



ELSEVIER

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

Dourine Reemergence in Italy

Paolo Calistri DVM, Valeria Narcisi DVM, Marcello Atzeni DVM, Fabrizio De Massis DVM, Manuela Tittarelli, Maria Teresa Mercante, Enzo Ruggieri DVM, Massimo Scacchia DVM

Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy

ARTICLE INFO

Article history:

Received 28 March 2012

Received in revised form

10 May 2012

Accepted 22 May 2012

Available online xxx

Keywords:

Dourine

Surveillance

CFT

Case definition

ABSTRACT

In 2011, five outbreaks of dourine were originally reported in Italy. The outbreaks occurred in two regions of southern Italy. From June to December 2011, a surveillance plan has been carried out in Italy to estimate the prevalence and geographical distribution of dourine infection in the equine population. Finally, two other new outbreaks were reported, and seven outbreaks were confirmed. This article describes the results of the surveillance plan, also taking into consideration the prevalence of infection observed in the different types of equine premises.

© 2012 Elsevier Inc. All rights reserved.

1. Introduction

Dourine is a chronic disease of solipeds, transmitted directly from animal to animal during coitus [1]. The causal organism is *Trypanosoma equiperdum*, a protozoan parasite related morphologically and serologically to *Trypanosoma brucei*, *Trypanosoma rhodesiense*, *Trypanosoma evansi*, and *Trypanosoma gambiense* [2].

T. equiperdum is the only trypanosome that is not transmitted by an invertebrate vector. It is difficult to distinguish *T. equiperdum* microscopically from other members of the subgenus *Trypanozoon* (*T. evansi*, *T. brucei*). In particular, *T. equiperdum* and *T. evansi* cannot be differentiated on the basis of morphological criteria.

The disease is characterized by edematous lesions of the genitalia, involvement of nervous system, and progressive emaciation, and it is ultimately fatal in most cases. Typical cutaneous lesions, from which the disease derives its name "dourine," have been described as

circular elevated plaques of thickened skin ranging in size from 1 to 10 cm in diameter, resembling money or "douros" [3].

In infected animals, trypanosomes are present, in low numbers, in lymph nodes and in edematous fluids of the external genitalia, in the vaginal mucus, and in cutaneous plaques. They are usually undetectable in the blood, but may be found in the urethral or vaginal mucus collected from preputial or vaginal washings or scrapings 4 to 5 days after infection [4].

Humoral antibodies are present in infected animals, irrespectively of the presence of clinical signs. Many authors have evaluated a range of techniques for the serological diagnosis of dourine, concluding that the complement fixation test (CFT) and the indirect immunofluorescent assay (IFA) are the most reliable and appropriate methods for indirect diagnostic procedures [5,6]. The CFT is used to confirm clinical suspicions and to detect latent infection. This technique is recommended by the International Animal Health Code for international trade. However, uninfected equines, particularly donkeys and mules, may give inconsistent or nonspecific reactions to CFT owing to the anticomplementary effects of their sera [7].

The IFA is used as confirmatory test and to resolve inconclusive results obtained by CFT [4].

Corresponding author at: Paolo Calistri, DVM, National Reference Centre for veterinary epidemiology, programming, information and risk analysis (COVEPI-Operational Veterinary Centre for Epidemiology, Programming and Information). Istituto Zooprofilattico Sperimentale, dell'Abruzzo e del Molise "G. Caporale", Campo Boario, 64100 Teramo, Italy.
E-mail address: p.calistri@izs.it (P. Calistri).

After an epidemic that spread throughout Europe soon after World War II, dourine disappeared from western Europe [5]. It still occurs in most Asian countries, as well as in Africa, Russia, the Middle East, and South America [3].

In Europe, the occurrence of dourine has been reported in Greece (1960), France (1958), Spain (1955), Bulgaria (1953), Croatia (1952), Czech Republic (1952), Hungary (1952), Poland (1951), and Portugal (1925) [8].

In Italy, no case was recorded after 1950, until a clinical case was observed in a stallion of a private stud in the Abruzzo region in August 1975 [5]. After this outbreak, dourine was confirmed in the Lazio, Umbria, Marche, Emilia Romagna, and Sicilia regions [9]. Since 1980, no further autochthonous cases were recorded except for a single case reported in 1996 [10].

In Italy, all male horses or donkeys used for reproduction, both in private or public premises, must be authorized by both the Agriculture and Veterinary authorities. Before being authorized for the stud, all male horses/donkeys must be tested for dourine, equine viral arteritis, glanders, equine infectious anemia, equine encephalitis, contagious equine metritis, and equine rhinopneumonitis [11].

In 2011, between May 23 and June 16, five epidemiologically linked outbreaks of dourine were confirmed in the Sicilia and Campania regions. In all outbreaks, cases were confirmed by CFT and IFA. The index case was a Friesian stallion on a farm in the Catania province, Sicilia region, which tested positive by CFT (1:640) and IFA ($\geq 1:640$) for dourine after serological controls for the authorization for stud. The stallion showed negative polymerase chain reaction (PCR) results. Trace-back investigations performed on the index case identified a CFT-positive (1:2560), IFA-positive ($\geq 1:640$), but PCR negative clinically affected mare in another premise of the Catania province. The mare had contacts with the stallion since March 2011 [12]. Further investigations permitted identification of two suspected Friesian stallions one CFT-positive (1:40), IFA-positive (1:80), and PCR-negative and one CFT-positive (1:5) IFA-negative ($< 1:80$), and PCR-negative Friesian stallions in a premise of the Napoli province (Campania region) and six further cases in two premises where these two stallions came from, both in the Napoli province: one CFT-positive (1:160), IFA-positive (1:160), and PCR-negative case in Casoria municipality and five CFT-positive (1:640, 1:320, 1:640, 1:1280, 1:160), IFA-positive ($\geq 1:640$, 1:160, 1:320, $\geq 1:640$, 1:160), and PCR-negative cases in Nola municipality.

Therefore, the Italian national veterinary authority decided to establish a national surveillance plan to achieve a reliable picture of dourine prevalence, and to promptly identify any further source of infection [13,14].

The aim of this study is to describe the methods and the results of the surveillance plan carried out from June to December 2011 in Italy, and to present a of the epidemiological situation of the disease.

2. Materials and Methods

2.1. Tested Animals

On June 10, 2011, the Italian veterinary authority decided to serologically test all stallions authorized for the 2011 mating season and all adult (> 2 years) male and

Table 1

Percentage of the farms tested for dourine in Italy (year 2011)

Region	Farms		
	Present*	Tested	%
Abruzzo	4.588	34	0.74%
Basilicata	2.849	494	17.34%
Calabria	1.890	259	13.70%
Campania	5.923	706	11.92%
Emilia Romagna	8.066	1077	13.35%
Friuli Venezia Giulia	1.043	33	3.16%
Lazio	13.143	891	6.78%
Liguria	3.652	64	1.75%
Lombardia	14.634	644	4.40%
Marche	3.813	357	9.36%
Molise	1.638	457	27.90%
Piemonte	9.781	399	4.08%
Puglia	5.650	1134	20.07%
Sardegna	5.990	224	3.74%
Sicilia	12.543	1292	10.30%
Toscana	7.333	539	7.35%
Trentino Alto Adige	1.510	258	17.09%
Umbria	4.051	1062	26.22%
Valle d'Aosta	—	—	—
Veneto	9.375	509	5.43%
Total	117.472	10.433	8.88%

* Source of data: National animal register.

female horses in nine regions (Abruzzo, Basilicata, Calabria, Campania, Lazio, Molise, Puglia, Sicilia and Sardegna) of central and southern Italy. Samples were collected during control activity carried out in the context of the surveillance plan for equine infectious anemia [13]. On June 30, 2011, the provisions were extended to the whole Italy [14].

2.2. Sample Collection

Blood samples were collected in sterile Vacutainer tubes (BD Vacutainer®, Franklin Lakes, NJ) and sent to the National Reference Centre for Foreign Animal Diseases at the "G. Caporale" Institute of Teramo. All samples were tested for anti-*T equiperdum* antibodies.

2.3. Serological Tests

All sera were tested by complement fixation test according to the method described in the World

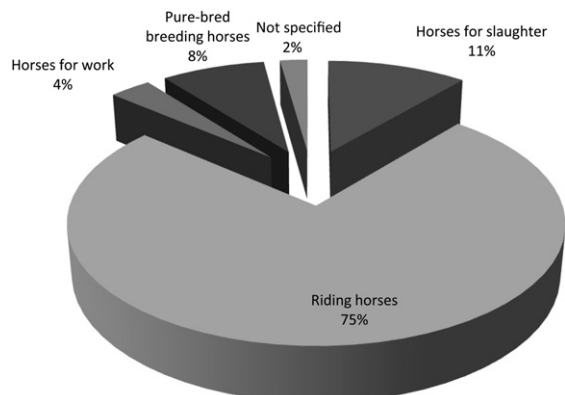


Fig. 1. Percentage of tested herds for dourine in Italy according to the category of husbandry (year 2011).

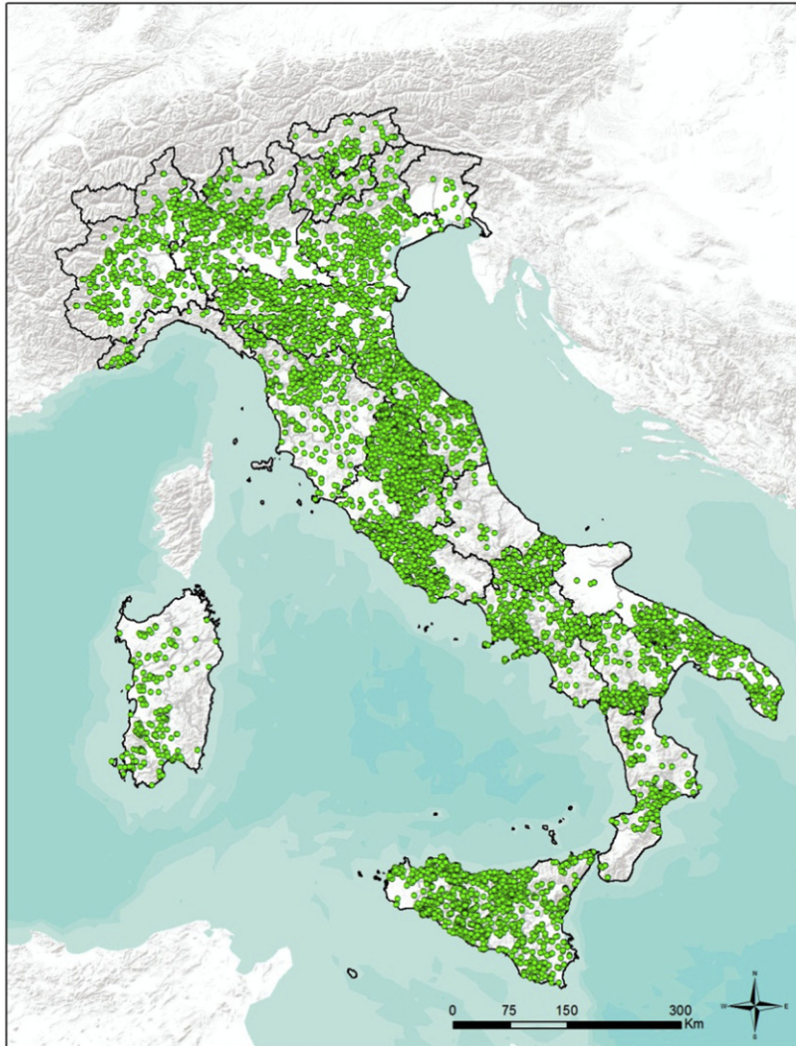


Fig. 2. Map showing the farm tested for dourine in Italy (year 2011).

Organisation for Animal Health (OIE): *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* chapter 2.5.3 [4]. A *T equiperdum* antigen deriving from Onderstepoort Veterinary Institute (*T.e.OVI*) was collected from rats infected intraperitoneally with infected blood diluted sodium citrate. The strain of *T.e.OVI* was cryopreserved in blood and adapted to rats. The rats were bled at the peak of parasitemia. The parasite concentration in the whole blood was estimated to be 1.8×10^8 trypanosomes/mL using Burker chamber, diluted in buffer, and freeze-dried in aliquots of 0.3 mL.

The sera were screened at a dilution of 1:5, and those showing >50% of fixation level at this dilution were considered positive.

Samples tested positive by CFT were then tested by IFA according to the methods described in the *OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* chapter 2.5.3 [4]. Sera diluted to $\geq 1:80$ showing strong fluorescence were considered positive.

2.4. Molecular Tests

Samples tested positive by CFT or IFA were tested by real-time PCR method, amplifying a highly repeated region specific for the *Trypanozoon* subgenus [15].

2.5. Case Definition

According to the surveillance plan, a suspect case was defined as an equine with positive serological test results, showing clinical signs compatible with dourine, or epidemiologically linked to a confirmed case of dourine.

In case of a serologically positive result, the following protocol was applied: all equines in the farms were clinically examined and serologically tested two times at 15 to 20 days of interval, and an accurate epidemiological investigation was performed.

A confirmed case of dourine was defined as an animal giving a positive result by CFT, IFA, or PCR, and (1) showing

clinical signs compatible with dourine, (2) showing an increase of serological CFT titer in two consecutive testing, or (3) epidemiologically linked with a confirmed case of dourine.

3. Results

From June to December 2011 in Italy, 10,433 of 117,472 (8.88%) existing farms have been tested. However, the percentage of tested farms of the existing ones was rather different among regions, varying from 0.74% in the Abruzzo region to 26.22% in the Umbria region. In relation to the type of husbandry, the majority of tested farms reared "riding horses" (75.10% of total tested farms), whereas 11.14% of total tested farms reared horses for meat production. (Table 1 and Figs. 1 and 2).

In total, 28,983 equines (89.23% horses and 10.77% donkeys) were tested for *T equiperdum* antibodies by CFT (Tables 2 and 3).

The overall number of CFT-positive animals was 140 horses (0.54%; 95% confidence levels, 0.46%-0.64%) and four donkeys (0.13%; 95% confidence levels, 0.05%-0.33%). However, applying the case definition, only 10 horses (0.03%; 95% confidence levels, 0.02%-0.07%) and no donkeys (95% confidence levels, 0.00%-0.12%) have been confirmed. Confirmed cases have been identified in two farms of the Campania and Puglia regions (Table 4 and Fig. 3). In particular, the first outbreak was confirmed on July 5, 2011, in the Caserta province, Campania region. One stallion showed positive CFT (+++1:160) and IFA (1:160) results; however, the PCR was not performed because the stallion died. One mare, with a major weight loss, showed positive CFT (1:5120), IFA (>1:640), and PCR results. The mare was moved, after Italian Ministry of Health authorization, to Istituto G. Caporale farm and died in October. Serological tests performed on all animals of the farm highlighted additional seven CFT-positive (1:5, 1:5, 1:10, 1:10, 1:10, 1:5,

1:10) and IFA- and PCR-negative animals, which are at present in a farm under restraint (Table 4). On November 25, 2011, a further outbreak was confirmed in the Bari province, Puglia region. The positive animal was a mare showing only slight weight loss and positive CFT (1:320), IFA (1:320), and PCR results; the other horses in the farm tested negative by CFT (Table 4). The animal was moved, after Italian Ministry of Health authorization, to Istituto G. Caporale farm to perform scientific studies.

Therefore, in Italy, seven dourine outbreaks were confirmed in total: three of them in farms of horses raised for meat production and the others in riding horse farms.

4. Discussion

Dourine was absent from the European Union for several years, and the last notified official case of dourine was in 1996 in Italy [10]. However, a serological suspect of dourine (not confirmed) was also reported in 1998 in the province of Padova, when a stallion tested positive by CFT performed according to the technique described by Caporale et al. [16].

Since then, and according to the Italian legislation, the serological control of animals was performed only in male horses and donkeys for the authorization for stud, and approximately 4000 stallions were tested in Italy every year for dourine, with no positive results [17].

In 2011, the reoccurrence of the disease in Italy led to the confirmation of seven outbreaks. Five epidemiologically linked outbreaks of dourine were identified in the Sicilia and Campania regions owing to back-trace investigations.

The main features such as prevalence, age, reproductive activity, and relationship between the affected animals evidenced from these outbreaks confirmed that the infection is transmitted directly from animal to animal during coitus. This supports that the disease was caused by *T equiperdum*, and not *T evansi* that is transmitted by mechanical vectors.

Table 2

Results of serological tests for *Trypanosoma equiperdum* antibodies by complement fixation test (CFT) in horses in Italy (June-December 2011)

Region	Horses								
	Tested	Positive	%	L.c.i. (95%)	U.c.i. (95%)	Confirmed	%	L.c.i. (95%)	U.c.i. (95%)
Abruzzo	101	2	0.02	0.61%	6.90%	0	0.00%	0.00%	2.89%
Basilicata	983	7	0.01	0.35%	1.46%	0	0.00%	0.00%	0.30%
Calabria	745	2	0.00	0.08%	0.97%	0	0.00%	0.00%	0.40%
Campania	2,793	24	0.01	0.58%	1.28%	8	0.29%	0.15%	0.56%
Emilia Romagna	3,088	19	0.01	0.40%	0.96%	0	0.00%	0.00%	0.10%
Friuli Venezia Giulia	62	0	0.00	0.00%	4.64%	0	0.00%	0.00%	4.64%
Lazio	2,261	8	0.00	0.18%	0.70%	0	0.00%	0.00%	0.13%
Liguria	47	0	0.00	0.00%	6.05%	0	0.00%	0.00%	6.05%
Lombardia	1,541	5	0.00	0.14%	0.76%	0	0.00%	0.00%	0.19%
Marche	809	2	0.00	0.08%	0.89%	0	0.00%	0.00%	0.37%
Molise	1,132	5	0.00	0.19%	1.03%	0	0.00%	0.00%	0.26%
Piemonte	478	2	0.00	0.13%	1.50%	0	0.00%	0.00%	0.62%
Puglia	2,801	19	0.01	0.44%	1.06%	1	0.04%	0.01%	0.20%
Sardegna	548	1	0.00	0.04%	1.01%	0	0.00%	0.00%	0.54%
Sicilia	3,294	20	0.01	0.39%	0.94%	0	0.00%	0.00%	0.09%
Toscana	1,435	4	0.00	0.11%	0.71%	0	0.00%	0.00%	0.21%
Trentino Alto Adige	776	2	0.00	0.08%	0.93%	0	0.00%	0.00%	0.38%
Umbria	2,215	13	0.01	0.35%	1.00%	0	0.00%	0.00%	0.14%
Veneto	753	5	0.01	0.29%	1.54%	0	0.00%	0.00%	0.40%
Total	25,862	140	0.54	0.46%	0.64%	9	0.03%	0.02%	0.07%

L.c.i., lower confidence interval; U.c.i., upper confidence interval.

Table 3

Result of serological test for *Trypanosoma equiperdum* antibodies by complement fixation test (CFT) in donkeys and other equines in Italy (June-December 2011)

Region	Donkeys and other equines								
	Tested	Positive	%	L.c.i. (95%)	U.c.i. (95%)	Confirmed	%	L.c.i. (95%)	U.c.i. (95%)
Abruzzo	21	0	0.00%	0.00%	12.73%	0	0.00%	0.00%	12.73%
Basilicata	62	0	0.00%	0.00%	464%	0	0.00%	0.00%	4.64%
Calabria	51	1	1.96%	0.47%	10.26%	0	0.00%	0.00%	5.60%
Campania	166	0	0.00%	0.00%	1.78%	0	0.00%	0.00%	1.78%
Emilia Romagna	456	2	0.44%	0.14%	1.57%	0	0.00%	0.00%	0.65%
Friuli Venezia Giulia	7	0	0.00%	0.00%	31.23%	0	0.00%	0.00%	31.23%
Lazio	322	0	0.00%	0.00%	0.92%	0	0.00%	0.00%	0.92%
Liguria	15	0	0.00%	0.00%	17.07%	0	0.00%	0.00%	17.07%
Lombardia	169	0	0.00%	0.00%	1.75%	0	0.00%	0.00%	1.75%
Marche	143	0	0.00%	0.00%	2.06%	0	0.00%	0.00%	2.06%
Molise	63	0	0.00%	0.00%	4.57%	0	0.00%	0.00%	4.57%
Piemonte	148	0	0.00%	0.00%	1.99%	0	0.00%	0.00%	1.99%
Puglia	283	0	0.00%	0.00%	1.05%	0	0.00%	0.00%	1.05%
Sardegna	66	0	0.00%	0.00%	4.37%	0	0.00%	0.00%	4.37%
Sicilia	566	1	0.18%	0.04%	0.98%	0	0.00%	0.00%	0.53%
Toscana	127	0	0.00%	0.00%	2.31%	0	0.00%	0.00%	2.31%
Trentino Alto Adige	11	0	0.00%	0.00%	22.09%	0	0.00%	0.00%	22.09%
Umbria	330	0	0.00%	0.00%	0.90%	0	0.00%	0.00%	0.90%
Veneto	115	0	0.00%	0.00%	2.55%	0	0.00%	0.00%	2.55%
Total	3,121	4	0.13%	0.05%	0.33%	0	0.00%	0.00%	0.12%

L.c.i., lower confidence interval; U.c.i., upper confidence interval.

After the first confirmed cases of disease, the Italian veterinary authorities had to face the problem of establishing a nationwide surveillance program. In particular, the performances of available serological methods, especially the specificity values of the CFT, suggested being prudent in the interpretation of monitoring results [4].

Subsequently, rather complex case definition and confirmation protocol were defined, coupling together serological and epidemiological findings. It is noteworthy that during surveillance activities, 140 horses tested positive using CFT, but only nine of them were confirmed as dourine cases. In all other cases, the epidemiological findings and the antibody titer trends excluded the presence of infection and attributed the positivity to aspecific reactions.

The results of the consequent surveillance activities performed from June to December 2011, with two more confirmed outbreaks in the Campania and Puglia regions, confirmed that the disease was apparently not widespread across the whole Italy. It was confined only in some southern Italian regions, although these results have to be

carefully interpreted in the light of the fact that the level of activities performed in some regions cannot be considered satisfactory (Table 1).

Because the surveillance plan for dourine mainly took benefit of sampling activities performed in the framework of the national control plan for equine infectious anemia [18], most of the tested animals belonged to "riding horse" farms, whereas a smaller number of farms with animals reared for meat production, normally excluded from equine infectious anemia control activity, was tested.

It is noteworthy that the surveillance activities did not detect the infection in any stallion for stud. On the contrary, the disease was detected in marketed horses, particularly those raised for meat production, or those belonging to owners allowing their animals to mate without any authorization and veterinary check.

Indeed, during surveillance activities, serious failures were found in the application of identification and registration rules for equines, which hampered the investigations for identifying epidemiologically related farms and potential sources of infection.

Table 4

Outbreaks of dourine notified in 2011 in Italy

Region	Province	Outbreak	Equidae			
			Present	Positive	Destination	With Symptom
Sicilia	Catania	Index case ^a	5	1	Isolated ^b	0
Sicilia	Catania	Epidemiologically linked to index case ^c	27	1	Slaughtered	1
Campania	Napoli	Epidemiologically linked to index case	8	2	1 Isolated ² 1 Slaughtered	0
Campania	Napoli	Epidemiologically linked to index case	3	1	Slaughtered	0
Campania	Napoli	Epidemiologically linked to index case	17	5	2 Isolated ² 3 Slaughtered	2
Campania	Caserta	Detected by serological controls ^d	59	9	2 Died 7 under distaint	1
Puglia	Bari	Detected by serological controls	49	1	Isolated ²	1
Total			168	20	/	5

^aThe initial case.

^bThe animal, after Italian Ministry of Health authorization, was moved to Istituto G. Caporale farm to perform scientific studies.

^cThe case was linked with the index case.

^dThe case was detected during surveillance plan activities.

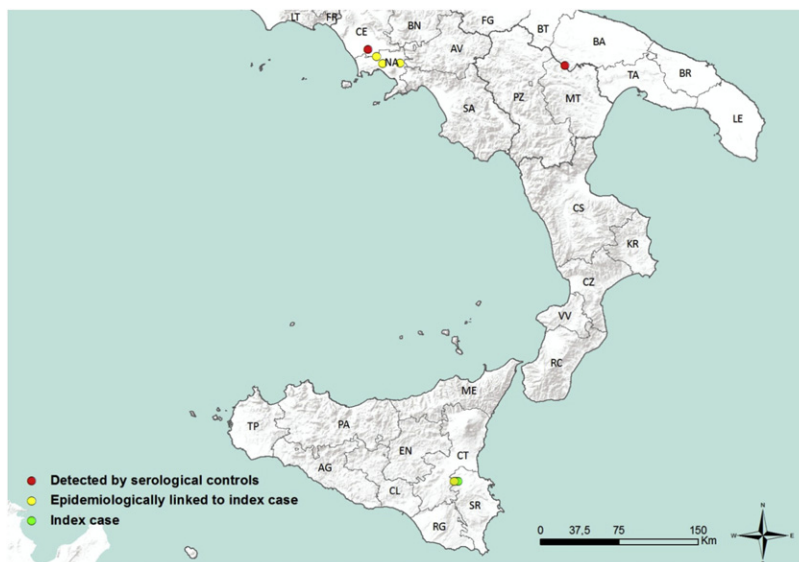


Fig. 3. Map showing geographic distribution of the outbreaks.

The uncontrolled mating and the infraction of existing veterinary provisions on animal movement control and animal identification were recognized as important factors in all outbreaks. In particular, the increasing request for horses for recreational use in the past years induced the proliferation of new nonprofessional horse breeders and traders, often buying and selling animals out of the official sanitary controls.

Recently, in the light of the results obtained from the surveillance activities, a modified surveillance plan has been issued by the Italian veterinary authority, to strengthen the veterinary controls on the movement of equines and to better investigate the health situation of horses for meat production. The surveillance activities are restricted only to some central and southern areas: Abruzzo, Basilicata, Calabria, Campania, Lazio, Molise, Puglia, and Sicilia regions.

Particularly, the new surveillance activities include the following: (1) the serological control of all marketed horses, 30 days before their exit from the farm of origin (excluding animals destined to sporting events, competitions, and exhibitions) and (2) the serological control at the abattoir of all animals showing clinical signs compatible with dourine and of those with irregularities in the accompanying documents.

In conclusion, the results of the surveillance activities highlighted the importance of strengthening veterinary control actions, especially in animals reared for meat production in the central and southern regions. The correct application of the existing identification and registration rules for equines proved also as an essential factor for an effective monitoring of health status of horse populations as well as for a better control of animal movements. In relation to the latter aspect, Italian veterinary authorities decided to obligatorily foresee the compilation by the veterinary services of an electronic form for each movement of horses. The results of premovement serological tests are also recorded in the electronic form permitting the cross-check between the local veterinary services

respectively competent to the farm of origin and destination. In the expectation of Italian veterinary authority, the application of this animal control system would be able to detect possible residual sources of infection, limiting the possibility of infection spread.

References

- [1] Wassal DA, Gregory RJF, Phipps LP. Comparative evaluation of enzyme-linked immunosorbent assay (ELISA) for the serodiagnosis of dourine. *Vet Parasitol* 1991;39:233-9.
- [2] De-Hua L, Hashimi H, Lun ZR, Ayala FJ, Lukes J. *Trypanosoma equiperdum* and *Trypanosoma evansi* are petite mutants of *T. brucei*. *Proc Natl Acad Sci USA* 2008;105:1999-2004.
- [3] Claes F, Buscher P, Touratier L, Godderis BM. *Trypanosoma equiperdum*: master of disguise or historical mistake? *Trends Parasitol* 2005;21:316-21.
- [4] OIE—World Organisation for Animal Health. Chapter 2.5.3: Dourine. In: *Terrestrial manual of diagnostic tests and vaccines for terrestrial animals*. Paris, France: OIE; 2008. p. 845-51.
- [5] Caporale V, Battelli G, Semproni G. Epidemiology of dourine in equine population of the Abruzzi Region. *Zbl Vet Med B* 1980;27:489-98.
- [6] Williamson CC, Stoltz WH, Mattheus A, Schiele CJ. An investigation into alternative methods for the serodiagnosis of dourine. *Onderstepoort J Vet Res* 1988;55:117-9.
- [7] Clausen PH, Chuluun S, Sodnomdarjaa R, Greiner M, Noeckler K, Staak C, et al. A field study to estimate the prevalence of *Trypanosoma equiperdum* in Mongolian horses. *Vet Parasitol* 2003;115:9-18.
- [8] OIE—World Organisation for Animal Health. World Animal Health Information Database (WAHID). Available at: <http://web.oie.int/wahis/public.php?page=home>. Accessed January 25, 2012.
- [9] Bellani L, Papalia S, Caporale EP. Report on dourine epidemiological surveillance and research in Italy. In expert consultation on research on trypanosomiasis. Food and Agriculture Organization; 1980. pp 16-18.
- [10] OIE Office International des Epizooties HandiSTATUS. Available at: <http://www.oie.int/hs2/report.asp>. Accessed December 20, 2011.
- [11] Italian Ministry of Health, General Direction for Animal Health and Veterinary Medicines. Law 15 January 1991, n. 30: Disciplina della riproduzione animale. G.U.R.I. January 29, 1991. Available at: <http://www.izs.it/IZS/Engine/RAServePG.php/P/332010010400/M/331510010400>.
- [12] Scacchia M, Cammà C, Di Francesco G, Di Provvido A, Giunta R, Luciani M, et al. A clinical case of dourine in an outbreak in Italy. *Vet Ital* 2011;47:473-5.
- [13] Italian Ministry of Health, General Direction for Animal Health and Veterinary Medicines. Note 10 June 2011 n. 10634: Focolai di Morbo Coitale Maligno. Misure suppletive sanitarie di controllo. Available

- at: <http://www.izs.it/IZS/Engine/RAServePG.php/P/332010010400/M/331510010400>.
- [14] Italian Ministry of Health, General Direction for Animal Health and Veterinary Medicines. Note 30 June 2011 n. 11872: Focolai di Morbo Coitale Maligno. Aggiornamento. Available at: <http://www.izs.it/IZS/Engine/RAServePG.php/P/332010010400/M/331510010400>.
- [15] Becker S, Franco JR, Simarro PP, Stich A, Abel PM, Steverding D. Real-time PCR for detection of *Trypanosoma brucei* in human blood samples. *Diagn Microbiol Infect Dis* 2004;50:193-9.
- [16] Caporale VP, Nannini D, Semproni G. Prove comparative tra la deviazione del complemento in provetta, deviazione del complemento miniaturizzata ed immunoelettro-osmoforesi nella diagnosi sierologica del morbo coitale maligno. *Atti Soc Ital Sci Vet* 1977;31:787-9.
- [17] Caporale V. Dourine in Italy. Standing Committee on the Food Chain and Animal Health (SCFCAH) Section: animal health & animal welfare. Presented on July 05-06, 2011. Accessed February 13, 2012.
- [18] Italian Ministry of Health, General Direction for Animal Health and Veterinary Medicines. 2010. Ordinanza 8 agosto 2010. Piano di sorveglianza nazionale per l'anemia infettiva degli equidi. G. U. R.I. September 18, 2010:219. Available at: <http://www.izs.it/IZS/Engine/RAServePG.php/P/344710010400/M/343410010300>.