



BENV

National Veterinary Epidemiological Bulletin



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EDITORIAL

The BENV as a tool for disseminating information

Dear all,

The new issue of Benv is now on line aiming at keeping you updated on the epidemiological situation of the main animal diseases in Italy and neighboring countries.

I would like to inform you that the space dedicated to the **Events** is now updated more often: you can consult this page monthly to search for training courses or conferences of your interest. In this context let me inform you that we are organizing an International Conference on “**Risk Analysis as a tool for the control of Animal Diseases and Zoonoses in the Mediterranean Basin**”. It will be held in Teramo from 5th to 7th of November. You all are kindly invited!

Regarding the epidemiological situation, our **News** have already reported the onset of outbreaks of highly pathogenic avian influenza in our country. The outbreaks were notified in the north of Italy, in Emilia Romagna region. In the section dedicated to “**In these months**” you can find a paper describing the epidemiological situation and the control measures adopted to face the emergency.

In the first days of September another disease has been reported in our country: after the outbreaks in Sardinia, the **serotype I of the bluetongue virus** has been reported for the first time in Sicily. More details on this and other outbreaks of animal diseases can be found in the sections of **Maps** and **Hand on Data** of Benv.

In the **Around us** section, you can find an article on **Bioinformatics**, a new topic that will be described also in the next two numbers of the bulletin. This new science has become nowadays an indispensable tool for epidemiological investigation and for the study of the distribution of animal disease.

In the same section you can find a short article about **MERS (Middle East Respiratory Syndrome)**, a disease caused by a new *Coronavirus* causing severe human cases in Middle East, in particular in Saudi Arabia. Further studies are underway to assess the zoonotic risk and the potential role of animal species in the infection transmission and the maintenance of the virus in the environment.

Another virus has been reported in Italy more than one year ago, the **new variant of the rabbit hemorrhagic disease**, named France from the place of its discover. In the section **In this months** you can find an article on this disease that is responsible of serious economic and healthy damages in the sector of rabbit production in Italy.

Finally, I report to you the establishment of the **National Reference Centre for veterinary urban hygiene and non-epidemic emergencies**, to which the bulletin dedicates a special paper in the section **In this months**. This reference

Centre is devoted to various aspects of non-epidemic emergencies and urban hygiene, from stray dogs and cats management to the implementation of information systems for the identification of pet animals, to predisposition of monitoring and surveillance plans on zoonoses and other non-epidemic animal disease. Therefore, the reference Centre has a transversal role in the field of veterinary epidemiology, being an important tool for daily activities in public and private health sector.

To contact us write to benv@izs.it , otherwise fill in the form on line in Suggestion section.

I wish you a pleasant reading.

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Simona Iannetti

National Reference Centre for Veterinary Epidemiology, Planning, Information and Risk Analysis (COVEPI)



IN THESE MONTHS

The main events of epidemiological interest in the last months in Italy and in the European Union

RHDV2: a new rabbit haemorrhagic disease virus

Origin and diffusion of RHD

Rabbit Haemorrhagic Disease (RHD) is one of the two rabbit diseases listed by OIE [1]. It is a **highly infectious and often fatal viral disease** of the European rabbit firstly described in China in 1984 [2]. It was then reported in East Asia (1985-86), continental Europe (1987-90), Central America (Mexico 1988, Cuba 1994), North Africa and Ocean Island (1988-89), Middle East (1990) and UK (1992). In Australia and New Zealand it was introduced as a biocontrol agent (1995). Cases have been reported in domestic rabbits in other parts of the world including the Americas i.e. USA and South America (2004-2005) and Canada (2011). On the whole, it has been detected in over 40 Countries and it is actually considered endemic in the Mediterranean Basin, i.e. where the natural host (*Oryctolagus cuniculus*) is commonly present in the wildlife.

Aetiology

The RHD virus (RHDV) is classified in the *lagovirus* genus of the family Caliciviridae [3,4]. RHDV is a non-enveloped single stranded RNA virus, showing a relevant genetic and antigenic variability, due to its high diffusion and environmental resistance. Yet, only one serotype is known, all the identified strains being antigenically and genetically similar. Starting from 1996 some variants were identified, including the main antigenic variant named RHDVa and some HA-neg strains. However, all these strains caused the same disease with identical epidemiological patterns. As they were antigenically similar, cross-protection was demonstrated with vaccines prepared with the classical RHDV strains. Nowadays, the RHDV isolates are grouped in five phylogenetically distinct clusters, showing both spatial and temporal trends [5,6,7].

RHDV2 characteristics

The firm belief of the existence of just one RHDV serotype was challenged in 2010 when a new RHDV-related virus with a discrete genetic profile (the amino acid identity was 89.2% with RHDV-RHDVa, and 76.7% with EBHS) and distinct phenotypic traits, was identified in France: first in wild animals and then also in industrial rabbit farms [8,9]. Further studies showed it also had a specific antigenic profile. The average mortality amounted to 20% in experimentally-infected rabbits, which is significantly less than that of classical RHDV [9]. Other typical features of RHDV2 include: the capacity to cause death also in RHDV-vaccinated does, in non-vaccinated fattening rabbits and in lactating rabbits from 15dd of age onwards, an incubation and course

of disease slightly longer than classical RHD. The antigenicity of this new virus was studied with a panel of monoclonal antibodies: the distinct antigenic profile of RHDV2 accounted for the partial and shorter cross-protection induced by RHD vaccines. The available data suggest that RHDV2 is not a variant of RHDV but a newly emerged virus [9], very close to a differently ranked serotype. Another distinctive phenotypic property of RHDV2 concerns its host susceptibility. In fact, classical RHDV can cause disease only in the European rabbit, and no other American, Asian or European lagomorphs of the genus *Romerolagus*, *Lepus* and *Sylvilagus* have been shown to be naturally or experimentally susceptible to it. Vice versa, the identification of RHDV2 as the cause in Sardinia of several outbreaks of acute hepatitis in rabbits and Cape hares (*Lepus capensis mediterraneus*) is the first report of a *lagovirus* causing fatal hepatitis in both rabbits and hares [10]. RHDV2 is now spreading in Europe as demonstrated by its detection in both Italy [11,12] and Spain during 2011 [13,14], and Malta and Portugal in 2012.

Detection and diffusion of RHDV2 in Italy

The intensive efforts that were put in the vaccination campaigns and other supporting The first outbreak caused by RHDV2 was diagnosed on June 2011 on a rabbit commercial farm in **Udine** province. It was followed within a month by a second, epidemiologically linked outbreak on another farm, located 10 km farther, run by the same owner. The RHDV2 strain identified in both outbreaks was the same (RHDVUd2011), and it was similar but not 100% identical to the strains originally detected in France [9]. The levels of mortality (10% in fattening rabbits and 20% among breeders) were lower than those observed in classical RHD (80-90%) and in French RHDV2-driven outbreaks in farmed rabbits (up to 60%). The lower mortality can be probably related to the intrinsic lower virulence of such a virus, but also to the prompt emergency vaccination of all the animals in the two farms and to the application of strict measures of direct prophylaxis. This intervention strategy was conducive to an effective disease control, with mortality levels back to the previous range after 3 weeks in the first farm. This strategy could totally prevent mortality and losses on the second farm, where the disease was not observed and the virus was detected in few rabbit carcasses sampled during the epidemiological survey.

Following the first RHDV2 detection, the Italian Ministry of Health immediately issued a note (prot. 21949 dated 6/8/2011), giving indications and recommendations to limit the diffusion and to control the disease. In particular, suspected cases of RHD had to be reported to local vet authorities, and organ samples or carcasses had to be delivered to the Istituti Zooprofilattici Sperimentali (I.I.ZZ.SS.) for laboratory diagnosis, genetic and antigenic typing of virus strains detected. At the same time, the National Reference Centre (NRC) for viral diseases of lagomorphs at IZSLER developed and updated the diagnostic methods for a reliable differential diagnosis: i) for virus detection, by means of a sandwich ELISA based on specific mAbs produced against RHDV2, and of distinct PCR protocols for RHDV and RHDV2; ii) for the detection of specific antibodies after disease or vaccination, and application of disease control measures, by means of RHDV and RHDV2-specific competition ELISAs and anti-isotype ELISAs (IgG, IgM and IgA), to detect early, natural infections as well as maternally-derived antibody. A concrete feedback of this approach and of the increased surveillance by institutional bodies was the delivery by IZS Sardinia, at the beginning 2012, of several samples from both rabbits (wild and farmed) and hares (in Sardinia there is a local species of cape hare, *Lepus capensis var mediterraneus*). All these samples were collected during 2011, examined and found positive for *lagovirus* using the RHDV/EBHSV ELISA kit distributed by IZSLER. As mentioned above, the typing and characterization of these positive cases enabled us to identify RHDV2 in both rabbits and cape hares [10]. This was true just of those samples taken between October and December 2011, whereas in samples taken previously only the classical RHDV was identified. Interestingly, the typical hare lagovirus (European Brown Hare Syndrome virus – EBHSV) has never been detected in Sardinia, nor in cape hares, nor in the few imported brown hares.

Few months later (January 2012) the third RHDV2 case was registered on a small rural farm in **Trento**. In this case, apart from a high mortality (60%) the particular feature was the contemporary detection of mortality due to the same virus in several wild rabbits living nearby and having direct contact with the domestic rabbits housed in external open-air fenced holdings. Considering the epidemiological importance of the

involvement of wild animals, which may lead to a much higher diffusion of the disease, the Ministry of Health decided to notify this outbreak to OIE WAHID, as already done in the case of the first outbreak.

Thereafter, more RHDV2 outbreaks were detected at the end of 2012. In November, two outbreaks were identified in **Lombardy** (North Italy): one in a big rabbit industrial farm in Monza-Brianza province (4.5% mortality) and the other in a very small and isolated rural farm in the mountains near the Como Lake (80% mortality). From that time onwards, the number of RHDV2 cases progressively increased showing a positive trend of diffusion all over the Country. The prevalence does not probably equal the levels of France and Spain, where at that time it already represented almost 100% of the RHD cases. However, such increasing numbers of cases were a concern for the industrial rabbit operations, that started to apply strict control and prevention measures, which may be summarized in two basic concepts: biosecurity and vaccination.

In **table I** is reported the number of suspect RHD cases for which organ samples were sent to the NRC at IZSLER for primary or confirmatory diagnosis during the last two years. The type of positive samples detected (classical RHDV, RHDVa or RHDV2) and the different origin and category of rabbits (rural/ backyard units, industrial farms or wild animals) are indicated. Two out of five RHDV2 outbreaks detected in the period 01/12/2012-31/3/2013 occurred in small backyard/rural units located in a small town near **Cremona** and in **Padua**, respectively, other two cases were in wild rabbits found dead in **Sicily** (Agrigento and Siracusa provinces), and the last case was diagnosed in an industrial farm in Cuneo province (25-35% mortality). During the same period, RHD outbreaks caused by the classical RHDV strain (3 cases) or by RHDVa (21 cases) were also registered. During the last period (1/4/2013 – 30/09/2013) six more RHDV2 outbreaks in different parts of Italy were notified. Of these, three were reported in April on an industrial farm in **Puglia** and in two rural units, respectively, the first near **Naples** and the others in **Varese** province. Two more outbreaks were detected in industrial farms, in May in Cuneo province and in September in Padua province, respectively. The last notified outbreak of this period occurred in July in a small rural farm in **Pistoia** province. Moreover, during this last period four cases due to classical RHDV (n.1) and RHDVa (n.3) were also observed in rural/backyard units.

Table I. Suspected RHD cases examined at IZSLER during the period 1/12/2012 - 30/9/2013

Period	N° cases	Origin	RHD pos.	RHDV	RHDVa (variante)	RHDV2 (Fra2010)
01/01/2012 30/11/2012	28*	26F, II, IS	14 50.0%	2 2 F	12 10F, II, IS	0*
16/06/2012 30/11/2012	24^	16F, 5I, 3S	7 29.2%	0	5 4F, IS	2 1F, II
01/12/2012 31/03/2013	21	9F, 10I, 2S	10 47.6%	1 II	4 3F, II	5 2F, II, 2S
01/04/2013 30/09/2013	31	28F, 3I	10 32.2%	1 1F	3 3F	6 3F, 3I
Total	104	79F, 19I, 6S	41 39.4%	4 3F, II	24 20F, 2I, 2S	13 6F, 5I, 2S

* not included the cases occurred respectively in Trento province and in Sardinia, but the two cases in Lombardy are included. W= wild rabbit; I = industrial; B = small rural/backyard unit)

Control measures adopted to control RHD outbreaks

Following the notification of a RHD outbreak, in compliance with Art. 1 of the Italian Codex for Veterinary Diseases (*Regolamento di Polizia Veterinaria – RPV*) and pending specific intervention measures, two different sets of indications and procedures were suggested depending on the type and characteristics of the holding i.e. rural/backyard unit vs industrial farm. Such measures should be adopted regardless of the virus type causing the disease (RHDV, RHDVa or RHDV2).

In the case of small rural or backyard units, it is advisable to humanely **kill the surviving rabbits** and to clean and disinfect the premises before repopulating with vaccinated rabbits after a suitable all-out period. In industrial rabbit farms, all the animals including young rabbits at weaning and those in the first half of the growing period should be immediately vaccinated. The others, close to the slaughtering age, may be transported to an abattoir in dedicated trucks and slaughtered as last batch of the day. The **emergency vaccination** should be performed, regardless of the level of protection achieved on farm by the previous vaccination, with a vaccine homologous to the RHDV strain identified during the outbreak. In fact, the use of heterologous vaccines (in particular classical RHD vaccines in the course of RHDV2 outbreaks) may exert a selective pressure on the viral strains, “pushing” the RHDV2 to further mutate its antigenicity and to definitively reach the status of “new serotype”. In such a case the heterologous vaccination will become totally ineffective. Since vaccines produced with RHDV2 strains are not yet commercially available in Italy, homologous vaccines can be obtained in the form of “autovaccines” produced and used in compliance with the Min. Decree n.287 of 17/3/1994. In addition to vaccination, the following direct prophylaxis actions should be adopted at the same time **to limit the diffusion of the infection**:

- I. registration of daily mortality for each category of animals;
- II. immediate removal of dead animals and stocking of frozen carcasses in dedicated refrigerated rooms. Then, the removal of infected materials should be performed periodically under security conditions with authorized trucks;
- III. live rabbits in exit are only intended for the abattoir as described above;
- IV. introduction of new breeders only if vaccinated twice;
- V. cleaning and disinfection of premises, equipment (e.g. cages and nests), plants (drinking and feeding) and instruments (e.g. for artificial insemination);
- VI. no exit of any material, equipment, tool or feed suspected of contamination;
- VII. permit of entry and exit to vehicles only after disinfection of the tyres and lower surface, and registration of any movement of vehicles and people;
- VIII. test for RHD, till the end of the outbreak, on samples randomly taken from dead animals showing suspected lesions or not clearly dead for other causes;
- IX. manure and sewage stocking for at least 45 days before removal and dispersal as fertilizer. The immediate removal is admitted only in the case of biogas production;
- X. pelts of regularly slaughtered rabbits may be used after treatments (drying or freezing plus inactivation with formaldehyde) according to the provisions of the **OIE Terrestrial Code**.

Moreover, in the territory where the outbreak occurred, it is highly recommended to apply similar rules and procedures also on all the farms which have a functional (same company) or geographic (high density farm areas) link with the infected farm. In particular:

- I. enhancement of all biosecurity measures and of hygienic and disinfection procedures normally applied;
- II. registration of daily mortality for any category of animals to keep a record of the health status;
- III. virological examination for RHD of any dead rabbit (particularly breeders) showing suspected lesions or not clearly referable to any other disease;
- IV. vaccination of breeders and restocking animals following the same vaccination scheme of infected farms;
- V. in the case of vaccination including also growing rabbits, the setup of a group of non-vaccinated rabbits as sentinels in order to trace the presence of specific antibodies and even the virus circulation through serological analyses;
- VI. the introduction of restocking animals only if younger than 7dd of age or of any age but vaccinated at least twice.

Finally, the RHD susceptibility of **wild lagomorphs**, i.e. not only wild rabbits but at least one *Lepus* species, imposes to strengthen the passive surveillance in the open areas. In particular, it should be emphasized to report to vet authorities any case of mortality among wild lagomorphs, including hares, by collecting the dead carcasses and delivering them to IZZSS for necropsy and laboratory analyses.

Conclusions

It cannot be excluded that the above data on the incidence of RHDV2 are just a rough representation of the real situation, since some outbreaks occurred especially in farmed rabbits from spring 2013 onwards, were not diagnosed and notified when the infection started to spread quickly. In fact, there is still a certain unjustified tendency to avoid reporting suspected cases, because it is believed that the compulsory notification entails too negative sanitary and economic consequences for the farmers. On the contrary, some positive experiences during the same period showed that it is possible to operate in a correct way for rapidly solving the outbreak without causing relevant economic losses and continuing the production cycles. This may be obtained through an **integrated approach**, enforcing the recommended measures and procedures for a correct management of outbreaks in strict co-operation with farmers, practitioners and the supervision of the State veterinary services. Therefore, farmers should not be frightened by a suspect of RHD. They ought to immediately **report the disease** to private and public vets, since only prompt and focused actions can limit the spread of the infection in the whole country.

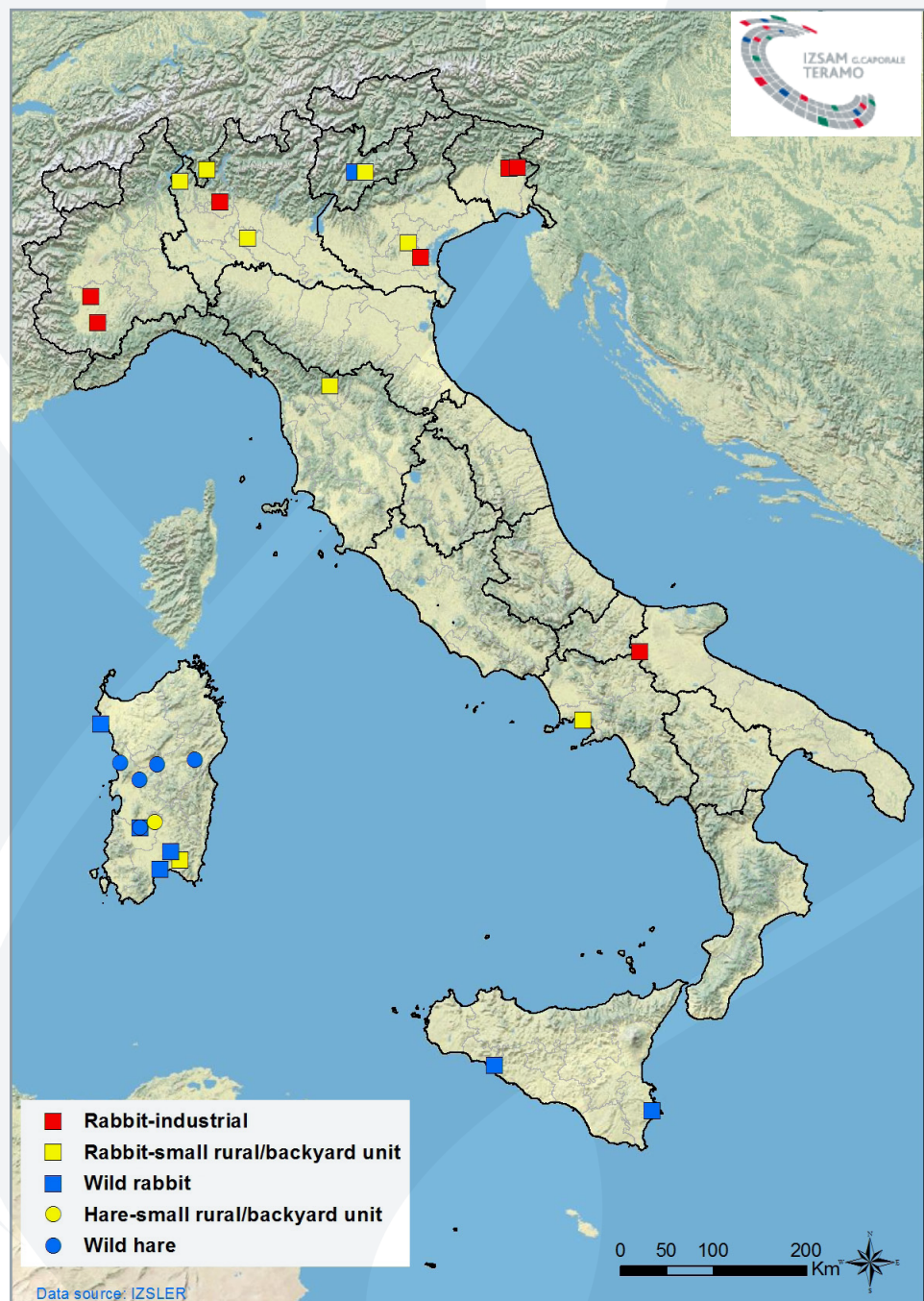


Figure 1. Distribution of RHDV2 in Italy from 2011 to 2013

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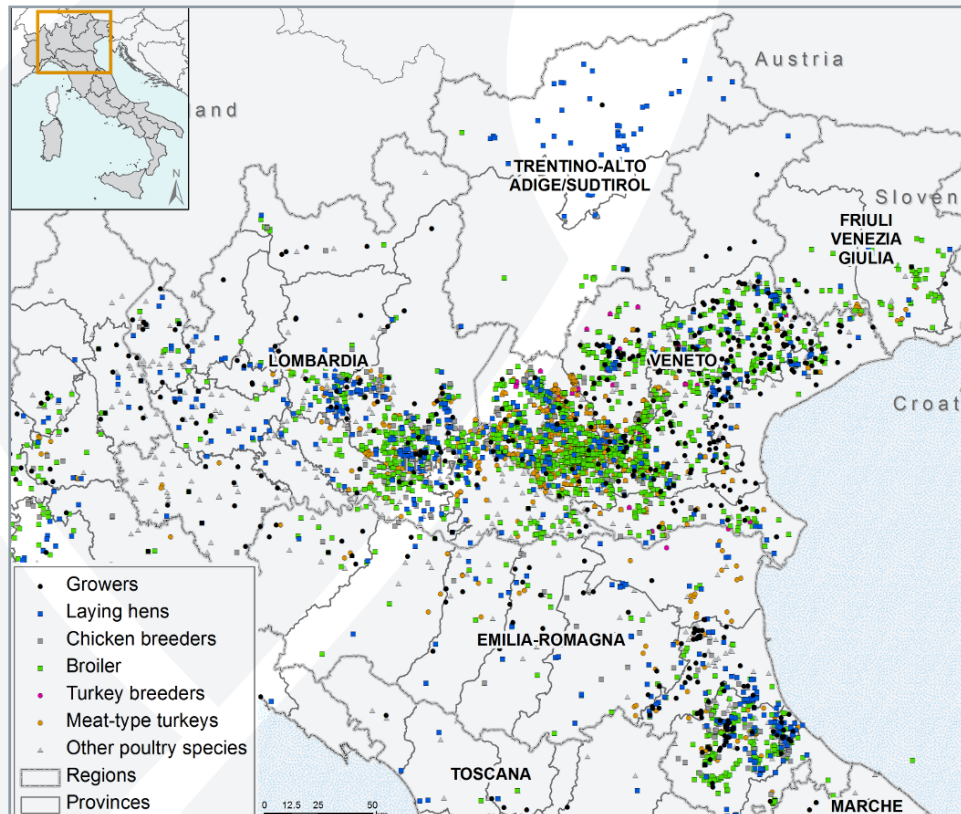
Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini"

H7N7 Highly Pathogenic Avian Influenza epidemic in Italy

Background

Since 1997 Italy has repeatedly experienced introductions of H5 and H7 Avian Influenza (AI) viruses both of the Low Pathogenicity (LP) and the Highly Pathogenic (HP) strains. Some of those introductions led to severe epidemics with huge economical losses to the whole Italian poultry sector (Capua *et al.*, 2008 Sartore *et al.*, 2010). The epidemic waves has mainly involved the **highly densely populated poultry areas (DPPA)** located in the northern Italian regions (**Figure 1**)

Figure 1.
Densely populated poultry areas
in north-eastern Italy



The Italian poultry production system

Since the late 1950, the Italian poultry industry has substantially grown. In particular, the system was developed through what is called “**vertical integration**”. Poultry companies own breeder farms, eggs selection premises and hatcheries, feed mills, and animals that are housed in farms belonging to the company or to other farmers. Moreover, to have a greater control on the poultry market, the companies schedule and manage also the phases related to the “secondary production”, i.e. slaughtering, poultry meat and table eggs transformation, and wholesale market.

The Italian poultry production system, similarly to other European Countries, is strongly driven by the presence of few integrating Companies which provide birds, feed, technology, and veterinary and technological support, and of large numbers of integrated farmers, who own the structures where birds are effectively reared.

From a geographical point of view, the development of the poultry production system is related to the orographic and environmental characteristics of the territory (with mild climate and limited temperature range being considered as most suitable to the intensive poultry farming). Moreover, the integrated productive structure require highly functional infrastructure (e.g. presence of motorways or railways) to guarantee a reliable connection between farms and premises that provide animals,

feed, and other support. Therefore most of the productive units are concentrated in well defined geographical areas, allowing reducing the expenses related to the management of the production system. The great majority of the Italian poultry farms is concentrated in the **north-eastern part of the country** (in the regions of Lombardy, Veneto and Emilia Romagna), in what is defined as a Densely Populated Poultry Area (DPPA). Due to the geographical position of the DPPA, on the migratory paths of wild waterfowls, AI viruses have been frequently introduced in the area. Nevertheless, the detailed knowledge of the territorial distribution of the poultry farms, the continuous monitoring of the circulating AI viruses, and the enforcement of strict control and biosecurity measures, allowed the early detection of the infected farms, therefore permitting the prompt implementation of measures to limit the uncontrolled spread of the disease in the Italian poultry population.

Re-emergence of HPAI in Italy

The last Highly Pathogenic Avian Influenza (HPAI) epidemic in Italy dated back to **1999-2000**. The epidemic was caused by a **H7N1 AI virus** that circulated as a LP strain for at least six months without any control measure, before mutating to a HP virus and spreading mainly to the industrial poultry sector in north-east Italy. The HPAI epidemic caused the death and/or culling of more than 16 million birds. Following the 1999-2000 epidemic, only LP viruses had been detected in industrial and rural farms (**Table I**), and in wild birds populations. Between 2007 and 2012 several LPAI cases were identified in different Italian regions, caused by viruses belonging to the H7 and H5 strains, mainly in rural farms. In 2012 H5N2 LP viruses were detected in a number of industrial meat-type turkey farms in the Lombardy region (Brescia province).

Table I. Avian Influenza viruses circulating in Italy in the 1997-2012 period

Year	Virus	Pathogenicity ¹	No. cases ²	Farm type
1997-1998	H5N2	HPAI	8	Industrial
1999	H7N1	LPAI	199	Industrial
1999-2000	H7N1	HPAI	413	Industrial
2000-2001	H7N1	LPAI	78	Industrial
2002-2003	H7N3	LPAI	388	Industrial
2004	H7N3	LPAI	28	Industrial
2005	H5N2	LPAI	15	Industrial
2007	H5N2	LPAI	2	Rural
	H7N3		17	
2009	H5N7	LPAI	4	Rural
	H7N3		4	
	H7		24	
2011	H5	LPAI	2	Rural
	H7		10	
2012	H5N2	LPAI	2	Rural
	H7		15	Rural and Industrial

¹ LPAI: Low Pathogenicity Avian Influenza; HPAI: Highly Pathogenic Avian Influenza

² From 2007, AI outbreaks were not aggregated in a limited area, but mostly spread throughout the whole National territory

In the second half of July 2013, an unexpected increase in the mortality rate was observed in a laying hens farm in **Ferrara province** (Emilia Romagna region, north-eastern Italy). Initially, the cause of this increase was attributed to the exceptionally high temperature experienced in that period. On 10 August 2013 the mortality rate was still increasing, and samples were sent on 13 August to the Istituto Zooprofilattico Sperimentale of Lombardy and Emilia Romagna in Forlì. On 14 August, the National

Reference Laboratory (NRL) for Avian Influenza and New Castle Disease confirmed the presence of a H7N7 HP virus in the premise. The infected farms reared **free-range laying hens** for the production of table eggs, and it was located in one of the largest **wetland** in Italy (in Ferrara province). The most reliable hypothesis, corroborated by laboratory data, is that the reared poultry came into contact with a **wild waterfowl** infected with a LP virus, which mutated to HP once introduced in the farm. The infected premise belonged to a large poultry company which has a wide number of poultry farms in several Italian regions (Piedmont, Lombardy, Veneto, Friuli Venezia Giulia, Emilia Romagna, Marche, Umbria, and Latium), with a total of 107 in which about 10 millions birds are reared.

In order to limit the risk of AI spread to the remaining part of the National territory, the Italian Ministry of Health, supported by the Istituto Zooprofilattico Sperimentale delle Venezie, enforced strict control measures including the ban of movements of birds and poultry products from Emilia Romagna to other regions. During the scheduled controls, on 19 August 2013, the virus was identified in another laying hen farm, belonging to the same poultry company in Bologna province. The second outbreak was confirmed by the NRL on 21 August 2013. Afterwards, other three industrial farms resulted infected (**Table 2, Figure 2**): two were epidemiologically related to the previous cases (same poultry company), while the third involved a meat-type turkey farm of a different company but located in strict proximity to previous outbreaks.

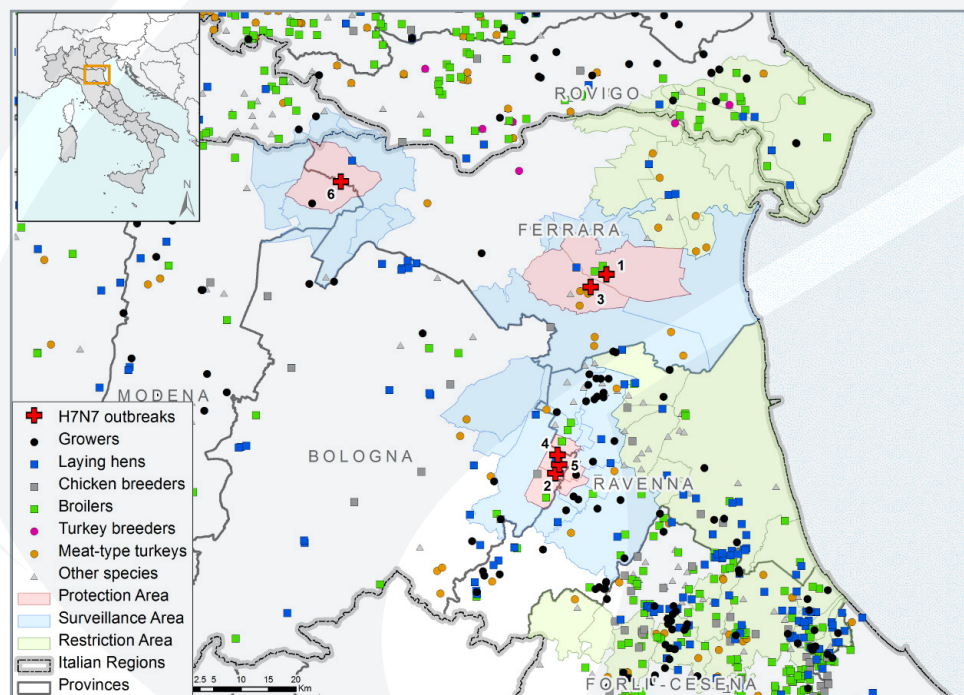
Table 2. Details on the H7N7 HPAI outbreaks occurred in Italy between 27 August and 5 September 2013

Outbreak ID	Province	Species	No. Birds	Confirmation date	Depopulation date ¹
1	Ferrara	Laying hens	128.000	14/08/2013	27/08/2013
2	Bologna	Laying hens	584.900	21/08/2013	08/09/2013
3	Ferrara	Meat-type turkeys	19.850	23/08/2013	27/08/2013
4	Bologna	Laying hens	121.705	28/08/2013	08/09/2013
5	Bologna	Pullets	98.200	04/09/2013	08/09/2013
6	Ferrara	Backyard farm	6	05/09/2013	06/09/2013

¹ Conclusion of the depopulation activities

Detailed epidemiological enquiries allowed to identify other farms that had contacts with the outbreaks, through eggs or feed transportation, or use of the same personnel.

Figure 2. Distribution of H7N7 HPAI outbreaks in the Emilia Romagna region; Protection, Surveillance, and Restriction areas are also reported. Outbreaks are labeled according to the ID reported in Table 2



A laying hens farm in the Rovigo province (municipality: Occhiobello), belonging to the same poultry company as the first outbreaks, was considered suspect of infection and, as for the Italian Law 218/88, the Italian Ministry of Health, in accordance with the Istituto Zooprofilattico Sperimentale delle Venezie, ordered that the birds were pre-emptively slaughtered.

An increased mortality rate was reported in the last week of August in a **backyard laying hens farm**. A carcass was submitted for analysis on 2 September 2013, and the confirmation of positivity to a H7N7 HP virus was given by the NRL on 5 September. Following this event, mandatory serological and virological controls were enforced on dealers and growers with a bi-weekly timeframe, both in the Emilia Romagna region and in the entire DPPA.

As provided in the Italian and EU regulations, all of the infected farms were subjected to depopulation, and destruction of poultry products. The depopulation activities led to the culling of about 1 million birds (**Table 2**). Up to 8 September 2013 all of the activities have been concluded (**Table 2**).

Due to the zoonotic risk, all of the personnel working in the infected farms were regularly checked for symptoms related to a possible H7N7 AI infection. Three humans cases were identified in personnel belonging to the crew charged with depopulating the first two outbreaks. All of the cases showed conjunctivitis, and in only in one patient influenza-like symptoms were present. No inter-human transmission has been detected.

Phylogenetical analyses detected a strong correlation of the H7N7 isolated virus with H7 LP viruses isolated between 2009 and 2013 in wild birds in Belgium, Poland, Czech Republic and Italy, and with H7 LP viruses sporadically isolated in reared poultry in 2010-2012 in northern Europe (The Netherlands and Germany) (97.5-98.4% homology for the HA gene, and 97.4-99.1% homology for NA gene). Moreover, the HA gene sequence derived from the virus isolated in the first outbreak resulted genetically similar to the HA gene sequence from a virus identified in a wild birds (*Anas crecca*) collected in southern Italy and submitted to the NRL in March 2013.

At present markers related to the adaptation of the virus to domestic poultry have not been identified, and there are no mutations associated to an increased virulence, or to a host species change.

Control Measures

Following the confirmation of the first 2013 H7N7 HPAI outbreak in Italy, on 14 August 2013, a series of control measures were implemented on the territory of Emilia Romagna region, to eradicate the disease in the shortest period, as provided in the Council Directive 2005/94/EC. The adopted measures included the ban on movements of living birds and table eggs in the region, and in a complete and defined functional separation between Emilia Romagna and the other Italian regions and EU Countries. All of the laying hens farms belonging to the poultry company involved in the first outbreak were subjected to weekly mandatory submission of serological and virological samples to the Istituto Zooprofilattico Sperimentale delle Venezie. Moreover, these farms need to transmit productivity and mortality data. Similar controls were enforced also in contact farms, as for the Clause 7 (2) of the Council Directive 2005/94/EC. Similarly, the laying hens farms located in the DPPA had to be tested every three weeks both serologically and virologically. On a National level, mandatory virological and serological tests have to be performed on pullets and laying hens before loading for transportation. Moreover, also a ban on introduction of table egg in reception facilities, to be transformed in egg-products, located in other farms. Additional biosecurity measures are also expected in industrial poultry farms.

As provided for in the Clause 16 (4) 2005/94/EC, a further restriction zone was defined external to the protection and surveillance areas (**Figure 2**), located where the greater risk of HPAI spread is expected. Laying hens, meat-type turkeys and breeders farms in the restriction zone are subject to virological and serological tests with a three-weekly timeframe.

In order to limit a further spread of the disease, **depopulation measures** were also applied to healthy poultry farms in the protection zone. On 25 September 2013,

with the conclusion of all of the control measures provided by the Italian Ministry of Health, and due to the positive epidemiological situation, most of the restriction measures were revoked.

Reference Legislation

European Legislation

- **2013/453/UE**: Commission implementing decision of 11 September 2013 amending Implementing Decision 2013/443/EU concerning certain protective measures in relation to highly pathogenic avian influenza of the subtype H7N7 in Italy
- **2013/443/UE**: Commission Implementing Decision of 27 August 2013 concerning certain protective measures in relation to highly pathogenic avian influenza of subtype H7N7 in Italy including the establishment of further restricted zones and repealing Implementing Decision 2013/439/EU
- **2006/437/CE**: Commission Decision of 4 August 2006 approving a Diagnostic Manual for avian influenza as provided for in Council Directive 2005/94/EC
- **2006/135/CE**: Commission Decision of 22 February 2006 concerning certain protection measures in relation to highly pathogenic avian influenza in poultry in the Community
- **Council Directive 2005/94/EC** of 20 December 2005 on Community measures for the control of avian influenza and repealing Directive 92/40/EEC.

Italian legislation

- **DGSA 17175-P-06/09/2013**. Avian Influenza - Executive Ruling containing further control and eradication measures to limit the Avian Influenza virus spread, including the implementation of a further restriction zone
- **DGSA 17181-P-06/09/2013**. Guidelines for the actualization of dispensation to the ban on movements from areas subjected to restriction measures related to HPAI outbreaks
- **DGSA 16501-P-29/08/2013**. Avian Influenza - containing further control and eradication measures to limit the Avian Influenza virus spread, including the implementation of a further restriction zone and repealing the Executive Ruling prot. 16208-P of 21 August 2013.

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The National Reference Centre for Urban Hygiene and Non-Epidemic Emergencies and its role in Veterinary Public Health

In recent years the need to coordinate and strengthen the intervention measures in veterinary public health has become more and more increasing both in the field of veterinary urban hygiene and in that of non-epidemic emergencies. The Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" (IZSAM) has built a solid experience in the management of epidemic emergencies, thanks to which forecasting models, methods for prevention, monitoring and control have been implemented, as well as operating procedures and evaluation instruments able to manage also non-epidemic emergencies.

Therefore, with the Decree of 19th march 2013 (published on the Italian official Gazette of 16th August 2013), the Italian Ministry of Health acknowledged the competences of IZSAM, establishing in Teramo the National Reference Centre for Urban Hygiene and non-epidemic emergencies.

This reference centre has the following tasks:

- to create a structured and permanent system of contacts in each one of the Istituti Zooprofilattici Sperimentali (IZSS);
- to provide the Italian Ministry of Health with assistance for planning and implementing surveillance, monitoring and control programmes of stray dogs and cats as well as in developing protocols and operative procedures;
- to define plans for surveillance and control of non-desired organisms of public health interest;
- in the field of veterinary public health, to draw up emergency plans and related operative procedures as far as animal health and welfare and food safety are concerned;
- to put in place every other useful activity in the field of urban hygiene and non-epidemic emergencies.

The Veterinary Urban Hygiene (VUH) is a branch of **veterinary public health**. Its first official definition goes back to 1977 at the WHO Expert consultation on "Veterinary public health and factory farms, animals in urban areas, and chemical residues in products of **animal origin**". **On that occasion**, the risk for public health linked to the presence of animals in urban areas was discussed the first time. Subsequently (1999), the VUH has been defined as "the activity dealing with those health aspects associated with human-animal-environment relationships in urban areas".

Over the last years, the number of animals living in urban areas has significantly increased. This has concerned not only the number of dogs and cats, but also the number of exotic species, such as turtles, reptiles, etc., as well as of synantropic animals such as pigeons, squirrels, starlings, jackdaws, mice, rats, foxes, seagulls, etc., which have chosen urban areas as their preferred habitat. These species can play an important role in the epidemiology of **zoonoses occurring in urban areas**. Our companion animals, after coming into contact with synantropic animals, can carry pathogens able to cross host barriers and affect humans.

Other problems related to the presence of animals in urban areas can be environmental fecalization, allergies, nuisance due to emanation of noise or odors, damage to objects, animals and people.

New aspects have been **then** added to the traditional problems. The VUH problems worsen in case of **environmental emergencies** such as natural (earthquakes, avalanches, flooding, volcanic eruptions etc.) or human induced (wars, chemical or radioactive pollution etc.). In this situations animals need to be rescued, cared for, managed from the health and welfare point of view. Furthermore, in emergency conditions, those who no longer have a home, or cannot longer use it, are generally housed in campsites, where there is a big concentration of people in a minimum space.

The difficulties to maintain an adequate environmental and personal hygiene, together with overcrowding and the lack of adequate hygienic tools, can lead to proliferation of vermin that can aggravate the existing conditions of discomfort and can also be responsible for the onset and propagation of outbreaks of transmissible diseases.

In the earthquake that involved L'Aquila and surrounding towns on April 6th, 2009, the Italian Civil Protection Agency appointed the IZSAM to coordinate supporting activities in the veterinary fields. The IZSAM, in cooperation with veterinarians of the local health units and private veterinarians, set up an organizational structure with the aim to guarantee the daily health activities and to face all requests related to sanitary aspects of veterinary interest.

All these topics need a strong **holistic-and epidemiologic approach** and a multidisciplinary and transversal collaboration.

The national reference Centre for Urban Hygiene and non-epidemic emergencies is placed in this context, with the aim of developing operating models and technologically advanced tools to face the effects of emergencies and quickly restore the previous situation. Among the tasks of the reference Centre, there is also the provision of **training programs** in the field of urban hygiene addressed to operators of local governments, to veterinarians of the local health units and to private veterinarians and volunteers. These training programs are foreseen to respond to the ever-changing and constantly increasing demand for veterinary training at national and international level. In the management of a health system, actually, the presence of trained personnel in its sectors is a pillar for the achievement of the objectives of the system itself.

Currently, the IZSAM is also involved at international level, in a research project of the Seventh Framework Programme dedicated to zoonoses transmissible to humans and to production animals and pets. The competence of the Institute - which in this project provides leadership in the field of epidemiology and risk analysis as well as in the field of knowledge management - is indeed supported as well by an extensive **network of collaborations** that includes all major stakeholders, national authorities, research centers, universities, non-governmental organizations, representatives of the world of production and of industry.

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HAND ON DATA

10th October 2013

Number of outbreaks reported to SIMAN in the I, II, III trimester 2013

Disease	Januray	February	March	April	May	June	July	August	September	Total outbreaks
African swine fever	1	3	6	6	45	32	19	1		113
American foulbrood of honey bees			2	2	12	12	9	8	4	49
Bluetongue	11	2		2			80	1563	1602	3260
Bovine leucosis	1	2	5	2		4	1	1		16
Bovine tuberculosis	44	33	47	64	54	74	40	11	22	389
Bovine viral diarrhoea							1	1		2
Brucellosis of cattle, buffalo, sheep, goats and pigs	69	53	74	93	93	78	66	24	33	583
Caprine arthritis/encephalitis	2	7	3	3	1		1			17
Contagious agalactia	7		8	12	16	20	16	1		80
Contagious bovine mastitis		1			3	1				5
Crayfish plague (<i>Aphanomyces astaci</i>)							1	1		2
Equine infectious anaemia	1		2	2	5	3	2	1		16
Erysipelas	2	1			1					4
European foulbrood of honey bees				2	3	2	2			9
High patogenicity Avian influenza in poultry								4	2	6
Infectious hematopoietic necrosis					1	1				2
Koi herpesvirus disease								1		1
Leptospirosis	2	1	3		1	1	3		2	13
Listeriosis	1									1
Low patogenicity Avian influenza in poultry		1								1
Lung adenomatosis									1	1
Non-typhoidal avian salmonellosis		1		5	3	3	2	2		16
Paratuberculosis				1						1
Pasteurellosis of cattle, buffalo, sheep, goats and pigs							1			1
Rabbit haemorrhagic disease	2		1	3	2				1	9
Salmonellosis (<i>S. abortusovis</i>)	11	2	1						1	15
Salmonellosis of animals				1			1			2
Schmallenberg disease	3		2	4	1				1	11
Scrapie		6	3	4	6	2	1		1	23
Swine vesicular disease			1							1
Viral haemorrhagic septicaemia (VHS)							1			1
West Nile Disease							25	42	6	73

Number of outbreaks reported by Regions to SIMAN in the I, II, III trimester 2013

Region	Disease	Trimester			Total outbreaks
		I	II	III	
ABRUZZO	American foulbrood of honey bees		1		1
	Bovine tuberculosis		1		1
	Brucellosis of cattle, buffalo, sheep, goats and pigs	1	1		2
	Crayfish plague (<i>Aphanomyces astaci</i>)			1	1
	Leptospirosis			1	1
APULIA	Bovine leucosis	2			2
	Bovine tuberculosis		3	3	6
	Brucellosis of cattle, buffalo, sheep, goats and pigs	7	16	12	35
	Equine infectious anaemia		1		1
	Non-typhoidal avian salmonellosis		2	1	3
	Rabbit haemorrhagic disease		1		1
BASILICATA	Salmonellosis of animals		1		1
	Bovine leucosis		1		1
	Bovine tuberculosis		1	2	3
	Brucellosis of cattle, buffalo, sheep, goats and pigs	10	3	5	18
	Schmallenberg disease			1	1
BOLZANO	Scrapie	1			1
	American foulbrood of honey bees		4	2	6
	Leptospirosis		1	2	3
	Schmallenberg disease	2	1		3
CALABRIA	Viral haemorrhagic septicaemia (VHS)			1	1
	Bovine tuberculosis	6	7	11	24
	Brucellosis of cattle, buffalo, sheep, goats and pigs	23	46	10	79
	Equine infectious anaemia		2		2
	Scrapie		1		1
CAMPANIA	Swine vesicular disease	1			1
	Bluetongue			1	1
	Bovine leucosis	1	3		4
	Bovine tuberculosis	8	17	2	27
	Brucellosis of cattle, buffalo, sheep, goats and pigs	29	53	16	98
	Equine infectious anaemia	1			1
	Listeriosis	1			1
EMILIA ROMAGNA	Rabbit haemorrhagic disease		1		1
	American foulbrood of honey bees	1	1	5	7
	Erysipelas	1	1		2
	High pathogenicity Avian influenza in poultry			6	6
	Leptospirosis			1	1
	Low pathogenicity Avian influenza in poultry	1			1
	Non-typhoidal avian salmonellosis	1			1
	Scrapie	1			1
FRIULI VENEZIA GIULIA	West Nile Disease			44	44
	Leptospirosis	2			2
	Non-typhoidal avian salmonellosis			1	1
	Scrapie		1		1

Region	Disease	Trimester			Total outbreaks
		I	II	III	
LAZIO	American foulbrood of honey bees		1	2	3
	Bluetongue			1	1
	Bovine leucosis	1	1	2	4
	Bovine tuberculosis	2	1	1	4
	Bovine viral diarrhoea			1	1
	Brucellosis of cattle, buffalo, sheep, goats and pigs			1	1
	Equine infectious anaemia	2	4	2	8
	Non-typhoidal avian salmonellosis		2		2
	Salmonellosis (S. abortusovis)	2			2
	Salmonellosis of animals			1	1
Scrapie		1		1	
LIGURIA	Schmallengberg disease		1		1
LOMBARDY	Bovine tuberculosis	1	3		4
	European foulbrood of honey bees		1	1	2
	Non-typhoidal avian salmonellosis		1	1	2
	Rabbit haemorrhagic disease		1		1
	Scrapie		1		1
	West Nile Disease			5	5
MARCHE	Bovine tuberculosis		1		1
	Equine infectious anaemia		1		1
	Non-typhoidal avian salmonellosis		4	1	5
	Rabbit haemorrhagic disease		1		1
	Scrapie	1	1		2
MOLISE	Bovine tuberculosis	1	1	1	3
	Brucellosis of cattle, buffalo, sheep, goats and pigs		1		1
PIEDMONT	Bovine tuberculosis	1	2		3
	Rabbit haemorrhagic disease	2	1		3
	Schmallengberg disease	1	3		4
SARDINIA	African swine fever	10	83	20	113
	Bluetongue	13	2	3205	3220
	Bovine tuberculosis	1			1
	Brucellosis of cattle, buffalo, sheep, goats and pigs		1		1
	Caprine arthritis/encephalitis	12	4	1	17
	Contagious agalactia	13	43	17	73
	European foulbrood of honey bees		1		1
	Leptospirosis	4		1	5
	Lung adenomatosis			1	1
	Paratuberculosis		1		1
	Pasteurellosis of cattle, buffalo, sheep, goats and pigs			1	1
	Rabbit haemorrhagic disease	1			1
	Salmonellosis (S. abortusovis)	12		1	13
	Schmallengberg disease	2			2
Scrapie	6	6	2	14	
SICILY	Bluetongue			40	40
	Bovine leucosis	3	1		4
	Bovine tuberculosis	102	148	52	302
	Brucellosis of cattle, buffalo, sheep, goats and pigs	126	143	78	347
	Contagious agalactia		1		1
	Equine infectious anaemia			1	1

Region	Disease	Trimester			Total outbreaks
		I	II	III	
TUSCANY	Bluetongue			2	2
	Bovine tuberculosis		1		1
	Bovine viral diarrhoea			1	1
	Brucellosis of cattle, buffalo, sheep, goats and pigs			1	1
	Koi herpesvirus disease			1	1
UMBRIA	Bovine leucosis	1			1
	Bovine tuberculosis		1	1	2
	Crayfish plague (<i>Aphanomyces astaci</i>)			1	1
	Equine infectious anaemia		2		2
	Erysipelas	2			2
	Non-typhoidal avian salmonellosis		1		1
	Scrapie		1		1
VENETO	American foulbrood of honey bees		2	2	4
	Bovine tuberculosis	2	5		7
	Infectious hematopoietic necrosis		1		1
	Non-typhoidal avian salmonellosis		1		1
	Rabbit haemorrhagic disease			1	1
	West Nile Disease			25	25

Animals involved in outbreaks reported to SIMAN in the I, II, III trimester 2013

Disease name	Animals involved	No. Of animal in the holding	No. Of diseased animals	No. Of died animals	No. Of culled animals	No. Of destroyed animas
African swine fever	Suidae	1713	331	246	1181	1409
American foulbrood of honey bees	Bees	677	132	21	72	84
Bluetongue	Ruminants	1042632	148504	35711	10	34038
Bovine leucosis	Ruminants	821	33	1	16	0
Bovine tuberculosis	Ruminants	26800	2940	9	1848	44
Bovine viral diarrhoea	Ruminants	494	14	13	0	0
Brucellosis of cattle, buffalo, sheep, goats and pigs	Ruminants	66137	6412	13	4758	13
Brucellosis of cattle, buffalo, sheep, goats and pigs	Suidae	30	5	0	0	0
Caprine arthritis/encephalitis	Ruminants	2836	171	0	0	0
Contagious agalactia	Ruminants	26838	3056	17	13	5
Contagious bovine mastitis	Ruminants	44	8	0	1	0
Crayfish plague (Aphanomyces astaci)	Acquatic animals		2	1	0	1
Equine infectious anaemia	Equidae	276	27	0	8	0
Erysipelas	Suidae	14946	7	3	4	6
European foulbrood of honey bees	Bees	204	46	10	79	109
High patogenicity Avian influenza in poultry	Birds	19850	19850	1300	19850	19850
High patogenicity Avian influenza in poultry	Poultry	932808	132013	4376	932807	932808
Infectious hematopoietic necrosis	Acquatic animals		9150	9150	0	9150
Koi herpesvirus disease	Acquatic animals		100	100	0	100
Leptospirosis	Domestic carnivores	7	3	3	0	2
	Equidae	13	1	0	0	0
	Ruminants	308	19	1	0	0
	Suidae	83	3	2	0	2
Listeriosis	Ruminants	83	1	1	0	0
Low patogenicity Avian influenza in poultry	Birds	222	0	1	182	183
Low patogenicity Avian influenza in poultry	Poultry	118	4	0	118	118
Lung adenomatosis	Ruminants	207	1	1	0	1
Non-typhoidal avian salmonellosis	Birds	9727	7527	0	0	0
Non-typhoidal avian salmonellosis	Poultry	859784	327199	27	79526	40026
Paratuberculosis	Ruminants	210	34	0	0	0
Pasteurellosis of cattle, buffalo, sheep, goats and pigs	Ruminants	207	1	1	0	0
Rabbit haemorrhagic disease	Lagomorphs	110887	5686	5750	9	495
Salmonellosis (S. abortusovis)	Ruminants	5560	183	1	0	0
Salmonellosis of animals	Ruminants	609	22	8	0	8
Schmallenberg disease	Ruminants	1136	22	4	0	1
Scrapie	Ruminants	6958	67	39	962	577
Swine vesicular disease	Suidae	4	4	0	4	4
Viral haemorrhagic septicaemia (VHS)	Acquatic animals		30	30	0	30
	Birds	34	34	0	0	0
	Equidae	224	29	0	1	1
West Nile Disease	Insects	112	112	0	0	0



A LOOK AT THE MAPS

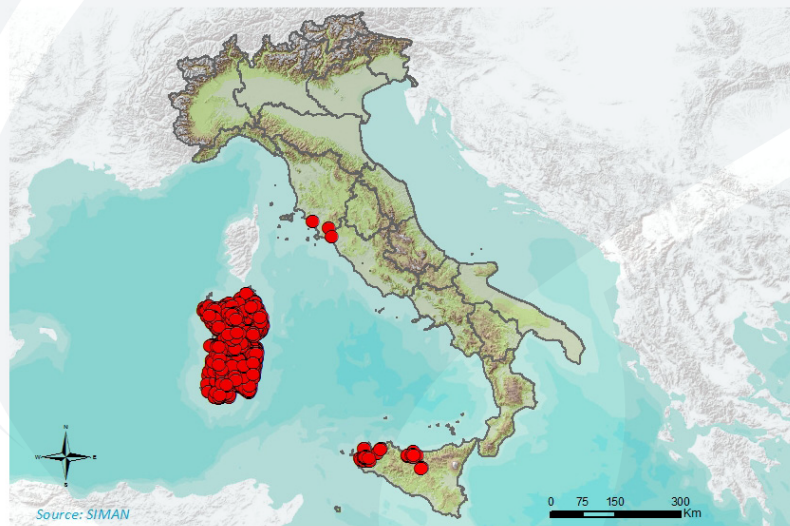
The geographical distribution of the main animal diseases reported to SIMAN in the I, II, III trimester 2013

Equine infectious anaemia



Geographical distribution of the outbreaks

Bluetongue



Geographical distribution of the outbreaks

Avian influenza, high patogenicity



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Geographical distribution of the outbreaks

Avian influenza, low patogenicity



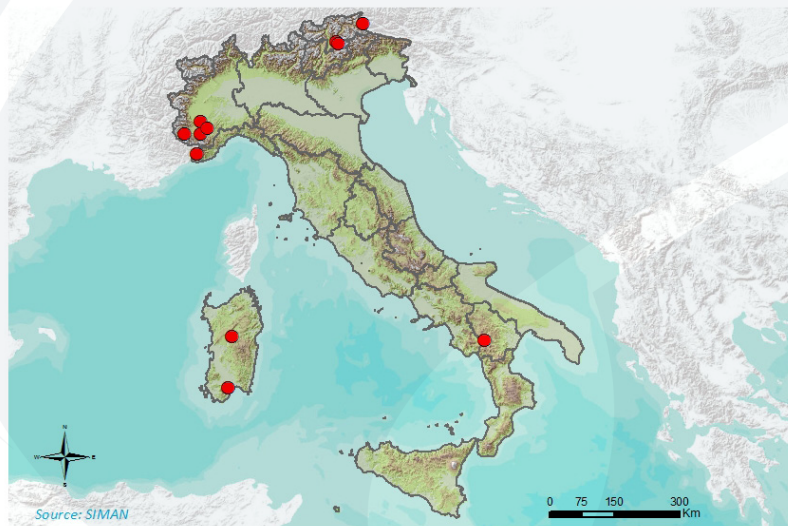
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Geographical distribution of the outbreaks

African swine fever



Geographical distribution of the outbreaks

Schmallenberg



Geographical distribution of the outbreaks

Swine vesicular disease



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Geographical distribution of the outbreaks

West Nile disease



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Geographical distribution of the outbreaks





AROUND US

The main events of epidemiological interest in the last months in the European Union and in the neighbour countries

Bioinformatics: a link between life sciences and computer science

In recent years a new science has rapidly developed: the Bioinformatics. It is an **interdisciplinary science** that involves biology, chemistry, mathematics, physics and informatics. Bioinformatics applies computational techniques to develop software and tools to generate useful biological knowledge.

The beginning of bioinformatics can be traced back to the end of Seventies and its evolution has been developed in parallel to the development of DNA sequencing technology.

The first attempt to define the concept of bioinformatics was in 1978 by Hogeweg & Hesper. They described bioinformatics as the study of information processes in biological systems. Currently, the term bioinformatics is often associated to the application of computational techniques for the analysis of bio-molecular data and it constitutes a new way of describing biological phenomena from a numerical and statistical point of view.

The sequences of biological molecules are constituted by character strings: the nucleotides of nucleic acids and the amino acids of the protein. This assumption is fundamental in molecular biology because it defines the meaning of biological information. For example, the alphabet of nucleic acids consists of: adenine, guanine, cytosine and thymine or uracil (the nucleotides of DNA and RNA), while for proteins the alphabet is composed by 20 letters (the 20 natural amino acids).

The tools of this new science are both the databases of bio-sequences and the software for analyzing and comparing them. With the advent of high throughput technologies such as NGS (Next Generation Sequencing), bioinformatics has become essential to the biological sciences.

NGS technologies are used to obtain biological data in a very fast way. In fact, in biological databases the number of nucleotide and protein sequences reduplicate every 18 months.

For example, for the database GenBank, managed by the National Center for Biotechnology Information (NCBI), the sequences were 606 in 1982; to date, instead, they are over 165 million. The complete sequences of the genomes of 25000 prokaryotes, 4000 viruses and 3500 eukaryotic have been also deposited.

The sequencing of complete genomes is rapidly increasing, so the most important challenge today is the interpretations of data for understanding an organism completely.

Currently, there are several open source software that are used for many types of analysis. The bioinformatics analysis include the assembly of genomic sequences, the comparison between these for epidemiological studies, the study of single nucleotide polymorphism, the study of virulence genes and drug resistance, the phylogenetic

relationships between the organism. This bioinformatics tools are designed for used on the web. There are platforms that can be integrated and updated by users with new applications.

Biological databases can be divided into primary or specialized. The **primary databases** are databases that collect primary sequences of nucleic acids and proteins; they contain information and generic annotation to identify the species from which they originated sequences and their functions.

The main databases of nucleic acid sequences are:

1. **EMBL data library** (European database established in 1980 in the European Molecular Biology Laboratory of Heidelberg, Germany)
2. **GenBank** (the NCBI – National Center for Biotechnology Information, founded in 1982 in Bethesda, USA)
3. **DDBJ** (DNA Data Bank of Japan, founded in 1986 from National Institute of Genetics Mishima in Japan).

Among the three biological databases there is an **international agreement**. In fact, the daily updates in each database are automatically transferred to the other. In addition, the three databases must share and make publicly available all data contained, but differing only for the format in which they are released.

UNIPROT is a catalog of information on **protein sequences**. The UniProt consortium, was founded in 2002 between: **PIR** (Protein Information Resource) was established by NBRF - National Biomedical Research Foundation – at Georgetown University Medical Center in Washington USA and developed with MIPS in Monaco; **SWISS-PROT** developed in Swiss by the Amos Bairoch group of SIB (Swiss Institute of Bioinformatics); **TREMBL** (Translated EMBL) included in EBI (European Bioinformatics Institute).

Specialized databases have been developed subsequently to the primary ones; they organize and interpret the information contained in the primary databases according to analytical criteria. In particular, specialized databases collect homogenous dataset from the point of view of taxonomic and functional databases available in the primary databases or in literature or derived from experimental analyzes, revised and annotated with information of added value. Biological data, collect by specialized databases, may therefore be of different types (domains and motifs protein, protein structures, genes, transcriptome, expression profiles, metabolic pathways, etc.).

Examples of specialized databases are:

- **wwPDB** (worldwide Protein Data Bank), database of 3D structure of proteins. It includes the atomic coordinates determined through X-ray crystallographic analysis, NMR analysis, ecc;
- database of genomic sequences, in this database for example there is GDB (human), **MGI** (mouse), **SGD** (yeast);
- database of genes and transcribed like **UniGene**, LocusLink, **dbEST**.

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The Middle East respiratory syndrome Coronavirus (MERS-CoV)

Middle East respiratory syndrome *Coronavirus* (MERS-CoV) is a severe respiratory human disease caused by a **novel beta-Coronavirus** which was identified for the first time in June 2012 in the **Kingdom of Saudi Arabia** (KSA) (1). Globally, from September 2012 to date, WHO has been informed of a total of 145 laboratory-confirmed cases of infection with MERS-CoV, including 62 deaths (2). The majority of human cases occurred in the KSA (119 cases with 51 deaths). Other cases have been notified in Jordan, Qatar and United Arab Emirates (UAE). France, Italy, United Kingdom (UK) and Tunisia experienced some imported cases in patients with a history of travels in the Middle East.

Coronaviruses are a family of viruses commonly infecting birds and mammals, including human beings. Human Coronaviruses usually cause mild to moderate **upper-respiratory tract illnesses**. Coronaviruses are named for the crown-like spikes on their surface. There are three main sub-groups of Coronaviruses, known as alpha, beta and gamma, and a fourth provisionally-assigned new group called delta Coronaviruses. The known Coronaviruses that can infect people are: alpha-Coronaviruses 229E and NL63 and beta-Coronaviruses OC43, HKU1, and severe acute respiratory syndrome *Coronavirus* (SARS-CoV), which causes a large epidemic between November 2002 and July 2003. SARS-CoV can infect people and animals, including monkeys, Himalayan palm civets, raccoon dogs, cats, dogs, and rodents. Molecular analysis of **SARS-CoV** from patients from various geographical regions allowed to better understand the virus sources and disease spread patterns (3). This analysis suggested that SARS-CoV was probably originated in bats and spread to people. A genetic link between the SARS-CoV in people and in civets revealed cross-host evolution (4).

Despite the common belonging to beta-Coronaviruses and the similarities in the clinical pictures in humans, SARS-CoV and MERS-CoV are characterized by clearly **different epidemiological features**. In particular, the spreading capacity across the human population is strongly different between these two viruses. In 2002- 2003 SARS-CoV globally caused 8273 human cases and 775 deaths in several countries. **MERS-CoV** has actually demonstrated a **minor capacity of spreading**, albeit a **higher case fatality rate**. This difference was also quantified by an epidemiological study which estimates the **basic reproduction number (R_0)** of MERS-CoV epidemic and compared it to that of pre-pandemic SARS-CoV. The MERS-CoV R_0 was calculated to be 0.69 (95% CI 0.50—0.92), whereas the R_0 for pre-pandemic SARS-CoV was 0.80 (0.54—1.13) (5).

In addition, molecular investigations on MERS-CoV highlighted some differences in the receptors for cell entry: SARS-CoV uses the angiotensin-converting enzyme 2 (ACE2) receptor for cell entry (6), whereas MERS-CoV employs the dipeptidyl peptidase 4 receptor (DPP4) (7), which has been extensively studied to better understand the pathogenesis of human infection as well as the possible animal host species (see below).

The primary source of MERS-CoV is actually not known. Epidemiological circumstances on the temporal and geographical distributions of notified human cases suggest the existence of other mechanisms than human-to-human transmission alone. Among the hypotheses on the possible sources of human infection, the direct contact with infected animals is under investigation.

The epidemiological investigations carried out in KSA on confirmed human cases coupled with the molecular analysis of viral isolates suggested the existence of an intermediary host as the source of human infection (8). Anyway the direct contact with animals (domestic or wild) was documented only in few cases, but no clear indication emerged on which animal species could be involved in the MERS-CoV transmission.

Samples have been collected in KSA from animals, both domestic (camels, sheep, goats) and wild (bats) in geographic zones where human cases were observed, but few results are currently available on the diagnostic investigations performed on these samples.

One fecal pellet sample collected from a *Taphozous perforates* (an insectivorous bat)

in KSA's Bisha region showed a genomic fragment with 100% nucleotide identity to MERS-CoV isolated from a human index case-patient (9). However several authors acknowledge that the identified genetic fragment was too short (190-nt RdRp) to draw any definitive conclusion on the proper identity of the isolated viral genome. Further analyses on molecular evolution of DPP4 genes in bats suggested the bat-origin of MERS-CoV (10).

In addition, two distinct studies performed in other countries seem to indicate a **high serological prevalence of infection in dromedary camels** (*Camelus dromedarius*). A first study revealed a 100% (50/50) serological prevalence in camels from Oman and 14% (15/105) in Spanish camels reared in Canary islands (11). The Omani camels were aged 8–12 years, belonged to different owners and were retired racing camels currently used for breeding. All Spanish positive camels were born and raised on the Canary islands and all animals were adults except one, which was 2 years old. In the same study other animal species were tested: cattle (n=80), sheep (n=40), goats (n=40) and other camelid species (n=34). No positive results were observed in these species for the presence of antibodies against MERS-CoV. All sera were tested by protein microarray using the receptor-binding S1 subunits of spike proteins of MERS-CoV and confirmed by virus neutralization.

In a second study (12) carried out in Egypt 103 out 110 (93.6%) dromedary serum samples tested positive by a new MERS-CoV spike neutralization assay. The dromedary camels were mostly imported from Sudan for slaughtering and were five to seven years old. Samples from other animal species (8 water buffaloes, 25 cattle, 5 sheep, 13 goats) were tested with negative results.

The most accredited hypothesis on MERS-CoV source considers the possibility that a new variant of beta-*Coronavirus* emerged in bats or other animal species populations where these viruses are common. According to this hypothesis this new variant, more pathogenic for human being, was spread (directly or indirectly) by **bats** and established in **camels** or other domestic animals as asymptomatic intermediate hosts, thus facilitating its transmission to human population. At moment this hypothesis must be seen as a pure speculation and no extrapolation may be done from the SARS-CoV experience to this new MERS-CoV infection.

As regards camels, the serological prevalence of camels in Spain (Canary islands), Oman and Egypt must be carefully evaluated. The serological tests used in these two studies are not validated for camel species and therefore their performances (sensitivity and specificity) are not known. Furthermore, a serological reaction does not imply that that animal is infectious or can play a role on virus shedding or transmission. Finally, the unusual high percentage of positive camels in these two studies and the absence of any evidence of MERS-CoV human cases in Spain, Oman and Egypt further suggest to carefully interpret these results.

Nevertheless the veterinary services of involved countries, in collaboration of the International Organizations (OIE, FAO and WHO), are involved in the investigations of possible animal sources, to better clarify the transmission chain of this infection and to put in place more appropriate and effective preventive measures.

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OFFICIALLY FREE TERRITORIES

Bovine tuberculosis: provinces and regions officially free according to the community legislation up to 10/04/2013

Decision EC	Province	Region
2003/467/EC	Bergamo	Lombardy
	Lecco	
	Sondrio	
	Ascoli Piceno	Marche
	Bolzano	Trentino Alto Adige
Trento		
2004/230/EC	Grosseto	Tuscany
2005/28/EC del 12-01-2005	Como	Lombardy
	Prato	Tuscany
2006/169/EC del 21-02-2006	Pescara	Abruzzi
2006/290/EC del 18-04-2006	All the provinces	Friuli Venezia Giulia
	All the provinces	Emilia Romagna
	Novara	Piedmont
	Verbania	Piemonte
	Livorno	Tuscany
Lucca		
Siena		
2007/174/EC del 20-03-2007	Belluno	Veneto
	Padova	Veneto
	Vercelli	Piedmont
	Pisa	Tuscany
	Pistoia	Toscana
2008/404/EC del 21-05-2008	All the provinces	Veneto
2009/342/EC del 23-04-2009	Oristano	Sardinia
2010/391/EC del 08-07-2010	All the provinces	Lombardy
2010/391/CE del 08-07-2010	All the provinces	Tuscany

Bovine tuberculosis

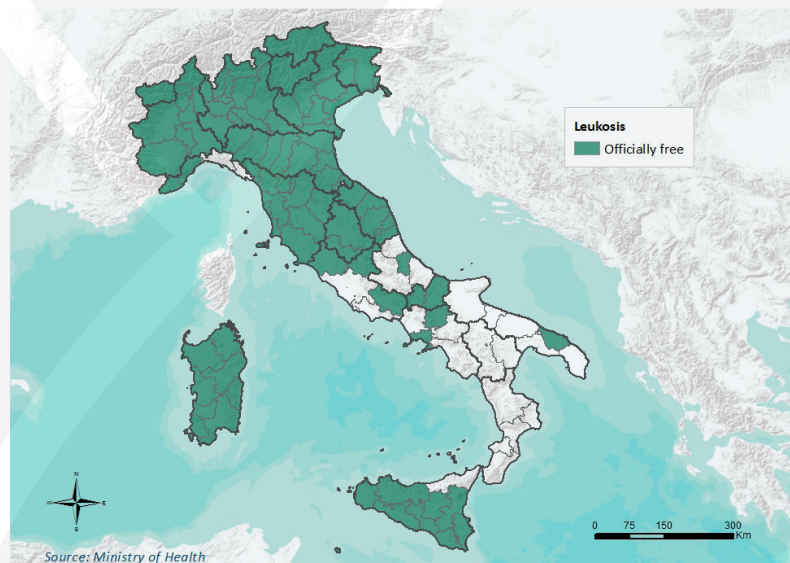


Bovine leucosis: provinces and regions officially free according to the community legislation up to 10/04/2013

Decision EC	Province	Region
2003/467/EC	Bergamo	Lombardy
	Brescia	
	Como	
	Lecco	
	Mantova	
	Sondrio	
	Varese	Marche
	Ascoli Piceno	
	Bolzano	Trentino Alto Adige
	Trento	
	Bologna	Emilia Romagna
	Ferrara	
	Forlì	
	Mcesena	
	Modena	
	Parma	
Piacenza		
Ravenna		
Reggio Emilia	Val D'Aosta	
Rimini		
Aosta		
2004/63/EC	Cremona	Lombardy
	Lodi	
	Milano	
	Arezzo	
	Firenze	
	Grosseto	
	Livorno	
	Lucca	
	Pisa	
	Pistoia	
	Prato	
Siena		
2005/604/EC	Alessandria	Piedmont
	Asti	
	Cuneo	
	Biella	
	Novara	
	Torino	
	Verbania	
Vercelli		
2005/28/EC	Pavia	Lombardy
2005/28/CE	Massa-Carrara	Tuscany
	Perugia	Umbria
	Terni	

Decision EC	Province	Region
2006/169/EC	Pescara	Abruzzi
	All the provinces	Friuli Venezia Giulia
	Frosinone	Lazio
	Rieti	Liguria
	Imperia	
	Ancona	Marche
	Macerata	
2006/290/EC	Pesaro	
	All the provinces	Molise
2007/174/EC del 20/03/2007	Savona	Liguria
	Oristano	Sardinia
2009/342/EC del 23/04/2009	All the provinces	Veneto
2013/177/UE del 10/04/2013	All the provinces	Sardinia
2010/391/EC del 08/07/2012	Benevento	Campania
	Napoli	

Bovine leucosis

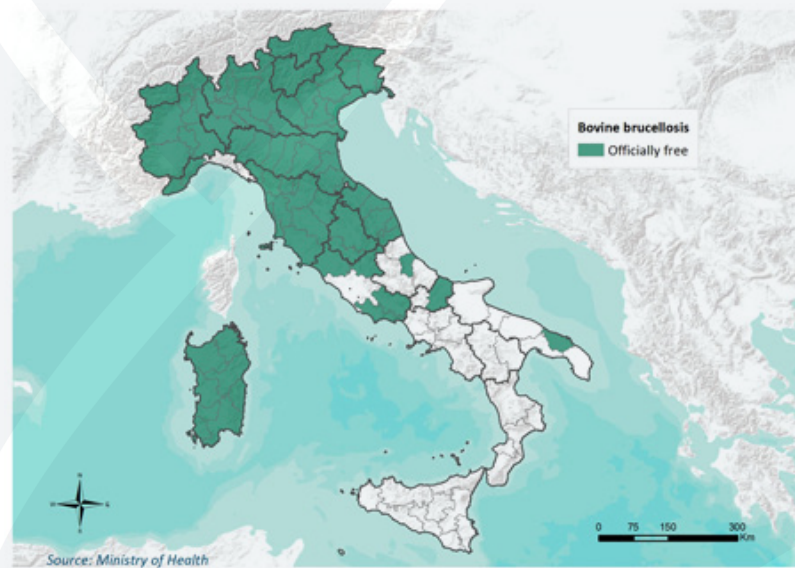


Bovine brucellosis: provinces and regions officially free according to the community legislation up to 10/04/2013

Decision EC	Province	Region
2003/467/EC	Bergamo	Lombardy
	Como	
	Mantova	
	Sondrio	
	Varese	
	Ascoli Piceno	Marche
	Bolzano	Trentino Alto Adige
	Trento	Trentino Alto Adige
	Bologna	Emilia Romagna
	Ferrara	
	Forlì	
	Cesena	
	Modena	
	Parma	
	Piacenza	
	Ravenna	Sardinia
	Reggio Emilia	
Rimini		
Cagliari		
Nuoro		
Oristano		
Sassari		
2004/63/EC	Cremona	Lombardy
	Lodi	
	Pavia	
2004/230/EC	Arezzo	Tuscany
	Grosseto	
	Livorno	
	Lucca	
	Pisa	
2005/28/EC	Brescia	Lombardy
	Prato	Tuscany
	Perugia	Umbria
	Terni	
2005/604/EC del 04/08/2005	Alessandra	Piedmont
2005/604/CE del 04/08/2010	Asti	
	Biella	
	Novara	
	Verbania	
	Vercelli	
2006/169/EC	Pescara	Abruzzi
	Tutte le province	Friuli Venezia Giulia
	Rieti	Lazio
	Imperia	Liguria
	Savona	Liguria

Decisione CEE	Provincia	Regione
2006/169/EC	Milano	Lombardy
	Pistoia	Tuscany
	Siena	Tuscany
2007/174/EC del 20/03/2007	Torino	Piedmont
	Firenze	Tuscany
	Tutte le province	Veneto
2008/97/EC del 30/01/2008	Brindisi	Apulia
	Tutte le province	Tuscany
2010/391/EC del 08/07/2010	Campobasso	Molise

Bovine brucellosis

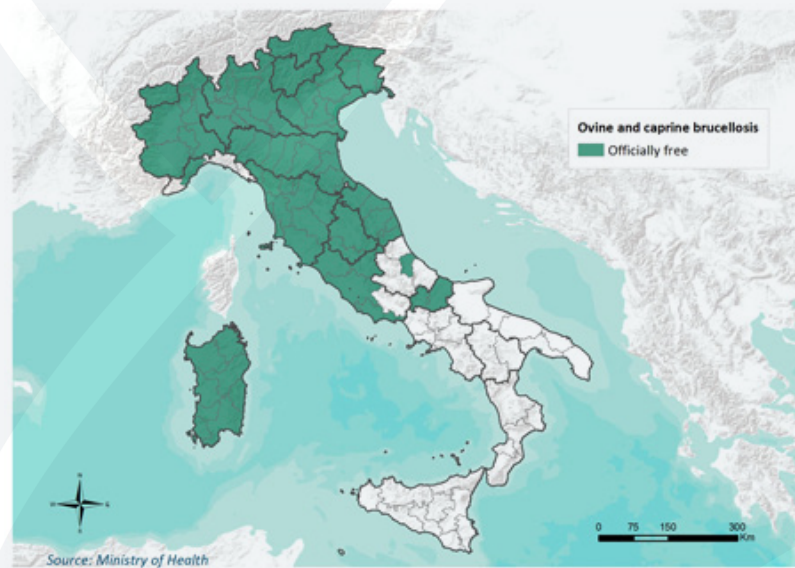


Sheep and goats brucellosis: provinces and regions officially free according to the community legislation up to 10/04/2013

Decision EC	Province	Region
2002/482/EC	Bolzano	Trentino Alto Adige
	Arezzo	Toscana
2003/237/EC	Cagliari	Sardegna
	Nuoro	
	Sassari	
2003/732/EC	Bergamo	Lombardia
	Brescia	
	Como	
	Cremona	
	Lodi	
	Mantova	
	Milano	
	Pavia	
	Sondrio	
	Varese	
2004/199/EC	Trento	Trentino Alto Adige
	Rieti	Lazio
2005/28/EC	Viterbo	Toscana
	Firenze	
	Livorno	
	Lucca	
	Massa-Carrara	
	Pisa	
	Pistoia	
	Prato	
	Siena	
	Perugia	
2005/764/EC del 28/10/2005	Terni	Toscana
	Grosseto	
2005/604/EC	Ancona	Marche
	Ascoli-Piceno	
	Macerata	
	Pesaro	
	Urbino	
	Alessandria	Piemonte
	Adti	
	Biella	
	Cuneo	
	Novara	
2006/169/EC	Torino	Friuli Venezia Giulia
	Verbania	
2010/391/EC del 08/07/2010	Vercelli	Molise
	Pecara	
2006/169/EC	All the provinces	Liguria
	Savona	
2010/391/EC del 08/07/2010	All the provinces	Molise

Decision EC	Province	Region
2008/97/EC del 30/01/2008	Roma	Lazio
	Latina	
	All the provinces	Veneto
2010/391/EC del 08/07/2010	All the provinces	Molise
2011/277/EC del 10/05/2011	All the provinces	Emilia Romagna
	All the provinces	Val D'Aosta

Ovine and caprine brucellosis





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