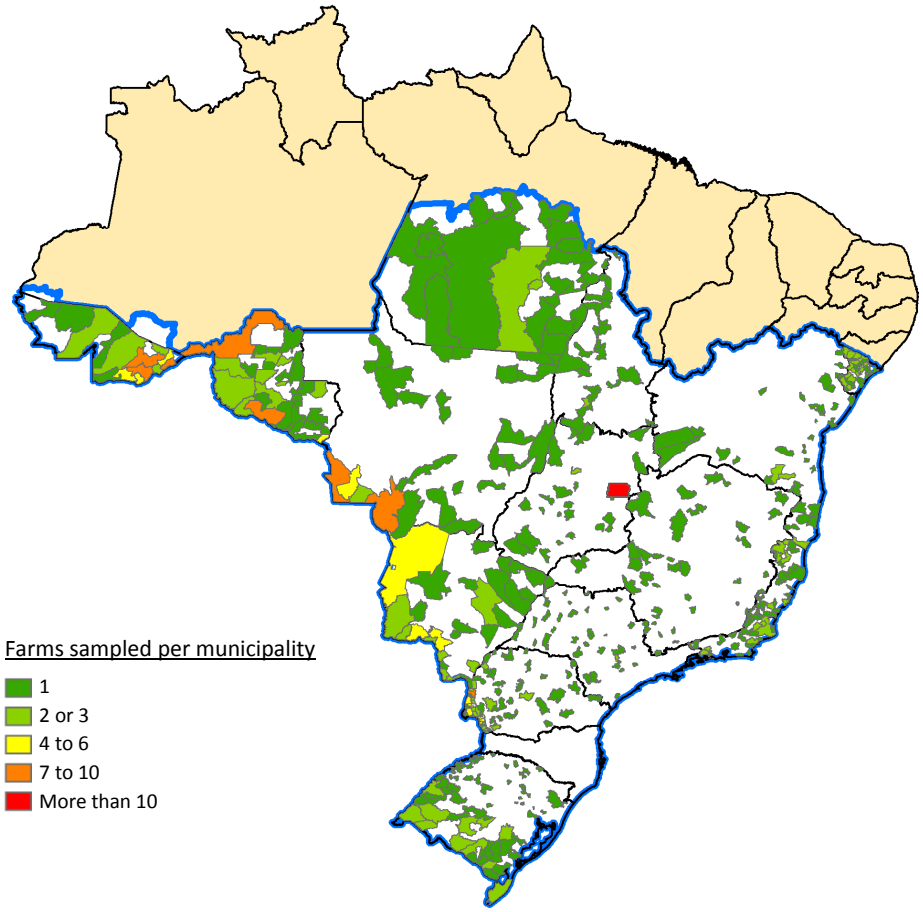

Figure 03. Distribution of the municipalities where farms submitted to sample collection are located

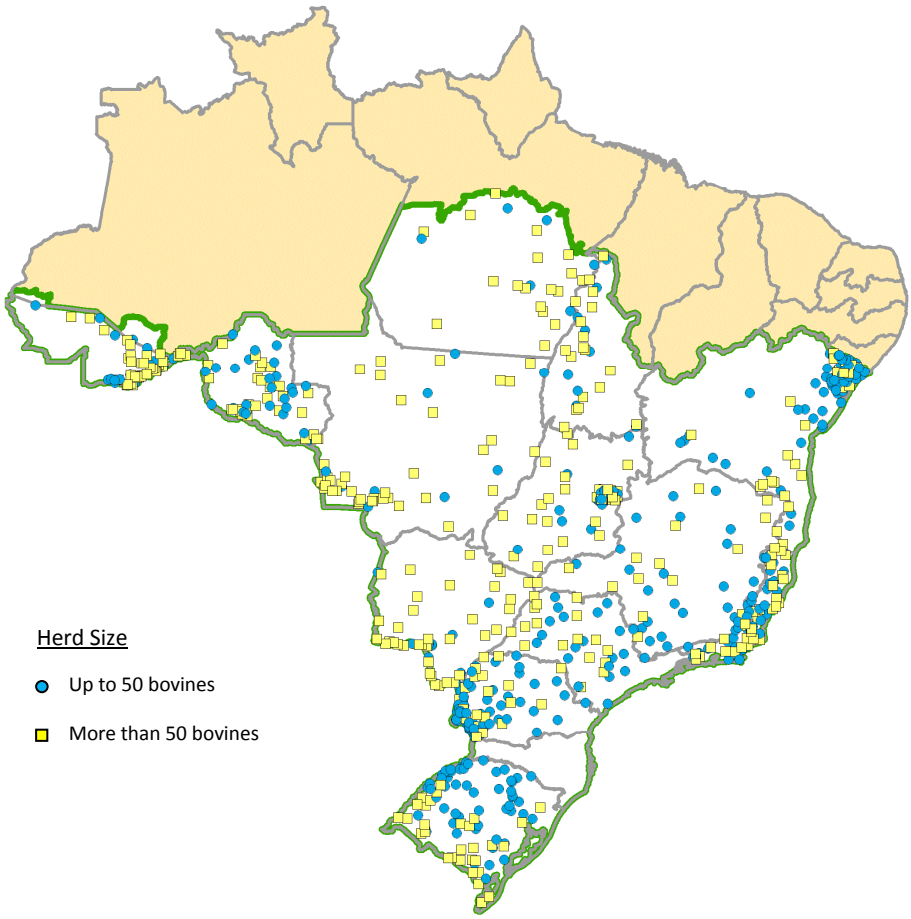

Figure 04. Geographic location of farms submitted to sample collection, according to the herd size

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Figure 5 allows us to compare, based on the animals' age, the percentage of bovine classified as protected, and the average number of stated vaccinated sampled bovine (considering only bovines that were born in the properties, in order to confer greater information reliability). As it had been expected, there was a direct relationship between the percentage of protected bovines, animal age, and number of vaccinations, thus indicating good consistency in terms of data and outcomes. In general terms as well, in the entire free zone, the percentage of protected bovines according to the type of virus may be observed in Figure 6. For animals between 6 and 12 months old, the average corrected prevalence varied between 70% and 73%, and for animals 13 to 24 months old, between 84% and 90%, figures that are within the expected levels for those age groups (70% to 75% and 80% to 85%, respectively).

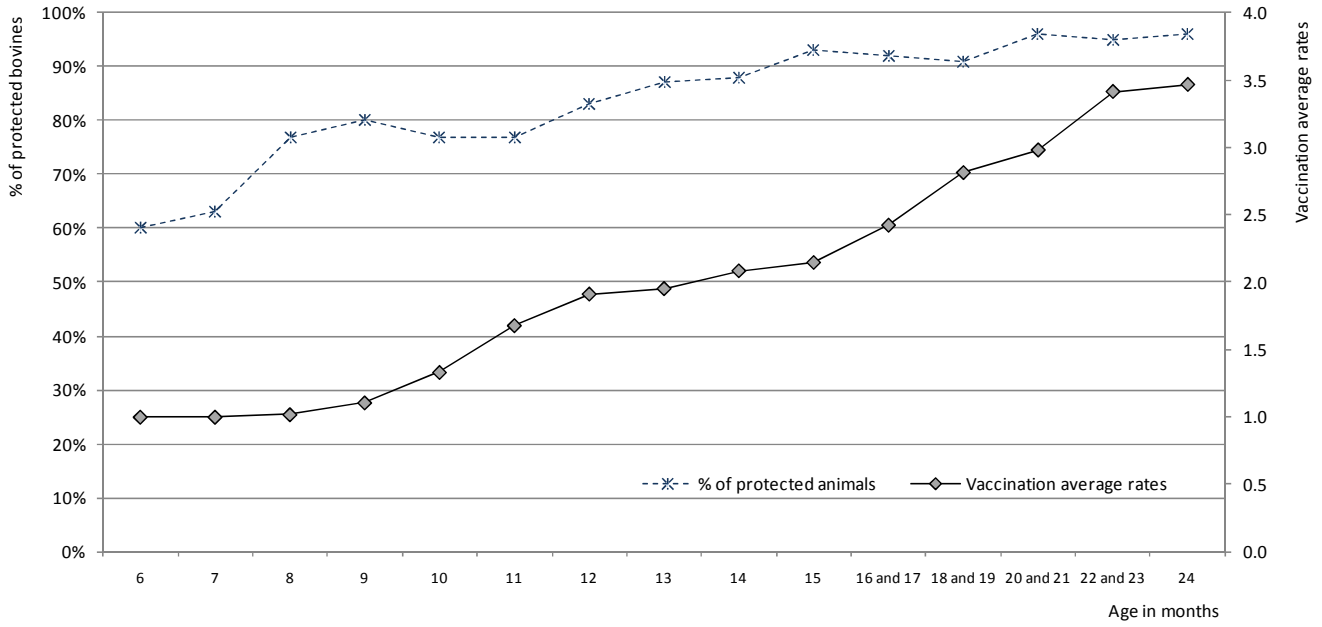

Figure 05. Observed prevalence of protected bovines and average vaccination against the foot-and-mouth disease, according to age, in months

Age	Vírus	Bovines		OP	CP (CI 95%)		
		Tested	Protected		Averege	LL	HL
6 to 12 m	O	2584	1725	67%	73%	68%	78%
	A	2584	1880	73%	71%	63%	62%
	C	2584	2017	78%	70%	62%	78%
13 to 24 m	O	1740	1482	85%	90%	87%	93%
	A	1740	1508	87%	84%	77%	90%
	C	1740	1588	91%	84%	77%	90%

OP = Observed prevalence; CP = Corrected prevalence
CI = Confidence interval; LL = Lowest limit of the CI
HL = Highest limit of the CI

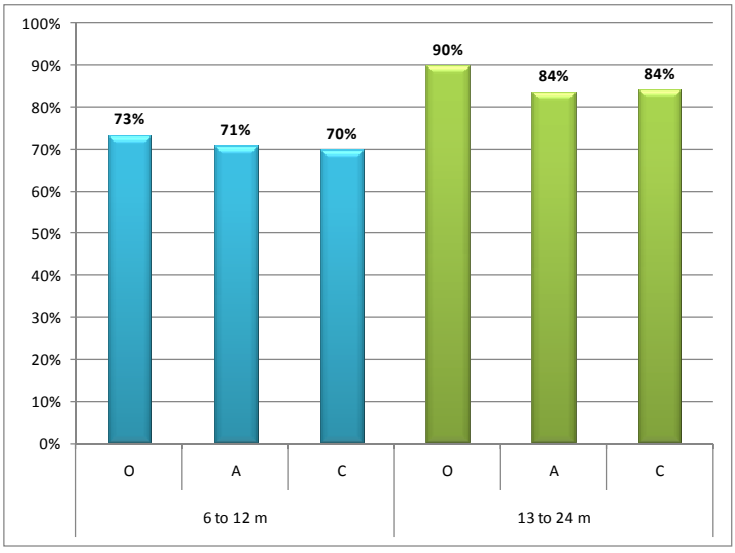

Figure 06. Observed and corrected prevalence of protected bovine, according to age group and type of virus

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Table 3 presents the outcomes of the observed (OP) and corrected (CP) prevalence found in each UF covered by the study, based on two by two and parallel evaluation of the tests carried out for viruses O, A and C. Complete tables, including the lowest and highest CP interval, with 95% of confidence, are available in annexes 1 to 3, respectively for the groups assessed “O or A”; “O or C” and “A or C”.

The average values regarding immunity coverage of the bovine population located in the free zone as a whole, measured during the final third stage of vaccination cycle, varied from 75% to 92%, depending on the cluster considered for the types of viruses addressed. Of the 16 UFs that make up the current free zone with vaccination, in 10 of them (62.5% of the total) we obtained figures above 80% for average immunity coverage, regardless of the types of viruses being assessed. The UFs which revealed the lowest immunity coverage averages are the Federal District (57% to 64%); Bahia (61% to 68%) and Sergipe (69% to 76%). Figures between 70% and 79% were recorded in Rio Grande do Sul, Rio de Janeiro and Espírito Santo.

Table 03. Observed prevalence (OP) and Corrected prevalence (CP) of protected bovine, based on a two by two and parallel evaluation of laboratory tests, per UF covered.

UF	6 to 12 month old bovines						13 to 24 month old bovines						Total (6 to 24 months)					
	Vírus O or A		Vírus O or C		Vírus A or C		Vírus O or A		Vírus O or C		Vírus A or C		Vírus O or A		Vírus O or C		Vírus A or C	
	OP	CP	OP	CP	OP	CP	OP	CP	OP	CP	OP	CP	OP	CP	OP	CP	OP	CP
AC	85%	84%	89%	91%	90%	96%	92%	94%	96%	98%	96%	98%	88%	88%	92%	96%	92%	99%
BA	60%	45%	61%	37%	61%	45%	82%	80%	86%	85%	86%	91%	71%	65%	73%	61%	73%	68%
DF	60%	51%	62%	40%	62%	47%	83%	81%	85%	81%	85%	88%	69%	64%	71%	57%	71%	64%
ES	80%	77%	81%	74%	76%	73%	82%	80%	82%	76%	78%	77%	81%	78%	81%	76%	77%	75%
GO	76%	73%	83%	79%	83%	86%	95%	96%	98%	97%	97%	97%	85%	84%	90%	92%	89%	96%
MG	82%	79%	83%	79%	84%	88%	94%	94%	94%	95%	92%	96%	86%	86%	87%	87%	87%	94%
MS	91%	93%	89%	90%	92%	98%	97%	98%	97%	99%	97%	99%	94%	96%	92%	96%	94%	99%
MT	77%	73%	80%	74%	80%	82%	97%	98%	98%	98%	98%	99%	84%	84%	87%	87%	87%	94%
PA	80%	77%	85%	83%	85%	89%	96%	97%	97%	97%	97%	97%	86%	86%	90%	92%	90%	96%
PR	77%	74%	81%	77%	81%	82%	89%	90%	91%	93%	90%	96%	82%	81%	85%	84%	85%	90%
RJ	79%	76%	80%	73%	80%	80%	80%	77%	85%	82%	85%	88%	79%	77%	82%	77%	82%	84%
RO	85%	84%	89%	90%	90%	97%	97%	98%	98%	98%	98%	99%	89%	90%	92%	96%	93%	99%
RS	71%	67%	73%	61%	73%	68%	84%	83%	87%	87%	87%	93%	77%	73%	79%	72%	79%	79%
SE	61%	53%	69%	53%	67%	57%	89%	89%	89%	89%	90%	94%	73%	69%	78%	72%	77%	76%
SP	85%	84%	84%	81%	86%	92%	94%	95%	93%	93%	94%	96%	88%	88%	87%	87%	89%	96%
TO	83%	81%	81%	75%	86%	91%	97%	99%	99%	97%	99%	97%	88%	88%	87%	86%	90%	97%
Total	**78%**	**68%**	**81%**	**70%**	**81%**	**84%**	**91%**	**86%**	**92%**	**87%**	**92%**	**88%**	**83%**	**75%**	**85%**	**77%**	**86%**	**92%**

As expected, the most critical figures were observed within the group of 6 to 12 months old bovines. In this age group, there is an estimated average immunity coverage ranging from 70 to 75%, however, lower figures were observed in the Federal District (40% to 51%); Bahia (37% to 45%), Sergipe (53% to 57%) and Rio Grande do Sul (61% to 68%). The average figures observed in the age group of 13 to 24 months old were considered, satisfactory; with lower rates recorded in Rio de Janeiro (77% for group O or A) and Espírito Santo (76% for group O or C and 77% for group A or C).

The outcomes on average prevalence found in bordering and non-bordering regions, of the six UFs where independent studies were carried out for the referred regions, are summarized in Table 4. Of the outcomes obtained, the greatest differences were observed in Rio Grande do Sul. In this State, the rates found in the border region are significantly higher than those of the rest of the UF (binomial test for prevalence of independent samples; $p = 0.0007$, age group of 6 to 12 months old; $p = 0.0284$, age group of 13 to 24 months old), clearly showing less immunity coverage in the inside areas of Rio Grande do Sul. In the other evaluated UFs, significant percentage differences in protected bovine were observed in some states, limited to clusters of viral tests and age groups, however, all of them taking place within satisfactory levels of immunity coverage.

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Table 04. Observed prevalence (OP) and Corrected prevalence (CP) of protected bovines, based on a two by two and parallel evaluation of laboratory tests, according to bordering and non-bordering regions.

State	Region	6 to 12 month old bovines						13 to 24 month old bovines						Total (6 to 24 months)					
		Vírus O or A		Vírus O or C		Vírus A or C		Vírus O or A		Vírus O or C		Vírus A or C		Vírus O or A		Vírus O or C		Vírus A or C	
		OP	CP	OP	CP	OP	CP	OP	CP	OP	CP	OP	CP	OP	CP	OP	CP	OP	CP
AC	Border	84%	82%	85%	83%	85%	90%	95%	95%	96%	96%	96%	97%	88%	88%	90%	92%	90%	96%
	Non-border	86%	85%	93%	95%	94%	97%	90%	90%	96%	96%	96%	97%	88%	88%	94%	97%	95%	98%
	Total	**85%**	**84%**	**89%**	**91%**	**90%**	**96%**	**92%**	**94%**	**96%**	**98%**	**96%**	**98%**	**88%**	**88%**	**92%**	**96%**	**92%**	**99%**
MS	Border	89%	89%	85%	83%	88%	93%	95%	95%	96%	96%	95%	97%	91%	92%	90%	91%	91%	97%
	Non-border	94%	95%	92%	94%	96%	98%	100%	98%	99%	97%	100%	98%	96%	98%	95%	98%	97%	99%
	Total	**91%**	**93%**	**89%**	**90%**	**92%**	**98%**	**97%**	**98%**	**97%**	**99%**	**97%**	**99%**	**94%**	**96%**	**92%**	**96%**	**94%**	**99%**
MT	Border	75%	71%	80%	73%	80%	80%	96%	96%	97%	97%	97%	97%	83%	81%	86%	86%	86%	92%
	Non-border	79%	75%	81%	75%	81%	82%	99%	98%	99%	97%	99%	97%	86%	85%	88%	88%	88%	94%
	Total	**77%**	**73%**	**80%**	**74%**	**80%**	**82%**	**97%**	**98%**	**98%**	**98%**	**98%**	**99%**	**84%**	**84%**	**87%**	**87%**	**87%**	**94%**
PR	Border	74%	69%	80%	73%	79%	79%	85%	83%	87%	86%	87%	91%	78%	75%	83%	79%	82%	85%
	Non-border	81%	78%	83%	79%	82%	85%	94%	95%	94%	95%	93%	96%	87%	86%	88%	89%	87%	93%
	Total	**77%**	**74%**	**81%**	**77%**	**81%**	**82%**	**89%**	**90%**	**91%**	**93%**	**90%**	**96%**	**82%**	**81%**	**85%**	**84%**	**85%**	**90%**
RO	Border	84%	82%	85%	83%	87%	92%	99%	98%	99%	97%	99%	97%	89%	90%	90%	92%	91%	97%
	Non-border	85%	85%	92%	95%	92%	97%	96%	96%	97%	96%	97%	97%	89%	89%	94%	97%	94%	98%
	Total	**85%**	**84%**	**89%**	**90%**	**90%**	**97%**	**97%**	**98%**	**98%**	**98%**	**98%**	**99%**	**89%**	**90%**	**92%**	**96%**	**93%**	**99%**
RS	Border	80%	77%	83%	79%	83%	86%	89%	89%	93%	94%	93%	96%	84%	83%	87%	87%	87%	94%
	Non-border	63%	55%	64%	43%	64%	50%	79%	76%	81%	76%	81%	83%	69%	64%	71%	57%	71%	64%
	Total	**71%**	**67%**	**73%**	**61%**	**73%**	**68%**	**84%**	**83%**	**87%**	**87%**	**87%**	**93%**	**77%**	**73%**	**79%**	**72%**	**79%**	**79%**
Total		**78%**	**68%**	**81%**	**70%**	**81%**	**84%**	**91%**	**86%**	**92%**	**87%**	**92%**	**88%**	**83%**	**75%**	**85%**	**77%**	**86%**	**92%**

The outcomes observed for immunity coverage of the bovine population, according the herd size, may be assessed in table 5. Such as we have observed in previous studies, immunity coverage of the bovine population in herds with more than 50 animals has shown to be greater than such coverage found in bovine populations of properties having up to 50 animals ($p < 0.0001$). Significant differences were observed in the Federal District ($p = 0.0187$); Minas Gerais ($p < 0.0001$); Paraná ($p = 0{,}0004$); Rio de Janeiro ($p = 0.0076$); Rondônia ($p = 0.0289$); Rio Grande do Sul ($p = 0.0186$); Sergipe ($p = 0.0175$) and São Paulo ($p = 0.0138$). The small number of observations of herds with up to 50 animals in Goiás, Mato Grosso, Mato Grosso do Sul, Pará and Tocantins, did not allow for an accurate evaluation of these states.

Table 05. Observed prevalence of protected bovine, according to size of herd based on a two by two and parallel evaluation of laboratory tests on the three virus strains

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UF	Bovine population in herds of up to 50 bovines			Bovine population in herds of more than 50 bovines		
	Tested	Protected	Observed prevalence	Tested	Protected	Observed prevalence
Acre	40	34	85%	345	321	93%
Bahia	60	41	68%	134	101	75%
Federal District	78	48	62%	118	91	77%
Espírito Santo	36	28	78%	160	133	83%
Goiás	14	11	79%	174	158	91%
Minas Gerais	48	32	67%	150	142	95%
Mato Grosso do Sul	26	25	96%	356	338	95%
Mato Grosso	18	15	83%	372	324	87%
Pará	12	10	83%	180	163	91%
Paraná	186	146	78%	228	207	91%
Rio de Janeiro	40	27	68%	154	132	86%
Rondônia	55	47	85%	337	316	94%
Rio Grande do Sul	112	80	71%	285	234	82%
Sergipe	96	68	71%	110	93	85%
São Paulo	42	33	79%	160	147	92%
Tocantins	18	14	78%	180	165	92%
Total	**881**	**659**	**75%**	**3,443**	**3,065**	**89%**

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7. Final comments

The outcomes reveal satisfactory immunity coverage for foot-and-mouth disease in the bovine population between 6 to 24 months old, located in most of the states within the FMD free zone with vaccination. However, with aim at improving the rates of immunity protection, vaccination stages shall be strengthened in some UFs, particularly toward the young animal population of the states of Bahia, the Federal District, Espírito Santo, Paraná (border), Rio de Janeiro, Rio Grande do Sul (non border) and Sergipe. Particularly in the state of Rio de Janeiro and the non-bordering region of Rio Grande do Sul, low levels of immunity protection were recorded within both age groups, thus revealing the need for reviewing vaccination campaigns in those locations.

In general, the bovine population of herds with up to 50 bovines presented the lowest immunity protection rates, revealing the need for the SVEs to strengthen and expand their strategies to provide for small rural properties, particularly in the Federal District (62%); Minas Gerais (67%); Bahia (68%); Rio de Janeiro (68%); Rio Grande do Sul (71%) and Sergipe (71%).

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Annex 1

Outcomes of the analysis in parallel for viruses O and A, by UF and region

UF	Region	6 to 12 month old bovinos						13 to 24 month old bovine						Total (6 to 24 months)					
		Total	Prot	OP	CP	LL	HL	Total	Prot	OP	CP	LL	HL	Total	Prot	OP	CP	LL	HL
AC	Border	117	98	84%	82%	73%	92%	76	72	95%	95%	89%	100%	193	170	88%	88%	82%	94%
	Non-border	113	97	86%	85%	76%	94%	79	71	90%	90%	81%	99%	192	168	88%	88%	81%	94%
	Total	**230**	**195**	**85%**	**84%**	**78%**	**90%**	**155**	**143**	**92%**	**94%**	**89%**	**99%**	**385**	**338**	**88%**	**88%**	**84%**	**92%**
BA	All state	99	59	60%	45%	37%	64%	95	78	82%	80%	69%	91%	194	137	71%	65%	56%	74%
DF	All state	118	71	60%	51%	39%	63%	78	65	83%	81%	70%	93%	196	136	69%	64%	55%	73%
ES	All state	113	90	80%	77%	66%	87%	83	68	82%	80%	68%	91%	196	158	81%	78%	71%	86%
GO	All state	102	78	76%	73%	61%	84%	86	82	95%	96%	91%	100%	188	160	85%	84%	77%	91%
MG	All state	119	97	82%	79%	70%	89%	79	74	94%	94%	88%	100%	198	171	86%	86%	77%	92%
MS	Border	115	102	89%	89%	81%	96%	77	73	95%	95%	89%	100%	192	175	91%	92%	87%	97%
	Non-border	115	108	94%	95%	90%	100%	75	75	100%	98%	94%	100%	190	183	96%	98%	95%	100%
	Total	**230**	**210**	**91%**	**93%**	**88%**	**97%**	**152**	**148**	**97%**	**98%**	**96%**	**100%**	**382**	**358**	**94%**	**96%**	**93%**	**99%**
MT	Border	123	92	75%	71%	60%	81%	75	72	96%	96%	91%	100%	198	164	83%	81%	74%	89%
	Non-border	121	95	79%	75%	65%	86%	71	70	99%	98%	93%	100%	192	165	86%	85%	79%	92%
	Total	**244**	**187**	**77%**	**73%**	**66%**	**81%**	**146**	**142**	**97%**	**98%**	**95%**	**100%**	**390**	**329**	**84%**	**84%**	**79%**	**89%**
PA	All state	115	92	80%	77%	67%	88%	77	74	96%	97%	91%	100%	192	166	86%	86%	80%	93%
PR	Border	130	96	74%	69%	59%	80%	84	71	85%	83%	72%	94%	214	167	78%	75%	67%	83%
	Non-border	113	91	81%	78%	68%	88%	87	82	94%	95%	89%	100%	200	173	87%	86%	80%	93%
	Total	**243**	**187**	**77%**	**74%**	**66%**	**81%**	**171**	**153**	**89%**	**90%**	**84%**	**96%**	**414**	**340**	**82%**	**81%**	**76%**	**86%**
RJ	All state	109	86	79%	76%	65%	87%	85	68	80%	77%	65%	89%	194	154	79%	77%	69%	85%
RO	Border	122	102	84%	82%	73%	91%	72	71	99%	98%	93%	100%	194	173	89%	90%	84%	95%
	Non-border	130	111	85%	85%	76%	93%	68	65	96%	96%	90%	100%	198	176	89%	89%	84%	95%
	Total	**252**	**213**	**85%**	**84%**	**78%**	**90%**	**140**	**136**	**97%**	**98%**	**95%**	**100%**	**392**	**349**	**89%**	**90%**	**86%**	**94%**
RS	Border	114	91	80%	77%	67%	87%	84	75	89%	89%	80%	98%	198	166	84%	83%	76%	90%
	All state	118	74	63%	55%	43%	67%	81	64	79%	76%	63%	88%	199	138	69%	64%	55%	72%
	Total	**232**	**165**	**71%**	**67%**	**58%**	**74%**	**165**	**139**	**84%**	**83%**	**76%**	**91%**	**397**	**304**	**77%**	**73%**	**67%**	**79%**
SE	All state	116	71	61%	53%	41%	65%	90	80	89%	89%	80%	97%	206	151	73%	69%	60%	77%
SP	All state	133	113	85%	84%	76%	92%	69	65	94%	95%	88%	100%	202	178	88%	88%	82%	94%
TO	All state	129	107	83%	81%	72%	90%	69	67	97%	99%	97%	100%	198	174	88%	88%	82%	94%
Total		**2,584**	**2,021**	**78%**	**68%**	**66%**	**70%**	**1,740**	**1,582**	**91%**	**86%**	**84%**	**88%**	**4,324**	**3,603**	**83%**	**75%**	**74%**	**77%**

Prot = total number of bovines considered to be protected; OP = Observed prevalence; CP = Corrected prevalence;
LL = Lowest limit and HL = Highest limit of the 95% Confidence Interval of the corrected prevalence

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Annex 2

Outcomes of the analysis in parallel for viruses O and C, by UF and region

UF	Region	6 to 12 month old bovinos						13 to 24 month old bovine						Total (6 to 24 months)					
		Total	Prot	OP	CP	LL	HL	Total	Prot	OP	CP	LL	HL	Total	Prot	OP	CP	LL	HL
AC	Border	117	100	85%	83%	71%	96%	76	73	96%	96%	88%	100%	193	173	90%	92%	84%	99%
	Non-border	113	105	93%	95%	87%	100%	79	76	96%	96%	89%	100%	192	181	94%	97%	93%	100%
	Total	**230**	**205**	**89%**	**91%**	**84%**	**98%**	**155**	**149**	**96%**	**98%**	**94%**	**100%**	**385**	**354**	**92%**	**96%**	**92%**	**100%**
BA	All state	99	60	61%	37%	20%	55%	95	82	86%	85%	71%	98%	194	142	73%	61%	48%	74%
DF	All state	118	73	62%	40%	23%	56%	78	66	85%	81%	65%	98%	196	139	71%	57%	44%	70%
ES	All state	113	91	81%	74%	59%	89%	83	68	82%	76%	59%	94%	196	159	81%	76%	65%	87%
GO	All state	102	85	83%	79%	65%	94%	86	84	98%	97%	91%	100%	188	169	90%	92%	85%	99%
MG	All state	119	99	83%	79%	66%	93%	79	74	94%	95%	85%	100%	198	173	87%	87%	79%	96%
MS	Border	115	98	85%	83%	70%	96%	77	74	96%	96%	88%	100%	192	172	90%	91%	84%	99%
	Non-border	115	106	92%	94%	86%	100%	75	74	99%	97%	90%	100%	190	180	95%	98%	93%	100%
	Total	**230**	**204**	**89%**	**90%**	**83%**	**97%**	**152**	**148**	**97%**	**99%**	**94%**	**100%**	**382**	**352**	**92%**	**96%**	**93%**	**100%**
MT	Border	123	98	80%	73%	58%	88%	75	73	97%	97%	89%	100%	198	171	86%	86%	76%	95%
	Non-border	121	98	81%	75%	61%	90%	71	70	99%	97%	90%	100%	192	168	88%	88%	79%	96%
	Total	**244**	**196**	**80%**	**74%**	**64%**	**85%**	**146**	**143**	**98%**	**98%**	**95%**	**100%**	**390**	**339**	**87%**	**87%**	**81%**	**93%**
PA	All state	115	98	85%	83%	70%	96%	77	75	97%	97%	90%	100%	192	173	90%	92%	85%	99%
PR	Border	130	104	80%	73%	59%	88%	84	73	87%	86%	71%	100%	214	177	83%	79%	68%	89%
	Non-border	113	94	83%	79%	65%	93%	87	82	94%	95%	87%	100%	200	176	88%	89%	80%	97%
	Total	**243**	**198**	**81%**	**77%**	**67%**	**87%**	**171**	**155**	**91%**	**93%**	**86%**	**100%**	**414**	**353**	**85%**	**84%**	**77%**	**91%**
RJ	All state	109	87	80%	73%	57%	89%	85	72	85%	82%	66%	97%	194	159	82%	77%	66%	88%
RO	Border	122	104	85%	83%	71%	96%	72	71	99%	97%	90%	100%	194	175	90%	92%	86%	99%
	Non-border	130	120	92%	95%	87%	100%	68	66	97%	96%	88%	100%	198	186	94%	97%	93%	100%
	Total	**252**	**224**	**89%**	**90%**	**84%**	**97%**	**140**	**137**	**98%**	**98%**	**94%**	**100%**	**392**	**361**	**92%**	**96%**	**93%**	**100%**
RS	Border	114	95	83%	79%	66%	93%	84	78	93%	94%	85%	100%	198	173	87%	87%	79%	96%
	All state	118	75	64%	43%	26%	60%	81	66	81%	76%	58%	93%	199	141	71%	57%	44%	70%
	Total	**232**	**170**	**73%**	**61%**	**49%**	**73%**	**165**	**144**	**87%**	**87%**	**77%**	**97%**	**397**	**314**	**79%**	**72%**	**64%**	**81%**
SE	All state	116	80	69%	53%	36%	70%	90	80	89%	89%	77%	100%	206	160	78%	72%	61%	84%
SP	All state	133	112	84%	81%	69%	94%	69	64	93%	93%	82%	100%	202	176	87%	87%	78%	96%
TO	All state	129	104	81%	75%	60%	89%	69	68	99%	97%	90%	100%	198	172	87%	86%	78%	95%
Total		**2,584**	**2,086**	**81%**	**70%**	**68%**	**72%**	**1,740**	**1,609**	**92%**	**87%**	**85%**	**89%**	**4,324**	**3,695**	**85%**	**77%**	**76%**	**79%**

Prot = total number of bovines considered to be protected; OP = Observed prevalence; PC = Corrected prevalence;
LL = Lowest limit and HL = Highest limit of the 95% Confidence Interval of the corrected prevalence

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Annex 3

Outcomes of the analysis in parallel for viruses A and C, according to UF and region

UF	Region	6 to 12 month old bovinos						13 to 24 month old bovine						Total (6 to 24 months)					
		Total	Prot	OP	CP	LL	HL	Total	Prot	OP	CP	LL	HL	Total	Prot	OP	CP	LL	HL
AC	Border	117	100	85%	90%	80%	100%	76	73	96%	97%	90%	100%	193	173	90%	96%	91%	100%
	Non-border	113	106	94%	97%	91%	100%	79	76	96%	97%	90%	100%	192	182	95%	98%	95%	100%
	Total	**230**	**206**	**90%**	**96%**	**92%**	**100%**	**155**	**149**	**96%**	**98%**	**95%**	**100%**	**385**	**355**	**92%**	**99%**	**96%**	**100%**
BA	All state	99	60	61%	45%	26%	63%	95	82	86%	91%	80%	100%	194	142	73%	68%	56%	80%
DF	All state	118	73	62%	47%	30%	64%	78	66	85%	88%	74%	100%	196	139	71%	64%	51%	77%
ES	All state	113	86	76%	73%	58%	89%	83	65	78%	77%	60%	100%	196	151	77%	75%	64%	87%
GO	All state	102	85	83%	86%	74%	99%	86	83	97%	97%	91%	100%	188	168	89%	96%	90%	100%
MG	All state	119	100	84%	88%	77%	99%	79	73	92%	96%	87%	100%	198	173	87%	94%	87%	100%
MS	Border	115	101	88%	93%	43%	100%	77	73	95%	97%	89%	100%	192	174	91%	97%	92%	100%
	Non-border	115	110	96%	98%	93%	100%	75	75	100%	98%	92%	100%	190	185	97%	99%	96%	100%
	Total	**230**	**211**	**92%**	**98%**	**94%**	**100%**	**152**	**148**	**97%**	**99%**	**95%**	**100%**	**382**	**359**	**94%**	**99%**	**97%**	**100%**
MT	Border	123	98	80%	80%	67%	93%	75	73	97%	97%	90%	100%	198	171	86%	92%	85%	99%
	Non-border	121	98	81%	82%	70%	95%	71	70	99%	97%	91%	100%	192	168	88%	94%	87%	100%
	Total	**244**	**196**	**80%**	**82%**	**72%**	**91%**	**146**	**143**	**98%**	**99%**	**95%**	**100%**	**390**	**339**	**87%**	**94%**	**89%**	**98%**
PA	All state	115	98	85%	89%	79%	100%	77	75	97%	97%	91%	100%	192	173	90%	96%	92%	100%
PR	Border	130	103	79%	79%	66%	92%	84	73	87%	91%	80%	100%	214	176	82%	85%	76%	94%
	Non-border	113	93	82%	85%	72%	97%	87	81	93%	96%	89%	100%	200	174	87%	93%	87%	100%
	Total	**243**	**196**	**81%**	**82%**	**73%**	**91%**	**171**	**154**	**90%**	**96%**	**91%**	**100%**	**414**	**350**	**85%**	**90%**	**84%**	**95%**
RJ	All state	109	87	80%	80%	66%	94%	85	72	85%	88%	75%	100%	194	159	82%	84%	75%	94%
RO	Border	122	106	87%	92%	83%	100%	72	71	99%	97%	91%	100%	194	177	91%	97%	93%	100%
	Non-border	130	120	92%	97%	91%	100%	68	66	97%	97%	89%	100%	198	186	94%	98%	95%	100%
	Total	**252**	**226**	**90%**	**97%**	**92%**	**100%**	**140**	**137**	**98%**	**99%**	**95%**	**100%**	**392**	**363**	**93%**	**99%**	**97%**	**100%**
RS	Border	114	95	83%	86%	75%	98%	84	78	93%	96%	88%	100%	198	173	87%	94%	87%	100%
	All state	118	75	64%	50%	33%	67%	81	66	81%	83%	67%	98%	199	141	71%	64%	51%	76%
	Total	**232**	**170**	**73%**	**68%**	**57%**	**80%**	**165**	**144**	**87%**	**93%**	**86%**	**100%**	**397**	**314**	**79%**	**79%**	**72%**	**87%**
SE	All state	116	78	67%	57%	40%	74%	90	81	90%	94%	85%	100%	206	159	77%	76%	65%	87%
SP	All state	133	115	86%	92%	83%	100%	69	65	94%	96%	87%	100%	202	180	89%	96%	90%	100%
TO	All state	129	111	86%	91%	82%	100%	69	68	99%	97%	90%	100%	198	179	90%	97%	92%	100%
Total		**2,584**	**2,098**	**81%**	**84%**	**81%**	**86%**	**1,740**	**1,605**	**92%**	**88%**	**86%**	**90%**	**4,324**	**3,703**	**86%**	**92%**	**90%**	**94%**

Prot = total number of bovines considered to be protected; OP = Observed prevalence; CP = Corrected prevalence;
LL = Lowest limit and HL = Highest limit of the 95% Confidence Interval of the corrected prevalence

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