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National Surveillance Plan for Equine Infectious Anaemia (EIA)

Report of the analytical activities conducted by the Network of the Official Diagnostic Laboratories (Istituti Zooprofilattici Sperimentali) in Italy during the period 2007-2011

The present document reports the activities conducted in Italy, during the period 2007-2011, related to the % ational Surveillance Plan for Equine Infectious Anaemia+. These activities were regulated by the following Italian Regulations:

- ORDINANZA 14 novembre 2006
 Disposizioni urgenti in materia di sorveglianza dell'anemia infettiva degli equidi.
- O.M. 18 dicembre 2007, Piano di sorveglianza nazionale per l'anemia infettiva degli equidi
- ORDINANZA 8 agosto 2010 Piano di sorveglianza nazionale per l'anemia infettiva degli equidi
- ORDINANZA 14 novembre 2006 Disposizioni urgenti in materia di sorveglianza dell'anemia infettiva degli equidi
- O.M. 18 dicembre 2007, Piano di sorveglianza nazionale per l'anemia infettiva degli equidi
- ORDINANZA 8 agosto 2010
 Piano di sorveglianza nazionale per l'anemia infettiva degli equidi

The following information refers to the activities conducted by the 10 Istituti Zooprofilattici Sperimentali (IIZZSS) for the serological diagnosis on equine samples collected in the relatives regions of jurisdiction.

The data processing was conducted on the basis of the information transmitted by each Institute to the National Reference Centre for Equine Infectious Anaemia (CRAIE), using specific and standard procedures. Data are transmitted by each Institute every trimester.

For the calculation of the denominators, samples in confirmation by the national reference centre, eventually tested in parallel using different techniques (ELISA, AGID Coggins, AGID OIE), were counted only once.

The results of the total number of samples analysed as well as the results of the confirmatory serological analyses conducted by the CRAIE-IZSLT are aggregated for each region. The results presented here are based on the total number of samples tested, because it was impossible, at national level, to identify with certainty the single equids analysed on the basis on the univocal identification (microchip . passport identification).

Also in 2011, even if the identification of equids was already compulsory, a high proportion of animals tested and transmitted to CRAIE were still without identification.

For the first time in 2011, data relative to the number of farms with equids tested and the outbreaks prevalence and incidence are also presented.

Activities conducted in Italy during the period 2007-2011

During the period 2007-2011, IIZZSS tested 1.141.643 samples of equids, while the samples confirmed as positive by CRAIE were 2.255.

Analyses of samples of Í Horsel species

Table 1 and figure 1 report the samples of horses tested by the IIZZSS and the distribution of samples per region.

During the period 2007-2011, 1.077.464 samples of horses were tested and 1.479 were confirmed as positive by CRAIE (0.14%).

Table 1: species horse	total number of blood samples tested and confirmed as positive by CRAIE during
the period 2007-201	classified per region

	20	007	2008		20	009	2010		2011	
REGION	TESTED	POSITIVES	TESTED	POSITIVES	TESTED	POSITIVES	TESTED	POSITIVES	TESTED	POSITIVES
ABRUZZO	7867	228	8994	205	7618	62	9427	55	11.152	46
BASILICATA	2195	6	2705	4	3279	4	3622	7	2.057	1
CALABRIA	1258	4	1100	2	2275	2	2136	1	1.693	2
CAMPANIA	9356	7	8752	12	8903	5	10620	15	5.067	6
EMILIA-ROMAGNA	14083	13	28221	6	21462	0	21874	5	7.927	1
FRIULI-VENEZIA GIULIA	2147	2	2164	1	2227	1	2595	3	1.322	0
LAZIO	28337	95	30940	151	31625	76	34757	31	30.807	22
LIGURIA	4496	0	5054		5098	0	6264	55	3.949	0
LOMBARDIA	43781	8	41223	3	35347	1	32943	0	23.186	2
MARCHE	7450	9	7183	2	7370	1	6783	3	2.832	0
MOLISE	1453	15	2676	17	2651	26	2988	7	3.177	4
PIEMONTE	24319	2	29430	2	26162	0	25998	1	14.597	0
PUGLIA	8059	17	8048	6	7308	13	8169	21	5.922	5
SARDEGNA	5595	0	5831	4	4936	0	5455	0	3.881	0
SICILIA	*	-	10982	4	12327	1	15144	8	7.620	0
TOSCANA	34153	47	28901	13	23939	1	22961	3	10.704	4
TRENTINO-ALTO ADIGE	7312	1	2926		2920	2	2638	0	1.377	0
UMBRIA	6192	12	7179	17	10503	13	11654	5	11.738	9
VALLE D'AOSTA	408	0	524		521	0	510	0	210	0
VENETO	13455	11	9973	5	7847	11	7721	3	4.947	4
TOTAL	221916	477	242806	454	224318	219	234259	223	154.165	108

* for the year 2007 no samples were received from Sicily

Figure 1: species horse . number of blood samples tested and confirmed as positive by CRAIE during the period 2007-2011



It appears clear that when comparing the year 2011 to the previous ones, a decreasing number of horses are tested. This decrease was due to the remodulation of the surveillance activities implemented in those regions demonstrating a low prevalence in the previous years (reduction of sampling from annual to biannual):

Piemonte Liguria Valle Dofosta Lombardia Emilia Romagna Veneto Friuli Venezia Giulia Trentino Alto Adige Toscana e Marche Puglia Sardegna e Sicilia

The raw prevalence referring to the number of positive samples during the observation period, goes from 0.21% (CI 95%, 0.19-0.23) in 2007, to 0.07% (CI 95%, 0.04-0.13) in 2011. Figure 2 shows the trends in prevalence during the years, with a clear significant decreasing pattern (chi squared test for linear trends: 220.7; p=0.00000).

Respect to the 2 previous years, the effective decrease in prevalence in horses refers to the year 2009. In the following two-year period the seroprevalence is basically constant, with a number of cases at national level of an apparently sporadic infection.

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Figure 2: species horse . annual prevalence of positive blood samples confirmed by CRAIE during the period 2007-2011



Horses

The annual prevalence of positive EIA horse samples at regional level also confirms a progressive decreasing tendency in the period 2007-2011. This is attested by the progressive resetting in time, of the result at regional level to the medium result at national level (red line in the figure). In 2011 all the regions showed a further decreasing of the raw prevalence.



Figure 3: species horse . prevalence of positive samples confirmed by CRAIE at regional level . year 2011

Analyses of samples of the species mule

Table 2 and in figure 4 report the samples of mules tested by the IIZZSS. Table 2 also reports the distribution of the samples per single region.

During the period 2007-2011, 11.789 samples of mules were tested. 736 were confirmed as positive by CRAIE (6.2%).

	20)07	20	008	20)09	20)10	2011	
REGION	TESTED	POSITIVES								
ABRUZZO	656	96	674	119	490	29	588	68	667	17
BASILICATA	16	0	13	0	18	0	21		16	0
CALABRIA	0	0	0	0	0	0	1		8	
CAMPANIA	117	3	50	11	77	1	74	4	39	6
EMILIA-ROMAGNA	16	3	14	0	28	0	14		25	
FRIULI-VG	0	0	1	0	5	3	7	5	3	
LAZIO	307	46	981	113	644	60	1004	27	739	8
LIGURIA	11	0	35	0	55	0	124	4	104	
LOMBARDIA	63	0	62	0	45	0	51		26	
MARCHE	216	21	222	13	210	2	170	5	136	1
MOLISE	20	0	10	0	7	0	21	2	27	
PIEMONTE	84	0	130	0	88	1	174	1	162	1
PUGLIA	19	0	25	0	36	2	39	3	42	1
SARDEGNA	1	0	0	0	0	0				
SICILIA	*	-	1	0	0	0			20	
TOSCANA	137	7	104	9	121	2	109	1	66	2
TRENT-A. ADIGE	6	0	1	0	2	0	6		1	
UMBRIA	95	6	300	12	362	6	365	10	294	5
VALLE D'AOSTA	0	0	1	0	0	0	4			
VENETO	8	0	10	0	14	0	12		12	
TOTAL	1772	182	2634	277	2202	106	2784	130	2.387	41

Table 2: species mule Ë total number of blood samples tested and number of samples confirmed as positive by CRAIE during the period 2007-2011 per region of origin

Figure 4: species mule . number of blood samples tested and confirmed as positive by CRAIE during the period 2007-2011



The prevalence of positive samples during the period of observation decreases from 10.27% (Cl95% 8.8-11.7) in 2007, to 1.7% (Cl95% 1.3-2.4) in 2011.

Figure 9 shows the change in prevalence of positive samples during the years, with a clear significant decreasing pattern (chi squared test for linear trends: 203.4; p=0.00000).

Figure 5: species mule - annual prevalence of positive blood samples confirmed by CRAIE during the period 2007-2011



The annual prevalence of positive EIA mule samples at regional level also confirms what is observed in horses, regarding the concentration of positive animals in the Italian Central regions.

To be noted is the result obtained in the Campania region, which even if characterized by a remarkable decreasing (50%) number of mules tested, respect to the previous years, 6 positive animals were confirmed.

Overall, during the period 2007-2011 a progressive decreasing tendency in the prevalence of positive samples was observed, with two points in which the flex was significant:

- 1. between 2008 and 2009 (Chi Squared Yates corrected; 52.7; p<0.00000)
- 2. between 2010 and 2011 (Chi Squared Yates corrected; 31.8; p<0.00000)

The relevant decrease of the prevalence observed during these two periods represents an important result of the application of the control measures implemented in the EIA outbreaks during the 5 years from which the surveillance plan became compulsory.



Figure 6 species : mule -prevalence of positive samples confirmed by CRAIE at regional level . year 2011 No mules were tested in Valle DqAosta and Sardegna regions in 2011.

Analyses of samples of Donkey

Table 3 and figure 7 report the results relative to samples of donkeys tested by the IIZZSS. Table 3 also reports the distribution of the samples per single region .

During the period 2007-2011, 52.390 samples of donkey were tested. 35 were confirmed as positive by CRAIE (0.07%).

[07			2010	200	20	10	20	44
	20	J07	20	008	20	109	20	010	20	711
REGION	TESTED	POSITIVES								
ABRUZZO	256	1	310	5	354	2	517	5	496	
BASILICATA	169		89		231	0	262		180	
CALABRIA	15		0		34	0	59		181	
CAMPANIA	101		124		111	0	177	1	165	
EMILIA-ROMAGNA	324	1	391	1	799	0	1486		783	
FRIULI-VENEZIA GIULIA	23		22		125	0	223		171	
LAZIO	1106		1039	3	1140	1	1573	1	1.712	1
LIGURIA	178		144		556	0	704	7	675	
LOMBARDIA	2162	1	0		1878	0	1523		667	
MARCHE	339		300		582	0	590		349	
MOLISE	28		23		51	0	55		84	
PIEMONTE	1548		965		2530	0	3357		2.028	
PUGLIA	231		169		467	0	421		475	0
SARDEGNA	150		17		85	0	151		231	
SICILIA			166		379	0	303	1	253	
TOSCANA	1724	1	1162		682	0	996		904	0
TRENTINO-ALTO ADIGE	246		14		113	0	149	1	103	
UMBRIA	218		372		809	1	1119		1.217	2
VALLE D'AOSTA	13		22		23	0	88		46	
VENETO	545	-	134		583	0	942	-	604	
TOTAL	9376	4	5463	9	11532	4	14695	16	11.324	3

 Table 3: donkey species - total number of blood samples tested and number of samples confirmed as positive by CRAIE during the period 2007-2011 per region of origin

**for the year 2007 no samples received from Sicily

Figure 7: species donkey . total number of blood samples tested and confirmed as positive by CRAIE during the period 2007-2011



Figure 8 shows the annual prevalence of positive blood samples confirmed by CRAIE during the 5-year period, 2007-2011.

Figure 8: species donkey - annual prevalence of positive blood samples confirmed by CRAIE during the period 2007-2011



Donkeys

It has to be noted that the number of samples tested each year is highly variable, reaching a minimum of executed analyses in 2008 (N=5.463) and a maximum in 2010 (N=14.695). This result suggests a disparity in the sampling intensity for donkeys through the 5 years of activity of the plan. This also suggests a probable lack in achieving the a 100% testing of the real population during one or more years of the surveillance plan.

The difference of the denominators (number of samples tested per year) could have determined distortions (bias) in the estimation of the annual prevalence of positive samples.

However, the low number of cases detected every year suggests a sporadic incidence of the infection in the species DONKEY

Risk comparison for EIA infection between horses and mules

During the 5-year period, 2007-2011 the cumulative prevalence of positive samples for mules was definitely higher (6.3%) than in horse (0.14%).

Using the data previously reported, the risk of contracting EIA in function of the species was estimated. This estimation was evaluated for each year using the Odds Ratio . O.R. calculation, by comparing the number of positive animals to the number of animals tested, both for horses and mules.

Table 4 reports the number of negative samples and the number of positive samples confirmed by CRAIE during the 5 years for mules and horses and the O.R. calculation (estimation of the risk for mules in comparison to horses). Figure 9 reports the O.R. for each year.

 Table 4: number of negative blood samples and number of positive blood samples confirmed by CRAIE during the 5 years for mules and horses, estimated prevalence and the O.R. calculation (CI 95%)

		sam	nples			CI	95%
		positives	negatives	prevalence %	O. R.	lower limit	higher limit
2007	horse	477	221.439	0,21	baseline		
2007	mule	182	1.590	10,27	53,14*	44,31	63,71
2008							
	horse	454	242.352	0,18	baseline		
	mule	277	2.357	10,52	62,64*	53,57	73,46
2000	horse	219	224.099	0,10	baseline		
2009	mule	106	2.096	7,38	51,75*	40,59	65,94
2010	horse	223	234.036	0,10	baseline		
2010	mule	130	2.654	4,81	51,41*	41,02	64,4
2011	horse	106	154.059	0,07	baseline		
2011	mule	41	2.346	1,72	25,4	17,7	36,5

Figure 9: mules and horses . 2007-2011 estimation of risk for EIA infection (Odds Ratio) - mules vs horses



Serology 2007-2011: Odds Ratio Mules vs Horses (samples)

For each year of the period 2007-2011, mules showed a significantly higher risk for EIA than horses. This higher risk was constant during the period 2007-2010, while decreased in 2011. Although the trend during the five years shows a significant decreasing pattern for both species (figure 2 and 8), the higher risk of infection for mules is constant during all the observation period.

It was therefore confirmed that a higher EIA prevalence is present in mules than in horses, therefore requiring the upholding of the control measures in existence. This appears necessary in order to achieve a further reduction of the incidence of the infection in this species, and avoid its possible risk of persistence. Tested mules, in fact, come from farms were they are used as draught animal power, usually managed in a rural environment with poor and generally non-existent biosecurity criteria. These conditions are optimal for the maintenance of a level of viral transmission capable of determining the persistence of reservoir.

Equids farms tested in 2011 and outbreak estimation

Table 5 reports the number of farms checked in 2011 in function of the species tested in the single premises. Therefore, the total number of farms tested, represents the effective number of farms controlled in 2011.

Table 5. Number	of farms with equids	checked per	region in 2	011 in f	unction of	the tested
species			_			

	N° of sin	gle-species	holdings	Ν			
REGION	Donkey	Horse	Mule	Donkey and horse	Mule and donkey	Other combinations	Total of holdings
ABRUZZO	110	2.080	54	92	99	11	2.446
BASILICATA	37	708	3	32	3	1	784
CALABRIA	21	515	4	29		0	569
CAMPANIA	20	1.100	1	38	5	1	1.165
EMILIA ROMAGNA	197	1.810	4	75	6	0	2.092
FRIULI-VENEZIA GIULIA	25	265		50	1	0	341
LAZIO	242	5.084	37	306	156	23	5.848
LIGURIA	199	804	9	102	33	11	1.158
LOMBARDIA	61	3.374	4	290	12	8	3.749
MARCHE	82	755	8	63	27	2	937
MOLISE	18	869	4	19	9	1	920
PIEMONTE	477	2.561	46	323	37	34	3.478
PUGLIA	34	1.276	6	95	17	2	1.430
SARDEGNA	30	1.535		26		0	1.591
SICILIA	20	2.273	3	59	12	4	2.371
TOSCANA	169	2.132	5	168	21	4	2.499
TRENTINO-ALTO ADIGE	28	447	1	45		0	521
UMBRIA	203	2.526	12	231	66	13	3.051
VALLE D'AOSTA	21	56		8		1	86
VENETO	96	1.181		240	5	3	1.525
Total	2.090	31.351	201	2.291	509	119	36.561

Table 6 reports the number of farms checked for EIA in 2011. The number was extrapolated from the 2011 surveillance plan database and refers to the farms with at least 1 equid checked in 2011, independently from the species.

The total number of farms with at least 1 confirmed positive equid in 2011 (prevalence) and the number of farms tested positive for the first time since 2007 (incidence) is also reported. For the counting of the farms with at least 1 confirmed positive equid, data from the confirmations carried out by CRAIE were considered. The incidence was calculated comparing the farms resulted positive in 2011 with those present in the CRAIE historic archive of outbreaks.

REGION	N°of tested holdings	N° of confirmed positive holdings	N°of new positive holdings	% prevalence	% incidence
ABRUZZO	2.446	37	25	1,51	1,02
BASILICATA*	784	1	1	0,13	0,13
CALABRIA	569	2	2	0,35	0,35
CAMPANIA*	1.165	10	10	0,86	0,86
EMILIA ROMAGNA*	2.864	1	1	0,04	0,04
FRIULI-VENEZIA GIULIA	341	0	0	0,00	0,00
LAZIO	5.848	19	15	0,32	0,26
LIGURIA	1.158	0	0	0,00	0,00
LOMBARDIA*	3.749	2	2	0,05	0,05
MARCHE	937	1	0	0,11	0,00
MOLISE	920	6	5	0,65	0,54
PIEMONTE*	3.478	1	1	0,03	0,03
PUGLIA*	1.430	6	6	0,42	0,42
SARDEGNA	1.591	0	0	0,00	0,00
SICILIA	2.371	0	0	0,00	0,00
TOSCANA	2.499	5	4	0,20	0,16
TRENTINO-ALTO ADIGE	521	0	0	0,00	0,00
UMBRIA	3.051	11	10	0,36	0,33
VALLE D'AOSTA	86	0	0	0,00	0,00
VENETO*	1.525	4	4	0,26	0,26
Total	36.561	106	86	0,29	0,24

Table 6. Number of farms with equids checked in 2011 per region.

The table shows that about 80% of the positive farms, represent new outbreaks, and therefore incident outbreaks. The other 20% represent farms already testing positive in the past. For some regions (marked with asterisk*), all outbreaks represents new cases.

The presence of clusters of outbreaks appears clear in the central-southern regions: Abruzzo, Molise, Lazio, Umbria, Campania e Puglia.

Important are again the results obtained in Campania, where an important reduction of tested equids in 2011 respect the other years (table 1 vs. table 2) was observed, although the prevalence remained basically the same, with an incidence of 0.9% in the farms tested. These results need further epidemiological investigations, in particular to understand if the positive results obtained were referable to equids/farms enrolled for the first time in the surveillance system. To confirm such a result it is basically difficult because of the inefficiency of the equid identification system, but imply, also for other regions, the possibility of the existence of a portion of Í hiddenÎ population, not yet registered and tested. This population could represent a risk for the persistence of the infection on the territory.

Localization of the farms with at least 1 positive equid confirmed by CRAIE in 2011

Figure 10 descriptively reports the geographic distribution of the farms with at least 1 confirmed positive equid. To obtain such results, the CRAIE historic archives were used, integrated with the information concerning the outbreaks reported on the National Disease Alert System. Thanks to this integration it has been possible to obtain the incidence of the outbreaks for 2011.

Figure 10: localization of the farms with at least 1 positive equid confirmed by CRAIE in 2011 and/or notified on the National Disease Alert System. In red INCIDENT OUTBREAKS

http://195.45.99.82:900/img/AIE italiaG2011.jpg

In 2011, previous reports about a higher prevalence of positive samples and farms in the Central



(+++) and Southern (+) regions were confirmed. Figure 11 reports the detail of the situation of the Central Italian regions.

Figure 11: localization of the farms with at least 1 positive equid confirmed by CRAIE in 2011 and/or notified on National Disease Alert System. In red INCIDENT OUTBREAKS E Central Italy http://195.45.99.82:900/img/AIE_italia_centG2011.jpg



Table 7: distribution of the farms with at least one confirmed positive equid in 2011 - OUTBREAK PREVALENCE

	N° of single-species holdings N° of mixed holdings						
REGION	Donkey	Horse	Mule	Donkey and horse	Mule and donkey	Other combinations	Total
ABRUZZO		27	10			0	37
BASILICATA		1				0	1
CALABRIA		2				0	2
CAMPANIA		4	6			0	10
EMILIA ROMAGNA		1				0	1
FRIULI-VENEZIA GIULIA						0	0
LAZIO	1	13	3		2	0	19
LIGURIA						0	0
LOMBARDIA		2				0	2
MARCHE			1			0	1
MOLISE		5	1			0	6
PIEMONTE			1			0	1
PUGLIA		5	1			0	6
SARDEGNA						0	0
SICILIA						0	0
TOSCANA		4	1			0	5
TRENTINO-ALTO ADIGE						0	0
UMBRIA	1	6	4			0	11
VALLE D'AOSTA						0	0
VENETO		4				0	4
Total	2	74	28	0	2	0	106

Table 8: distribution of the farms with at least one confirmed positive equid in 2011 - OUTBREAK INCIDENCE

	N° of sing	le-species l	holdings	N°	N° of mixed holdings			
REGION	Donkey	Horse	Mule	Donkey and horse	Mule and donkey	Other combinations	Total	
ABRUZZO		20	5			0	25	
BASILICATA		1				0	1	
CALABRIA		2				0	2	
CAMPANIA		4	6			0	10	
EMILIA ROMAGNA		1				0	1	
FRIULI-VENEZIA GIULIA						0	0	
LAZIO	1	12			2	0	15	
LIGURIA						0	0	
LOMBARDIA		2				0	2	
MARCHE						0	0	
MOLISE		4	1			0	5	
PIEMONTE			1			0	1	
PUGLIA		5	1			0	6	
SARDEGNA						0	0	
SICILIA						0	0	
TOSCANA		4				0	4	
TRENTINO-ALTO ADIGE						0	0	
UMBRIA	1	6	3			0	10	
VALLE D'AOSTA						0	0	
VENETO		4				0	4	
Total	2	65	17	0	2	0	86	

Synthetic analysis of the problems of the national data flow system

The trends observed in the population of animals and farms checked in Italy in the period 2007-2011 show a progressive and constant decrease in occurrence of EIA prevalence, although clusters of infection are still confirmed in central-southern Italy. This picture suggests the efficacy of the control measures implemented, with a consequent decrease of the source of the infection, represented by the infected animals.

Also, considering that the mechanisms of transmission of the infection are rather inefficient (iatrogenic infection-mechanic vectors insects), the reduction of contagious sources is encouraging regarding the positive evolution of the epidemiological situation of EIA in Italy.

However, the strength in the application of the surveillance on the whole national population of equids is still the principal critical point.

In the previous report, regarding the period 2007-2010, the testing of the whole equid population, in harmony by the Regulation Act, 2006, was achieved only partially in 2007, and probably totally only in the two-year period, 2007-2008. Considering that the first testing started in 2007, it is reasonable to assume that the prevalence obtained for the first 2 years was constituted by % prevalence+of the sum of all previous positive samples. These would presumably represent animals never tested before, that would have acquired the infection in years previous to 2007.

In the same report, it was reasonably assumed that animals tested positive in the first two years would have been removed and therefore not tested again. The evaluation of the incidence in the second two-year period (2009-2010) was therefore interpreted as a better expression of the incidence of the infection, owing to 2 possible reasons:

- detection of positive equids tested negative in the past, but contracting the infection from 2008
- 2) detection of new cases due to newly enrolled animals .

The analysis of the 2011 data seems to confirm this last hypothesis.

Although the result of the incidence of new outbreaks for 2011 (year in which this analysis has been conducted for the first time) should be more objectively confirmed on the basis of the official archive of notified outbreaks received by the Ministry of Health in 2007, the data from the historic archive of CRAIE seem to confirm this trend.

In particular, most of these outbreaks, especially those occurred in the central-southern regions, would be identified with farms detaining equids, enrolled for the first time in the surveillance plan.

This hypothesis, although partially supported by information collected on the field, is of difficult evaluation because of the insufficient implementation of the individual identification system of equids and because of the inefficiency of the equine registry system.

It has to be recalled that in this regard, a first evaluation would be obtained from a more objective analysis of the intensity of the application of the surveillance on the population of equids farms present and registered in the single regions, in correlation with the number of farms checked during the year.

This represents an objective, in the analysis of the process, that CRAIE wants to carry out in 2012.