

UNIVERSITÀ DEGLI STUDI DI MILANO
Dipartimento Di Scienze Veterinarie E Sanità Pubblica



DOCTORAL SCHOOL
“HEALTH E ANIMAL PRODUCTION:
SCIENCE, TECHNOLOGY AND BIOTECHNOLOGY”

PhD COURSE OF
“VETERINARY HYGIENE AND ANIMAL PATHOLOGY”
Cycle XXVII

**Health monitoring of wildlife for the protection of human, livestock
and environment health**

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Academic Year
2013-2014

INTRODUCTION

The risk of spreading pathogens among wildlife, humans and livestock is increasing in recent years (Jones et al., 2008). This increased risk is being driven by multiple factors related to changes in human society (e.g., mobility, recreation), animal husbandry (e.g., intensification, range farming), natural habitat (e.g., forest cover, connectivity), and climate change (e.g., mean temperature, rainfall) (Gortázar et al., 2014). Until these underlying factors are addressed, it is important to carry out effective surveillance activities for early warning of disease emergence. This requires disease surveillance of not only humans and livestock, but also of the reservoir for many of these diseases: wildlife. “Wildlife reservoir” is a deceptively simple term for a complex entity. In contrast to humans (one species), domestic animals (about 50 species), wild mammals and birds alone comprise more than 1100 species in Europe. Many of these species move freely across country boundaries, and some, as birds, migrate long distances, not only within Europe, but also to and from Asia and Africa. Wild animals harbor a multitude of viruses, bacteria, parasites, that can be considered as emerging zoonosis (Kruse et al., 2004; Kuiken et al., 2005; Jones et al, 2008) and many of which have yet to be discovered. It is poorly understood which of these pathogens have the potential to transmit to livestock and humans. Nevertheless, in some specific ecological situation the role as reservoir for wildlife for specific pathogens was described. Considering the old world, tuberculosis in badger in United Kind as well as wild boar in Spain and Portugal, rabies in fox in central Europe and Classical Swine Fever in wild boar in Germany could be considered of clear examples (Gortázar et al. 2007; Martin et al., 2011). In addition to the difficulties in managing diseases in wildlife, the demonstrated ability of pathogens to infect a wide range of hosts underline the risk for disease emergence in both humans and domestic animals. Pathogens that infect more than one host species are by definition likely to be encountered in several host populations, some of which may constitute infection reservoirs. Therefore, a key issue in the design of control measures for multi-host pathogens is

defining what is meant by reservoirs of infection and developing surveillance for their identification (Haydon et al., 2002). As result, an effective surveillance system for wildlife diseases in Europe therefore needs to be broad in its coverage of host species and pathogens, and international in its geographical scope, considering that more than 70% of emerging zoonoses are hosted in wild animals (Kruse et al., 2004; Kuiken et al., 2005; Jones et al., 2008).

From a general point of view, the emergence of pathogens in humans and domestics is dependent on interactions between the final host and reservoir and/or vector hosts or their environment (Figure 1, center). The extent of such interactions is influenced by the prevalence of zoonotic pathogens in the animal reservoir or vector populations, which is in turn influenced by these populations' health and immune status. In addition, the population dynamics of humans, animal reservoirs, and vectors drive ecological processes that govern pathogen abundance and spread, both within and among species (Gortázar et al., 2014). Moreover the role of environment is relevant, influencing the relation between host and pathogen as well as diseases transmission. In fact, environment can influence the epidemiological role of different species/population, since a reservoir is considered as one or more epidemiologically connected populations or environments in which the pathogen can be permanently maintained and from which infection is transmitted to the defined target population (Haydon et al, 2002).

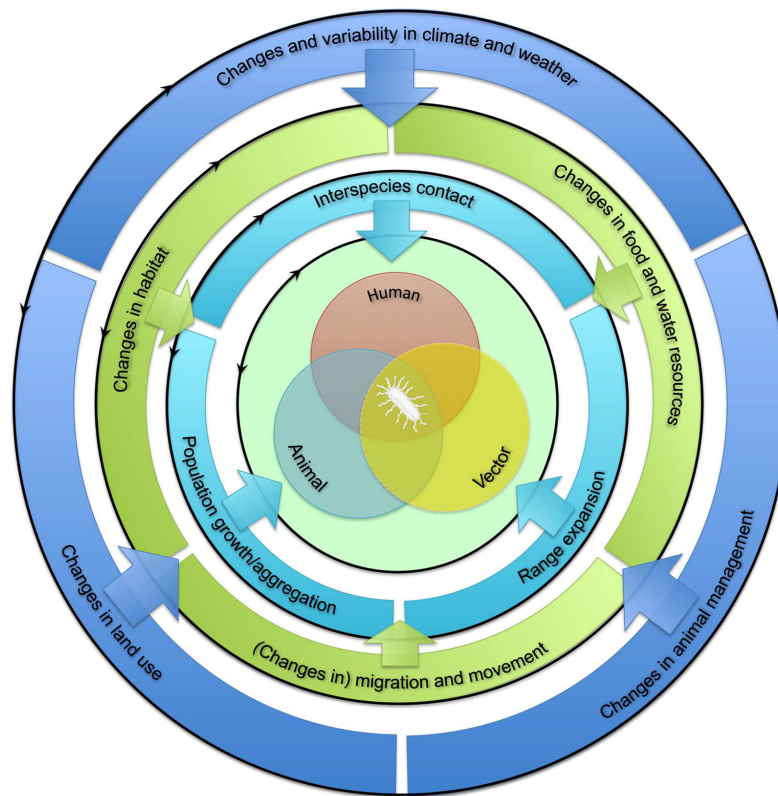


Figure1. Framework for the classification of drivers of human exposure to animal pathogens

(from Gortázar et al., 2014).

Pathogens present in free-living wild animals, and the diseases they cause, may be important for several different reasons, as mentioned above, and they are classically split into three different groups:

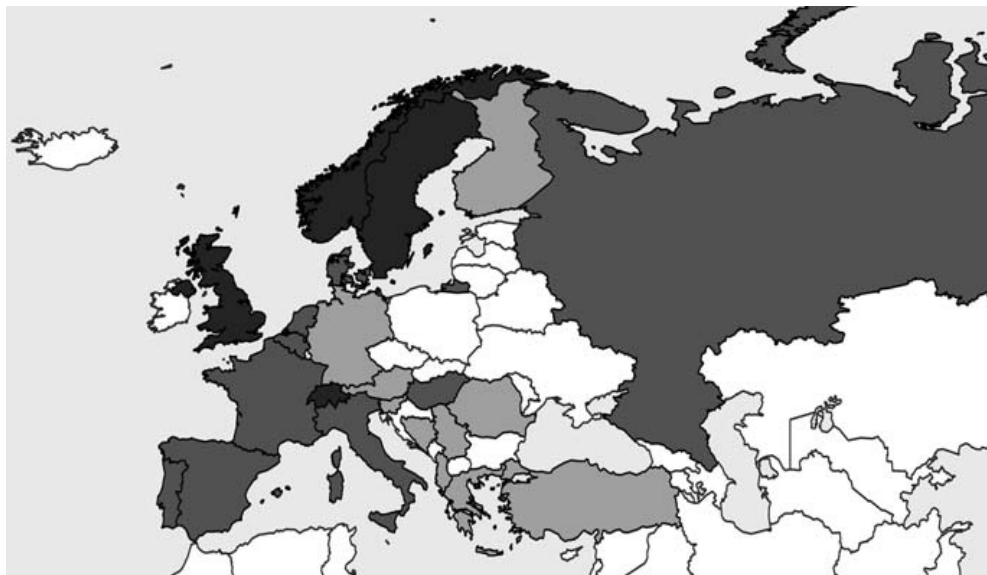
- Pathogens in wild animals may affect human health: Wild animals can be direct sources of infection for people with pathogens that can cause disease in humans. Pathogens carried by wild animals can be very important to human health and to public health and food safety programs. Effective public health programs require a complete understanding of the epidemiology of zoonotic pathogens in wild animals, as well as in humans and domestic animals.
- Pathogens in wild animals may affect the health of domestic animals: Many pathogens can infect both domestic animals and wild animals. Programs to control these pathogens

in domestic animals can fail if the programs do not take wildlife into account. Wild animals may be reservoirs for pathogens of domestic animals that affect international trade in animals and animal products. Some examples from this list would include bovine tuberculosis and classic swine fever or Aujeszky disease.

- Pathogens in wild animals may have important effects on wild animal populations dynamics: Pathogens and diseases can have a wide range of impacts on wild animals, ranging from subtle but important effects, such as reduced reproduction and life span or increased predation rates, to population declines from lethal disease.

The management and control of disease in wildlife present many challenges. Symptoms and signs of disease in wildlife are not as readily observed as in domestic animals, and specimens for laboratory analysis are more difficult to collect, thus making early detection and response to disease outbreaks slow to implement. The only approach suitable for the control of wildlife diseases is surveillance, that it can be divided in general surveillance (the pathological examination of animals found dead or moribund) and targeted surveillance (the testing of animals for the presence of specific pathogens) (Artois et al., 2009). Effective wildlife disease surveillance depends on knowledge of the population size of wild animals, as well as the geographical distribution over time of the population. Such knowledge is required in order to design appropriate sampling protocols for disease surveys, to develop disease contingency plans, to assess the risk of disease transmission to other host species, and to guide wildlife management strategies in general (Acevedo et al. 2008). At European scale, many national and regional disease monitoring programs include free-ranging wildlife (Fig. 2). These programs are usually proactive measures aimed at generally supporting national domestic animal and wildlife health, international trade in animals and animal products and protecting public health. Part of any national or international strategy for monitoring wildlife disease should include the capability to investigate mass mortality or morbidity events, investigate new disease syndromes, identify and

categorize new pathogens, and monitor the status of known diseases within wildlife populations. These different goals can be achieved through specific surveillance schemes, classically divided into active and passive surveillance. Targeted (or active) surveillance for known diseases of economic or public importance amongst wildlife, that is an increasingly well recognized need at the national and international level. General (or passive) surveillance as the reports of illnesses or deaths involving many animals from a free-living population representing the initial alert to the likely presence of a new disease agent. Early intervention and investigation of such unusual or unexpected disease events is essential to the goal of determining the cause and significance of the outbreak.



White: no data / Dark grey: comprehensive general wildlife health surveillance / Medium grey: partial general wildlife health surveillance (wide range of programs but restricted in various ways) / Light grey: no general wildlife health surveillance, but some degree of targeted surveillance for a few specified diseases

Fig. 2. Map of Europe depicting the level of wildlife health surveillance according to a self-evaluation of the participating countries (n = 25) (Kuiken et al., 2011)

Wildlife disease control begins with surveillance, knowing which diseases are present, their past and current distribution and the trends in their prevalence. In addition to surveillance, three basic forms of disease management strategies for wildlife are known: prevention of introduction of disease, control of existing disease or almost impossible eradication (Wobeser 2002). Top ranking wildlife diseases to research on are those where wildlife has a high probability of substantially affecting regional disease status, and the disease has a strong impact on human health, economy, wildlife management and conservation.

Most of the scientific studies dealing with infectious pathogens in wildlife describe how is require an effective collaboration with “hunter world”, as sampling on carcasses of hunted animals. Many surveillance programs on wildlife diseases in Europe were performed in cooperation with national hunting associations and governmental agencies. Hunters play an important role in these programs because they spend time into the field where they can observe wildlife in different seasons and they are concerned with the health of the wild animals they hunt and consume (Mörner and Fischer; 2011). These programs are an integrated part of the environmental monitoring programs and a large number of animals are submitted every year, mainly by hunters and landowners. Similar programs currently are in place in many countries in Europe (Kuiken et al., 2011). In outbreaks of FMD, Bovine Tuberculosis (TB), Classical Swine Fever and other important diseases in domestic and/or wild animals, cooperation between hunters and animal health authorities is critical. That was clearly demonstrated in the outbreak of highly pathogenic H5N1 influenza virus in 2006 when valuable disease surveillance information was developed by testing tens of thousands of samples from hunter-killed waterfowl in Asia, Europe, and North America. In addition, in the USA, samples collected from hunter-killed wild cervids provide important data on disease distribution (including first detection), as well as the

impacts of disease management efforts on TB and chronic wasting disease there they occur in wildlife (Mörner and Fischer; 2011).

PROJECT AIMS

In Italy, as the rest of Europe, changes in land use and in wildlife management practices have influenced the population dynamics of wildlife, facilitating the growth in numbers of some species, as wild ungulates (Pedrotti et al., 2001; Carnevali et al., 2009), amplified the health risk for livestock, for man and also for the biodiversity (Rizzoli et al., 2009; Daszak et al., 2010). On the other hand land use change have been caused a dramatic effect on population dynamics of small game species like hare, that showed a progressive decline in population densities in Italy as in the rest of Europe during the last decades (Edwards et al. 2000).

In Lombardy the interest in the detection of wildlife infectious diseases has considerably grown in the last years, and monitoring control programs in wildlife have been carried out for more than 10 years. This interest raised from the high value of biodiversity present in the Region. Lombardy counts many protected areas: the most important are the Stelvio National Park (the largest Italian natural park), with typically alpine wildlife and the Ticino Valley Natural Park, instituted in 1974 on the Lombard side of the Ticino River to protect and conserve one of the last major examples of fluvial forest in Northern Italy, with typically plain wildlife. The remaining territories are divided into public hunting areas, private hunting areas and small protected areas where hunting is not allowed.

Based on previous health monitoring experiences, in December 2012 a regional monitoring program of wildlife diseases was adopted in order to standardize the activities to increase the knowledge of the health status of wild populations (D.D.G., 5 December 2012 - n. 11358; http://www.izsler.it/izs_bs/allegati/3097/piano%20monitoraggio%20regionale.pdf). This is the second (the first has been already active in Emilia Romagna Region; <http://www.alimenti-salute.it/materiali.php?id=19>) example of an integrate system of wildlife surveillance at National level. The surveillance of wildlife diseases has been implemented harmonizing activities toward

relevant wildlife diseases providing a common approach in all the areas of the Region involved in the surveillance.

In Brescia Province, wildlife disease monitoring programmes were initially - in 1998 - implemented and organized by IZSLER at the request of the hunters' association as a consequence of the first outbreaks of European Brown Hare Syndrome. This attention let us to plan both active surveillance on target host species and passive surveillance, since hunters (27,139 in Brescia Province; hunting season 2012-13) can be considered as an *infinity of eyes*. In addition they are interested in the health of wildlife and local conservation brown hare syndrome (EBHS). Since this lesion, at the beginning of each year, the veterinary services, as they inspect all hunted animals. Specific diagnostic protocols were developed for each host species, in particular for those that are under specific hunting planning, such as wild ungulates, hare and red fox. Different contexts can influence the diseases transmission between wildlife, livestock and human and can increase the spread of pathogens. The adoption of monitoring programs, as routinely work, is needful to identify changes in their occurrence and also to detect unusual wildlife mortality.

The first goal of the present thesis was to select diseases with a high probability of substantially affecting provincial and regional disease status, and diseases that has a strong impact on human health, economy, wildlife management and conservation, on the light of the diseases surveillance on wildlife of Brescia Province (Figure 3), in particular on red deer (*Cervus elaphus*), wild boar (*Sus scrofa*), hare (*Lepus europaeus*). I selected this three species because of their significance in terms of population number, distribution in Brescia province, possible epidemiological role and economical and hunting value. Specific activities aimed to collect biological samples and epidemiological data were organized in order to evaluate the possible source of infection or the cause of the circulation of pathogens in specific areas, in particular for bovine tuberculosis in red deer and Aujeszky disease in wild boar, and the spatial/time exposure

of the hare populations to European Brown Hare Syndrome (EBHS) infection. Another step was standardize an appropriate methods of sampling biological material, in particular blood from hunted hares, in order to correctly interpret the EBHS sero-epidemiological results.

As reported in the introduction, the collaboration with hunters is basilar for the implementation of surveillance programs on wildlife diseases. The improvement of such collaboration with new strategy of involvement in Brescia Province is another objective of PhD activities.

Summarizing:

✓ Focus on:

- Zoonotic diseases: I selected **Bovine Tuberculosis** (TB), which is a chronic bacterial disease that affects species of mammals. Although the classical tuberculosis in human is not caused by the same species of *Mycobacterium* as for cattle and wild animals, bovine tuberculosis (*M. bovis* and *M. caprae*) can spread from animals to humans, known as a significant zoonosis. Bovine tuberculosis can be transmitted to humans by contact with infected animals or by consumption of contaminated food. Wildlife can play an important role in the epidemiology of this disease. Thus, the knowledge of TB from wildlife is important to ensure the efficacy of eradication programs.
- Diseases with an high economic impact for livestock: I selected **Aujeszky's disease** (AD), which is one of the most economically important infectious diseases of swine for which wild and domestics suids are the natural hosts. Although for AD it has been shown that the prevalence in wild boar populations was not a significant risk factor for the level of AD prevalence in the coexisting pig farms, there are studies that suggest the opposite. Intact, wild boar can act as

reservoirs for pathogens shared with their related domestic species, being able to transmit and maintain them even without the presence of the domestic reservoir.

- Diseases with an high demographic impact for wild animals: I selected **European Brown Hare Syndrome**, which is a species-specific highly contagious disease of hare with extremely high rates of morbidity and mortality, with a preferential susceptibility of adult animals, especially breeders. For this reason this disease can cause dramatic decrease of densities in hare populations, when it appears.
- ✓ Develop/standardize appropriate methods of sampling/diagnostic techniques specific for wildlife
- ✓ Educational training on diseases in wild animals of the stakeholder, as hunters.

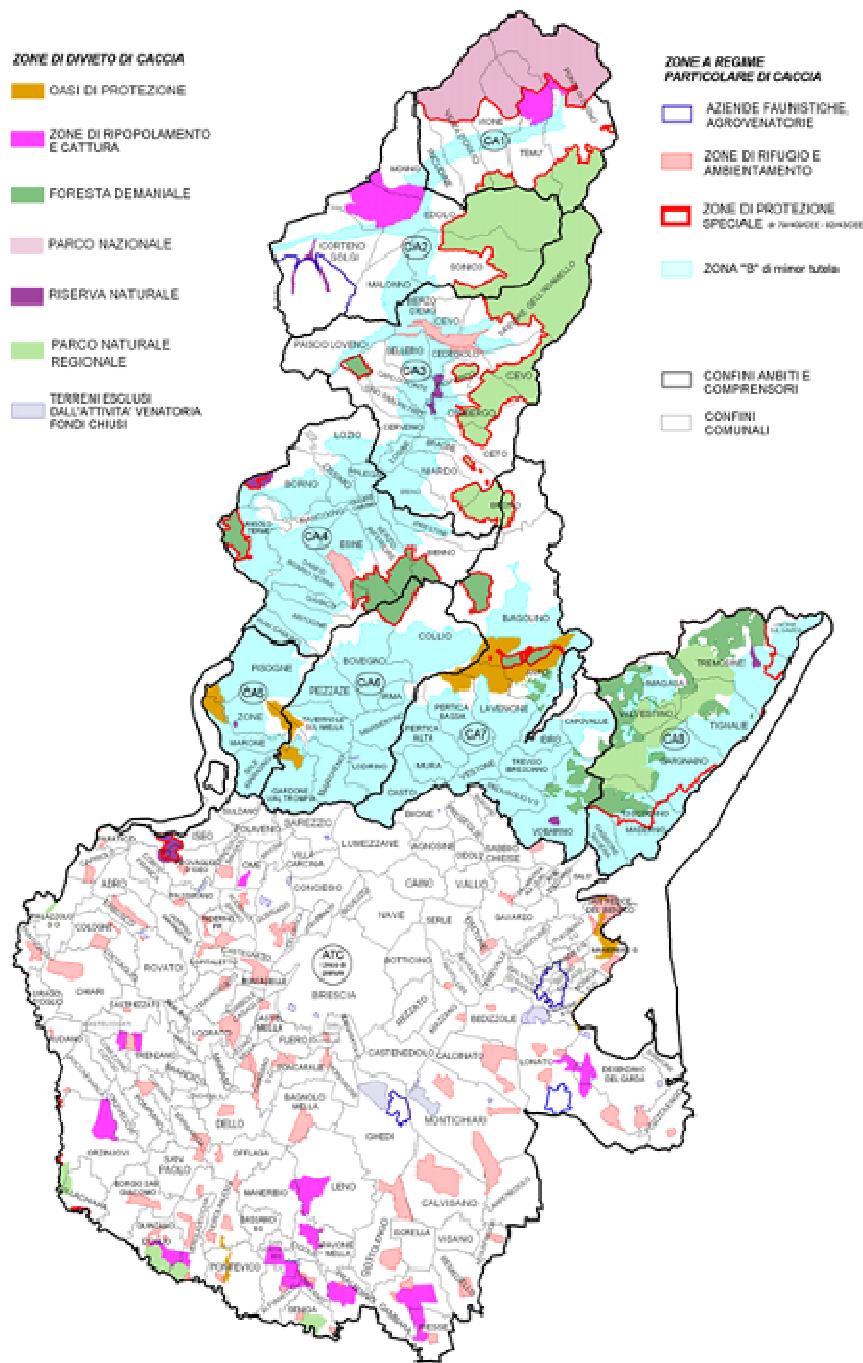


Figure 3. Public hunting areas, private hunting areas and protected areas of Brescia Province.

ZOONOTIC DISEASES

Bovine tuberculosis is a chronic bacterial disease of animals and humans caused by *Mycobacterium bovis*. In a large number of countries bovine tuberculosis is a major infectious disease among cattle, other domesticated animals, and certain wildlife populations. Transmission to humans constitutes a public health problem. It should be noted that other members of the *M. tuberculosis* complex, previously considered to be *M. bovis*, have been accepted as new species despite identical 16s RNA sequences and over 99.9% identity of their genome sequences. These include *M. caprae* (Aranaz et al., 2003) that in some countries is considered to be a primary pathogen of goats. These new species are known to be zoonotic and, in central Europe, has been identified as a common cause of bovine tuberculosis. Disease caused by *M. caprae* is not considered to be substantially different from that caused by *M. bovis* and the same tests can be used for its diagnosis.

Mycobacterium caprae, a member of the *Mycobacterium tuberculosis Complex* (MtbC), is a zoonotic pathogen that causes **tuberculosis (TB)** in livestock and wild animals (Aranaz et al., 2003). It has been isolated mainly in Central Europe from humans (Kubica et al., 2003), cattle (Prodinger et al., 2002; Erler et al., 2004; Boniotti et al., 2009), goats (Aranaz et al., 1999) and wildlife, including red deer (*Cervus elaphus*) (Prodinger et al., 2002; Rodríguez et al., 2011; Schoepf et al., 2012) and wild boars (*Sus scrofa*) (García-Jiménez et al., 2013).

The assessment of the epidemiological role of wildlife is crucial for the implementation of effective control measures to eradicate TB in cattle (Prodinger et al., 2005; Gortázar et al., 2011). Recently, the red deer was revealed as a maintenance host for *M. caprae* infections in the Tyrol region (Austria), where the disease prevalence ranged from 0 to 23% in a population with a density of 5.6 animals/km² (Schoepf et al., 2012). *M. caprae* is responsible for over 10% of the TB outbreaks in cattle herds in Italy; nevertheless, most of the northern Italian regions were officially declared TB-free ten years ago. During the same period, wild ungulate populations

have increased significantly, but *M. caprae* has been detected only in cattle (Boniotti et al., 2009).

The aim of this study was to ascertain through targeted surveillance whether TB infections were present in free ranging red deer sampled from an Italian alpine area, collecting 53 animals. Calculation of sample size was based on estimated population sizes derived from the official hunting bags, and performed using WinEpiscopeH 2.0 software (Thrusfield et al., 2001), with the aim of detecting infection and assuming a prevalence of 5% with 95% confidence level. The area is located in Valcamonica (Brescia province), an alpine valley characterized by a high red deer density (16 animals/km²) estimated from kilometric abundance index obtained by spotlight data (Whipple et al., 1994). Most of livestock are managed by traditional transhumance, that is a seasonal droving of grazing livestock between the valleys in winter and the high mountain pastures in summer, with spatial interactions with wild ungulates. Such area was declared officially free from bovine tuberculosis (OTF) in cattle in 2010 (Unpublished Official Data). Here *M. caprae* was previously identified from cattle during TB outbreaks involving eight herds in 2001 and one herd in 2010. This last outbreak had not compromised the OTF legal status of the area. As shown by epidemiological investigations and confirmed by molecular typing of the isolates, the first TB outbreak cluster was linked to the introduction of infected dairy cows from Austria and Germany through a livestock collection center (Boniotti et al. 2007). The local trade of those animals from the collection center and the grazing of cattle on common summer pastures likely facilitated the TB diffusion into the eight affected herds. In 2010, *M. caprae* of the same genotype was re-isolated in the same area from TB lesions observed in an indigenous dairy cow of a different herd. This case was not connected to the introduction of the TB-positive animals from endemic countries and no other risk factors were discovered.

Between August and December 2011, to investigate the presence of *M. caprae* in red deer, we examined the viscera (lungs, liver and intestine) and lymph nodes (Lnn) of 53 hunted animals (Table 1).

Table 1: Sex and age of sampled red deer (*Cervus elaphus*)

	Male		Female		Calf	Total
	< 5 y	5 - 9 y	< 2 y	> 2 y	< 1y	
	12	11	7	16	8	53

At necropsy, we observed small granulomatous, tuberculosis-like lesions in just one liver of a female aged 12 years (P=1.30%; 0.0 - 7.1 C.I. 95%) (Figure 4). This organ and two Lnn pools for each deer were subjected to a bacteriological examination and Real-time PCR analyses.



Figure 4. Small granulomatous tuberculosis-like lesions in the liver of the hind of 12 years of age

The first Lnn pool was composed of retropharyngeales Lnn, the second one of tracheobronchialis, mesenteriales, and mediastinales Lnn. Standard procedures of mycobacterial culturing were used. Briefly, the samples were homogenized, decontaminated, and inoculated onto solid selective media, Löwenstein-Jensen and Stonebrink (Heipha Diagnostika, Heidelberg, Germany), and a liquid medium, Modified Middlebrook 7H9 broth (BBL MGIT, Becton, Dickinson and Company, Milan, Italy). Bacteriological cultures were positive only from the liver and first Lnn pool of the deer with lesions. We employed a Real-time PCR assay using the Artus *M. tuberculosis* TM PCR commercial kit (Qiagen) for the direct detection of MtbC mycobacteria. DNA was extracted and purified by mechanical lysis with glass beads followed by affinity column purification using the PureLink TM Genomic DNA mini Kit (Invitrogen). The Real-time PCR assay permitted the detection of MtbC bacteria only in the liver of the deer showing TB-lesions.

Identification of the isolates was confirmed by PCR-RFLP analysis of the *gyrb* gene through *RsaI* and *SacII* restriction enzymes. The genotyping of isolates was performed by Spoligotyping and MIRU-VNTR analysis using the following 12 markers: ETR-A, -B, -C, -D, -E, MIRU-26, VNTR 2163a, 2163b, 4052, 3155, 1895 and 3232 (Boniotti et al., 2009).

The isolate was identified as *M. caprae* by *gyrb*-RFLP analysis. The genotype profile, according to spoligotyping and MIRU-VNTR analyses, was SB0418, 5-3-5-2-3-4-8-5-3-4-3-11. This profile corresponds to the “Lechtal genotype”, which was the most frequently detected in the TB outbreaks in Austria and Germany in cattle and red deer (Prodinger et al., 2002; Eler et al., 2004). In addition, it was the same genotype identified in the 2001 and 2010 cattle herd’ TB outbreaks reported in the sampling area. Interestingly, during this period, neither tuberculin skin testing, done every two year to all cattle in the region, nor surveillance at the abattoirs, performed in agreement with the Italian and EU legislation for TB eradication program (respectively DM 592/95 and DLgs 196/99, Council Directive 64/432/EEC, Annexes A, I and

B), revealed any TB infections among cattle in the study area. Therefore, the absence of TB reports in cattle suggest the lengthy undisclosed persistence of the Lechtal genotype in the study area. The absence of classical TB lesions and the presence of only one lesion in the liver do not permit to evaluate the stage of infection. The possible migration of the red deer from the Tyrol infected areas is unlikely considering the distance (over 200 km) between the two areas that is much more than the natural migratory behaviour of deer. In addition, no positive animals was found in border Italian sample areas (Bergamo, Sondrio, Trento, Bolzano and Brescia) and the most prominent clusters of infection (hot spot) were detected in Austrian and German sampling regions (Fink et al., EMIDA TBAlpineWildlife). Therefore, since no likely sources of infection were identified and considering that cattle graze on common pastures with red deer during the summers, the possibility that the red deer act as a maintenance host of *M. caprae* in the environment from the first to the last TB outbreak in cattle could be not excluded.

In contrast to the TB epidemiological situation of neighbouring alpine countries (Schoepf et al., 2012), the sporadic detection in northern Italy (one infected red deer on 53 sampled), notwithstanding the high density red deer population, could suggest that the wildlife management measures applied in the study area reduced the risk of TB spreading. Management options were designed with the aim to improve the local wildlife management and hunting rules. In particular, the two main factors influencing the TB diffusion were addressed (Schoepf et al., 2012). First, differently from what normally practiced in Tyrol (Schoepf et al., 2012), artificial feeding and salt licks for wildlife were not permitted, limiting aggregation that occurs in particular during winter at the feeding sites. Secondly, hunters securely disposed of offal from hunted animals, after evisceration at the slaughterhouse, to reduce the likely presence of infected materials in the field (Zanella et al., 2012).

This is the first report of a *Mycobacterium caprae* infection in wildlife in Italy. Thus, targeted surveillance should be performed in the future to monitor the presence and diffusion of TB as well as the efficacy of the applied control measures.

DISEASES WITH AN HIGH ECONOMIC IMPACT FOR LIVESTOCK

Aujeszky's disease (pseudorabies) is a highly contagious, economically significant disease principally affecting wild and domestic pigs (Müller et al. 2011). Aujeszky disease can result in trade restrictions from regions where it is endemic, as a consequence, eradication programs in swine and surveillance programs on wild boar are ongoing. In fact wild boars can act as reservoir for Aujeszky disease virus (ADV) and may represent a potential threat for domestic animals (Müller et al. 2011). Experimental studies with isolated ADV strain from wild boar suggest the virus is of low virulence resulting in more latent or subclinical infections, although mild and reversible disease could be induced (Pannwitz et al. 2012). Thus far, few clinical ADV cases in wild boar have been described, apparently associated with combinatory effects of age, genetic disposition, immune status and other factors. The wild boar-domestic pig interface represents one of the clearest examples of wildlife-domestic interface, where wildlife can act as reservoirs for pathogens shared with their related domestic species, being able to transmit and maintain them even without the presence of the domestic reservoir (Boadella et al., 2012).

From 2006 to 2012 sera from 2493 wild boar hunted in Brescia province were collected and tested with a competitive ELISA for ADV-gE (IZSLER home-made kit). To deeper investigate the presence of ADV, also in latent form, 635 tonsils samples were analyzed with real time-PCR described by Yoon et al., 2005.

One hundred and fourteen wild boar (4.5%) tested positive in the gE-ELISA, 7 animals (2.9%) for hunting season 2006/07, 10 (2.2%) for 2007/08, 11 (2.1%) for 2008/09, 42 (9.4%) for 2009/10, 19 (3.9%) for 2010/11 and 25 (6.7%) for 2011/12, respectively. The PCR resulted positive in 8 cases (1.22%).

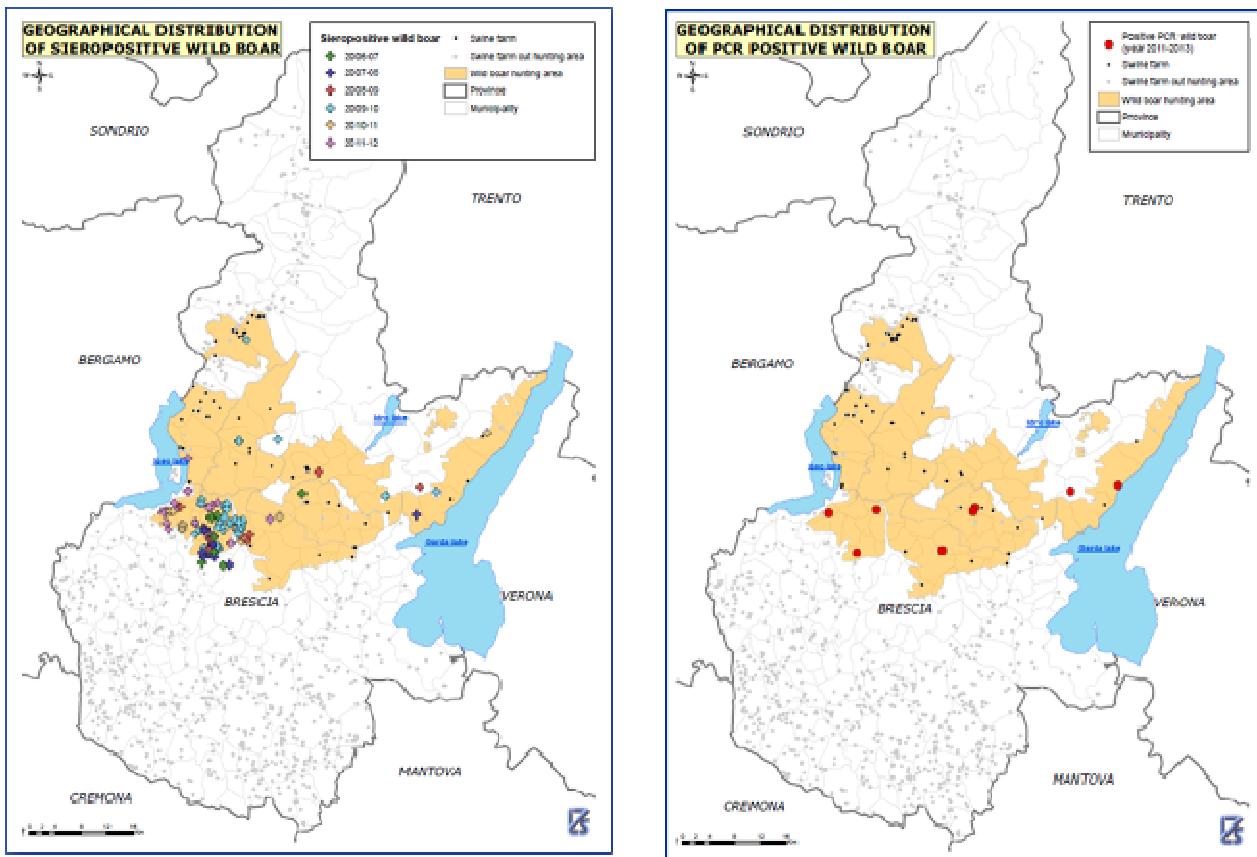


Figure 5. Geographical distribution in Brescia province of seropositive and PCR positive wild boars.

The obtained data demonstrate that ADV is present and persistently circulating in wild boars of monitored area, even if the infection is present only in an isolated population, located in an area without industrial swine herds (Figure 5). Even that, familiar swine farming is present in the study area, but epidemiological role of this system of farming on ADV presence in wild boar is not clear. In any case, the infection in wild boar is present since 2006 and seroprevalence rates higher than 9.4% have never been reached, indicating that it can be considered maintenance host for the infection and then ADV maintenance in wild boars could be independent of the presence of pig farms.

DISEASES WITH AN HIGH DEMOGRAPHIC IMPACT FOR WILD ANIMALS

The European brown hare (*Lepus europaeus*) is an important game species in Italy and is subjected to a specific hunting management which relies on restocking to try to maintain hare populations in hunting areas at densities sustainable with harvest. To stabilize population declines, restocking programmes using allochthonous individuals have been carried out in several European countries. Bulgaria, Slovakia, Hungary and Poland have traditionally functioned as source populations for restocking operations in Central and Western Europe. Despite these efforts, in the last decades, a progressive decline in the hare population has occurred in Italy and Europe generally (Edwards et al. 2000). One of the several causes is the occurrence of **European Brown Hare Syndrome (EBHS)** (Duff and Gavier-Widén, 2012). EBHS is a species specific highly contagious disease, first described in Sweden in 1980 (Gustaffson et al. 1989) and now considered endemic in all European countries, including Italy (Paci et al. 2011).

The first EBHS reported in Northern Italy (Province of Brescia) occurred in 1988, coinciding with the identification of the virus (EBHSV), which belongs to the genus lagovirus of the Caliciviridae family (Lavazza and Vecchi 1989, Capucci et al. 1991, Wirblich et al. 1994). Since its first appearance, EBHSV has evolved in different genotypes and sub-types but at present they all belong to a unique serotype.

When introduced into the native brown hare population EBHSV infection achieves almost 100% morbidity. Mortality is about 50% in the adult age class, but absent in young individuals less than about 40-50 days old. These younger individuals acquire the infection and develop a long lasting immunity with low-medium antibody titres, without exhibiting any clinical signs (Scicluna et al. 1994; Zanni et al. 1993). When the infection is endemic and constantly circulates in a hare population, individuals can acquire the infection in early life and almost all seroconvert

before becoming susceptible to the disease and the mortality due to EBHS on the host's dynamics seems to be almost negligible (Zanni et al. 1993). The conditions for the endemic circulation of the virus require a constant recruitment of a sufficient number of new receptive individuals, and, if these are represented by new born hares, the virus is maintained endemically with low mortality outbreaks and most adult individuals will be seropositive with low titres. Lavazza et al. 1997 estimated this condition could be satisfied when hare population reached densities greater than 15 hares/km². On the contrary, if the virus has no endemic transmission into the hare population, the infection dynamics will be characterised by recurrent outbreaks with high mortalities. These outbreaks begin when the population immunity is low (high number of seronegative individuals) and native hares are exposed to a newly reintroduced virus. The hares surviving this epidemic will develop protective immunity against the virus and show high titres and until the seroprevalence and mean titres proportion of the population decreases below the threshold density of transmission, about 15 hares/km², the population will be resistant to a new outbreak.

The Italian territory is divided into public hunting areas, private hunting areas and protected areas where hunting is not allowed. Some protected, but open (i.e. not fenced), areas are managed as "breeding for restocking" grounds (BfRG). In such agricultural areas brown hares are free to reproduce and during the winter a portion of them are caught and relocated into hunting areas for restocking purposes, taking care to maintain a high density in the BfRG (the percentage of hares caught and transferred is dependent on the estimated density). Specific monitoring plans, based on the assessment of the immunological status of the population through serological anti-EBHSV titres, have been implemented in most Italian provinces during restocking operations (Paci et al. 2011). However, limited data is available on the infection's temporal trend and disease occurrence and on the effects of different wild hare population characteristics at density values greater or lesser than 15 hares/km².

Therefore, through the analyses of serological data collected in seven BfRG areas during seven consecutive hunting seasons (2006-2007–2012-2013), this study aims to describe the in-field temporal dynamics of EBHSV infection in wild European brown hares (*Lepus europaeus*) populations and to test the influence of density on EBHS seroprevalence. In particular, the present study was performed using the blood from hares caught in seven different BfRG locations in the province of Brescia. Hare densities were estimated each autumn using spotlight counts along transecting lines (Barnes and Tapper 1985, Tizzani et al. 2013, Santilli et al. 2014). Three study areas (Quinzano, Montichiari and Ghedi Military Airport) are characterized by density values constantly higher than 15 hares/km² (min: 17 hares/km²; max: 70 hares/km²), whereas the other four areas (San Felice, Bagnolo Mella, Lonato and Calcinato) had density values consistently less than 15 hares/km² (min: 4 hares/km²; max: 14 hares/km²).

A total of 512 blood samples (~0.5 mL, serum) were collected from the ear vein during the relocation procedures performed each year at the end of the hare hunting season (December/January) (Table 2).

Table 2. Number of tested hares, EBHS seropositive results and prevalence of blood samples collected in seven different BfRG during seven years

Area/Year	2006-07	2007-08	2008-09	2009-10	2010-11	2011-12	2012-13	Total
Bagnolo	10 (10)	8 (6)	8 (4)	6 (2)	N.D.	12 (4)	10 (2)	54 (28)
	100.0 %	75.0 %	50.0 %	33.3 %		33.3 %	20.0 %	51.8 %
Calcinato	N.D.	N.D.	8 (7)	9 (9)	9 (3)	11 (5)	11 (8)	48 (32)
			87.5 %	100.0 %	33.3 %	45.4 %	72.7 %	66.7 %
Lonato	7 (7)	7 (7)	6 (6)	7 (4)	N.D.	N.D.	6 (4)	33 (28)
	100.0 %	100.0 %	100.0 %	57.1 %			66.7 %	84.8 %
Military Airport	N.D.	16 (13)	11 (5)	9 (3)	N.D.	23 (12)	N.D.	59 (33)
		81.2 %	45.4 %	33.3 %		52.2 %		55.9 %
Montichiari	N.D.	20 (18)	21 (21)	21 (21)	16 (15)	13 (5)	12 (8)	103 (88)
		90.0 %	100.0 %	100.0 %	93.7 %	38.5 %	75.0 %	85.4 %
Quinzano	37 (37)	20 (20)	13 (13)	16 (11)	18 (11)	12 (3)	11 (11)	127 (106)
	100.0 %	100.0 %	100.0 %	68.8 %	61.1 %	25.0 %	100.0 %	83.5 %
San Felice	16 (12)	12 (4)	11 (1)	8 (0)	12 (1)	19 (7)	10 (4)	88 (29)
	75.0 %	33.3 %	9.1 %	0.0 %	8.3 %	36.8 %	40.0 %	32.9 %
Total	70 (66)	83 (68)	78 (57)	76 (50)	55 (30)	90 (36)	60 (37)	512 (344)
	94.3 %	81.9 %	73.1 %	65.8 %	54.5 %	40.0 %	61.7 %	67.2 %

All samples were analysed to detect anti-EBHSV antibodies using a competitive ELISA (cELISA) test. This method, developed in-house, is based on the competition between the antibodies, anti-EBHS, adsorbed into the solid phase and those possibly present in the serum sample for a prefixed and limited concentration of the antigen (EBHSV) present in the liquid phase. The tracers used in the reactions are HRP-conjugated monoclonal antibodies (MAbs) against EBHS (Capucci et al. 1991). The detailed protocols are fully described in the “OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals” (Capucci and Lavazza, 2008). This cELISA test has a high specificity since it mainly measures antibodies, which bind with high avidity, directed against highly specific antigenic determinants on the viral external surface.

A generalized linear mixed model (GLMM) with a binomial distribution and logit link function was used to estimate the effects of yearly population density on EBHS prevalence, with the different areas analysed included as a random factor (Goldstein, 2011). In the GLMM, the density was categorized as equal to one when its value was more than 15 hares/km² and equal to zero otherwise (Lavazza et al., 1997). Moreover, in each area, the Cochran-Armitage trend test (Armitage, 1955; Agresti, 2002) was performed to verify the trend of prevalence over time. A linear regression model was used to assess the relationship between annual prevalence and mean titres after transforming the serological titres into their reciprocal values. All analyses were performed using *R* Software (version 3.0.0; R Development Core Team 2013). The package *lme4* was used to develop the GLMM with a binomial distribution. The statistical significance level was set at $\alpha = 0.05$.

Out of the 512 tested, 344 (67.2%) tested positive for EBHSV antibodies (Table 1). The annual seroprevalence ranged from 94.3% (2006–07) to 40.0% (2011–12), while mean titres fluctuated from 167.12 in 2006–07 to 23.75 in 2011–12 indicating a significant decline (Figure 6).

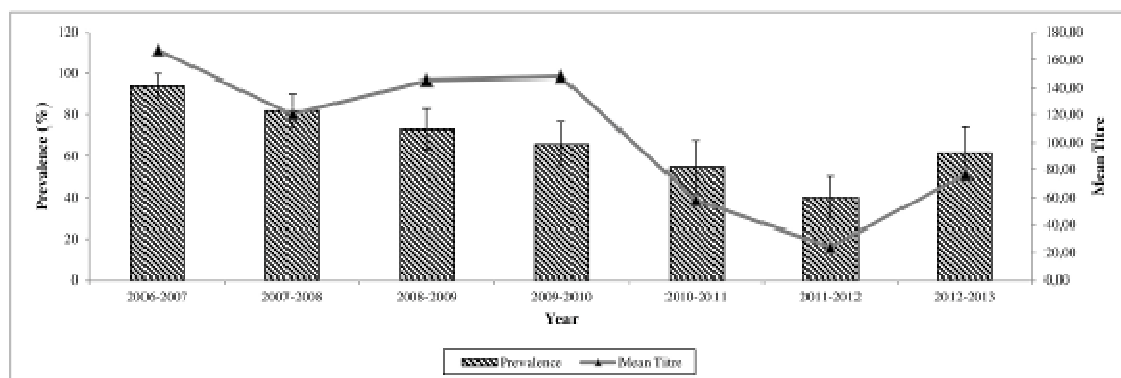


Figure 6. Disease prevalence and mean anti-EBHSV antibody titres' recorded during the seven years of European Brown Hare Syndrome (EBHS) survey.

The GLMM showed that both yearly occurrence and population density explains the seroprevalence of EBHS in hares ($p < 0.05$). The 3.303 (95% CI =1.059 to 10.301) density odds ratio indicates that disease prevalence is higher in areas with a population density greater than 15 hares/km², while the 0.521 (95% CI = 0.433 to 0.627) yearly odds ratio indicates a reduced prevalence over the years.

The Cochran-Armitage test showed a decreasing trend for seroprevalence in two high-density areas, Quinzano and Montichiari, and in two low-density areas, Bagnolo Mella and Lonato. However, no trends were highlighted in the other high-density area, Military Airport, or in the other two low-density areas, San Felice and Calcinato (Table 4). The absence of a significant density and year interaction indicates that the temporal trend between populations of different density areas was consistent.

Table 3. Trend of European Brown Hare Syndrome seroprevalences analyzed using the Cochran-Armitage trend test for each northern Italian “breeding for restocking” area. High density = >15ind/km², Low density = <15ind/km².

Area	Density	Chi Squared	Df	P value
Quinzano	High	21.62	1	< 0.001
Military Airport	High	2.71	1	0.099
Montichiari	High	14.87	1	< 0.001
San Felice	Low	3.43	1	0.0636
Bagnolo Mella	Low	16.37	1	< 0.001
Lonato	Low	6.29	1	0.01
Calcinato	Low	2.56	1	0.109

A positive relation between seroprevalence and mean titres was observed, so that for a each unit increase of prevalence, the mean titre increased by 2.487 (95% CI = 0.998 to 3.975) (F statistic=18.45 df=1; p value= 0.007).

The present study highlights both a temporal decline in the EBHS seroprevalence and a positive relationship between seroprevalence and hare density. The serological results demonstrated the widespread presence of EBHS in the study areas throughout the whole period considered and active circulation of EBHSV with seropositive animals found in every study area. The annual disease prevalence ranged from 94.3% in 2006–2007 to 40.0% in 2011–2012. Studies investigating long temporal trends of EBHS serology are generally lacking, and our results disagree with those of a previous study, covering a three year period, performed in Germany, where the overall seroprevalence was 29% and stable (Frölich et al. 2003). This difference might be due to the limited number of populations analysed in Germany (n= 2) and to the long-lasting persistence of EBHS antibodies, which may require longer temporal spans to highlight changes.

EBHSV is easily transmitted mainly by oro-faecal and respiratory routes and, due to its high resistance in the environment, alternative routes and carriers may be involved such as humans, insects, and birds as well as by contaminated equipment and tools (Frölich and Lavazza 2008). In addition, infection via consumption of contaminated vegetation is also likely, with the virus being secreted and excreted, and also being spread in the droppings of predators that have consumed infected hares. In fact, lagoviruses have a high environmental resistance and may remain infectious for 3–4 months in the field (Henning et al. 2005; Frölich and Lavazza 2008). Since hares are still the only recognized host of EBHSV supporting viral replication, the hare populations' density could influence the amount of virus excreted and its circulation due to both direct and indirect transmission. The direct influence of hare density in EBHSV transmission was indicated by the deterministic model of the natural diffusion of EBHS (Lavazza et al. 1997). In

particular, this model hypothesized that in areas where hare density is greater than 15 hares/km², the virus is endemically maintained in the population. Under this condition, most young hares acquire the infection before the age of 50 days. On the contrary, in areas where the hare density is lower than 8 hares/km², the yearly virus transmission is reduced and irregular. The results of the present survey proved that in areas with a density greater than 15 hares/km² the probability for a single hare to be seropositive is 3.3 higher than in low-density areas (<15 hares/km²). However our results partially contrast with the deterministic model that predicts an endemic stability for areas in which the brown hare density is greater than 15 hares/km². In fact we found that the seroprevalence declined progressively in both high- and low-hare density areas. This last result was unexpected since, according to the model, the antibody levels should be respectively steady in hare population above 15 hares/km² and variable or declining in less dense populations. Results indicate how in every BfRG tested there was not a situation of real endemic stability.

The decreasing seroprevalence observed during this study could indicate the emergence of new EBHS epidemics. Indeed, looking at the past, the periodic occurrence of epidemics is a typical pattern of the temporal and geographic dynamics of EBHS. Thus, the epidemiological situation that allows new epidemics to develop is characterized by the decrease of seroprevalence. Moreover, in populations with low seroprevalence, the detection of young hares with high serological titres could indicate the beginning of a new epidemic outbreak. Unfortunately this survey did not assess this hypothesis because it was not possible to sample young animals but only sub-adult and adult. In fact, hares were live-captured during winter restocking procedures and released immediately in hunting areas, making it impossible to understand the relationship between average titre and the age-structure of the population. Additionally, the exact determination of the timing of virus exposure through the detection of different classes of immunoglobulin's is not possible, in contrast with rabbits for which anti-isotype IgA/IgM/IgG ELISA are used (Cooke et al. 2000).

In conclusion, these results suggest that population density influences the virus maintenance in the hare population with a temporal decline of the EBHS seroprevalence and circulation of the virus. These findings contribute to improve the understanding of the epidemiology of EBHSV, a disease considered as one of the main causes of the hare population decline in Europe (Duff and Gavier-Widén 2012). In particular, since the eradication of EBHS in a wild population is not feasible, the strategy of promoting the endemic stability of the virus through density-dependent mechanisms is the only way of minimizing the EBHS impact. However this method of EBHS control appears more difficult to realize in practice than in theory due to the very high brown hare density most likely required to make it effective. A density value greater than 15 hares/km² is difficult to achieve and to be maintained during the year, especially when the wild population is not correctly managed and the environmental carrying capacity is limited. This situation is complicated by the simplified agro-ecosystems present in Brescia Province, as in the rest of the Po valley, that cannot maintain high density hare populations even at a short time scale. Agricultural intensification has had dramatic effects on farmland biodiversity and has caused declines in many taxa. Habitat changes are thought to be one of the main causes of the decline in numbers of European hares, throughout Europe. Increasing habitat heterogeneity at the farm scale may benefit hares, especially in highly homogeneous, intensively managed landscapes. However, managers of pastoral farmland should aim to increase habitat heterogeneity at the within-habitat (or within-field) scale in particular, to provide better cover throughout the year. Agri-environment schemes should target the regeneration of heterogeneity in pastoral landscapes, by encouraging changes such as an increase in fallow land and a reduction in livestock density. Such shifts in management are likely to benefit both hares and farmland biodiversity in general. As to be stressed, that hunting organizations of Brescia Province are involved in improving the biodiversity and the typical hare habitat.

DEVELOP/STANDARDIZE APPROPRIATE METHODS OF SAMPLING OR DIAGNOSTIC TECHNIQUES SPECIFIC FOR WILDLIFE

There is a need to develop diagnostic tests appropriate for wildlife species. Many tests designed for domestic mammal samples do not have the same levels of sensitivity and specificity when used in wild species (Gortázar et al., 2007; Artois et al. 2009;). Whereas tests aimed at directly detecting the pathogen usually give similar results in both domestic and wild animals, indirect tests such as ELISA – which are based on detecting the immune response of the host to the pathogen and thus depend on the recognition of specific proteins associated with that response – may not deliver reliable results (Artois et al. 2009).

Validation of diagnostic tests in wildlife is associated with a number of challenges such as the difficulty to obtain enough positive and negative controls, the lack of gold standards, the large number of animal species and limited financial resources. Nevertheless, efforts made to overcome these problems are increasing. For example: the EWDA Wildlife Health Surveillance Network has initiated the edition of Diagnosis Cards recommending diagnostic techniques appropriate for wildlife testing (<http://www.ewda.org>); the WildTech Project aims at developing new technologies for the improvement of wildlife health surveillance (<http://www.wildtechproject.com>); and the APHAEA project will propose harmonized methods for diagnostic investigations in wildlife (<http://www.aphaea.eu>).

New diagnostic tools may also contribute to overcoming difficulties related to sampling conditions and the limitations of traditional diagnostic tests. For this reason a practical and usefully way of sampling blood and the relative laboratory analyses were standardized.

As reported, the first outbreaks of the disease in North Italy in the 1990s forced the adoption of serological surveillance in protected areas (Lavazza e Vecchi, 1989), in order to control the dynamics of hare populations in relation to the spread of the virus. Thereafter the

periodical reoccurrence of cases imposed the adoption of a more complete program with the aim of monitoring the whole hare metapopulations, including those dwelling in hunting territories. In fact, hunting activity may facilitate the spread of the virus and also natal dispersal rates of animals, higher in hunting areas than in non-hunting areas, may contribute to the diffusion of the disease (Lavazza et al., 1996). In addition to the examination of carcasses of dead animals for viral detection, such monitoring activity takes advantage from serological survey, i.e. by checking the presence of antibodies to EBHSV in both hares shot during hunting activity (from September to December) or those captured in restocking areas at the end of the hunting season (December-January), before being moved and released in hunting areas.

Since different types of blood sampling may be adopted according to each situation (Portejoie et al., 2009), we planned to compare the serological titres obtained by testing with competitive enzyme-linked immunosorbent assay (cELISA) the “classical” serum from blood taken from ear vein with samples of blood dried onto blotting paper (Figure 7) and also bloody samples taken directly from the heart cavities during necropsy.



Figure 7. Example of blotting paper

The major aim was to establish the utility of each sampling method for verifying the health status of hares and particularly to check the possibility to get data from low density areas. From 2005 to 2012 we analysed the following samples: a) serum from venous blood and blotting paper of 305 animals; b) blotting paper and cardiac blood of 182 animals; c) serum from venous blood and cardiac blood of 95 animals. Two small squares of approximately 6x6 mm of side were cut from dried blotters and placed in 100 µl of phosphate-buffered saline (pH 7,4) for 1 night and then 32µl of the eluted was recovered for ELISA testing (Capucci et al., 1991; Capucci and Lavazza 2008).

Table 4. European Brown Hare Syndrome serological results according to different sampling methods of blood collecting.

Titre class	A) Blood venous sera		B) Blotting paper		C) Heart clutch	
	N°	%	N°	%	N°	%
Neg	140	35.0	252	51.7	122	44.0
≤40	132	33.0	169	34.7	127	45.8
>40<640	121	30.3	56	11.5	26	9.4
≥640	7	1.8	10	2.1	2	0.7
Total	400	100	487	100	277	100

Hearts were collected by hunters during evisceration and delivered to the laboratory, where the bloody cardiac fluid (7 µl) was obtained by pressing the heart (Table 4). Even if blotting paper and cardiac blood slightly underestimate the EBHSV antibody titres, if compared to the titre of serum from venous blood, both these “alternative” sampling methods may be useful for field studies (Portejoie et al., 2009). In particular blotting paper appears easier in sampling, storing and transporting also for hunters. In addition the slightly underestimates of antibody titres do not prevent to correctly interpret the sero-epidemiological results with regard

to the understanding of spatial/time exposure of the population to EBHS and the ability of single hares to resist the EBHSV infection.

EDUCATIONAL TRAINING OF THE STAKEHOLDER

A wildlife disease program cannot function unless there are a sufficient number of properly educated people to work within the program. Such required personnel include technical personnel with knowledge and skills specifically in wildlife pathogens and diseases, but fundamental in order to execute the monitoring plan at capillary level is the involvement of the hunters.

Yearly, the results of activity are show to hunters and hunters association at local conference. Moreover, the annual activity of sampling is planned in collaboration with representatives of hunters. Specific courses have been organized in order to educate hunters for correct sampling methods and for process of wild game meat after slaughter.

Additionally, thanks to the collaboration of the Brescia division of the Federazione Italiana Della Caccia (F.I.D.C.) five different brochures were made in order to share knowledge on both wildlife diseases and the regional monitoring program (Figure 8). The topics were selected based on the evidence of the presence of the same diseases at the local level in the last years, like cysticercosis in hare, sarcoptic mange in foxes and trichinellosis in wild boar. An additional brochure on the monitoring scheme of wildlife in Lombardy region was created in order to share information regarding the activity and the possibility given to hunters to participate at the surveillance. These brochures were spread in all the 245 local division of this hunting association.

Sintomatologia

Il ciclo di incubazione è variabile, il periodo di incubazione è di 10-15 giorni, il periodo di incubazione è di 10-15 giorni, il periodo di incubazione è di 10-15 giorni.

Sintomatologia epidemiologica

La prima segnalazione della presenza della malattia è stata fatta nel 1970 in un cane di razza Labrador Retriever, in un allevamento di cani da caccia di razza Labrador Retriever, in un allevamento di cani da caccia di razza Labrador Retriever.

Per ulteriori informazioni

Dr. Paolo Di Marco, Veterinario di Medicina Veterinaria, Via S. Maria Maddalena, 100, 00187 Roma, Tel. 06/49811111, Fax 06/49811112, E-mail: info@medvet.it

COME INTERVENIRE

Consigli generali

Il cane deve essere isolato e sottoposto a quarantena. È necessario evitare il contatto con altri cani e con l'uomo. È importante evitare il contatto con altri cani e con l'uomo.

Consigli generali

È importante evitare il contatto con altri cani e con l'uomo. È importante evitare il contatto con altri cani e con l'uomo.

Per ulteriori informazioni

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LA CISTICERCOSE NELLA LEPRE



Federazione Italiana della Caccia

Settore Provinciale di Arezzo

IN COLLABORAZIONE CON

ISTITUTO ZOOPIROLOGICO SPERIMENTALE DELLA SANITA' PUBBLICA "ROSSI" DI ROMA

Redazione e impaginazione: ANTONIO DI MARCO, Settore Dipartimentale di Arezzo, Via S. Maria Maddalena, 100, 00187 Roma, Tel. 06/49811111, Fax 06/49811112, E-mail: info@medvet.it

LA CISTICERCOSE

Causa

La cisticercose è causata dal verme *Parascaris leishmani*, che si trasmette attraverso le feci.

Specie colpite

Le specie colpite sono i cani e i gatti, in particolare i cuccioli.

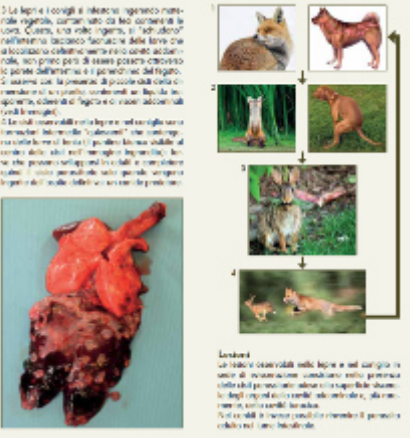
Ciclo biologico

Il ciclo biologico della cisticercose è molto breve, con un periodo di incubazione di pochi giorni.

Per ulteriori informazioni

Dr. Paolo Di Marco, Veterinario di Medicina Veterinaria, Via S. Maria Maddalena, 100, 00187 Roma, Tel. 06/49811111, Fax 06/49811112, E-mail: info@medvet.it

LA CISTICERCOSE



Lesioni

Le lesioni causate dalla cisticercose sono localizzate principalmente nel sistema digerente.

Per ulteriori informazioni

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Sintomatologia

Il sintomo principale della rogna è la presenza di croste e croste gialle, che si formano sulle zone colpite.

Lesioni

Le lesioni causate dalla rogna sono localizzate principalmente sulle zone colpite.

Per ulteriori informazioni

Dr. Paolo Di Marco, Veterinario di Medicina Veterinaria, Via S. Maria Maddalena, 100, 00187 Roma, Tel. 06/49811111, Fax 06/49811112, E-mail: info@medvet.it

COME INTERVENIRE

Consigli generali

È importante isolare il cane e sottoporlo a quarantena. È importante evitare il contatto con altri cani e con l'uomo.

Consigli generali

È importante evitare il contatto con altri cani e con l'uomo. È importante evitare il contatto con altri cani e con l'uomo.

Per ulteriori informazioni

Dr. Paolo Di Marco, Veterinario di Medicina Veterinaria, Via S. Maria Maddalena, 100, 00187 Roma, Tel. 06/49811111, Fax 06/49811112, E-mail: info@medvet.it

LA ROGNA NELLA VOLPE



Federazione Italiana della Caccia

Settore Provinciale di Arezzo

IN COLLABORAZIONE CON

ISTITUTO ZOOPIROLOGICO SPERIMENTALE DELLA SANITA' PUBBLICA "ROSSI" DI ROMA

Redazione e impaginazione: ANTONIO DI MARCO, Settore Dipartimentale di Arezzo, Via S. Maria Maddalena, 100, 00187 Roma, Tel. 06/49811111, Fax 06/49811112, E-mail: info@medvet.it

LA ROGNA

Causa

La rogna è causata dal fungo *Microsporum canis*, che si trasmette attraverso le spore.

Specie colpite

Le specie colpite sono i cani e i gatti, in particolare i cuccioli.

Ciclo biologico

Il ciclo biologico della rogna è molto breve, con un periodo di incubazione di pochi giorni.

Per ulteriori informazioni

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LA ROGNA



Lesioni

Le lesioni causate dalla rogna sono localizzate principalmente sulle zone colpite.

Per ulteriori informazioni

Dr. Paolo Di Marco, Veterinario di Medicina Veterinaria, Via S. Maria Maddalena, 100, 00187 Roma, Tel. 06/49811111, Fax 06/49811112, E-mail: info@medvet.it

Sintomatologia

Il sintomo principale della sindrome della lepre bruna europea è la presenza di croste e croste gialle, che si formano sulle zone colpite.

Lesioni

Le lesioni causate dalla sindrome della lepre bruna europea sono localizzate principalmente sulle zone colpite.

Per ulteriori informazioni

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COME INTERVENIRE

Consigli generali

È importante isolare il cane e sottoporlo a quarantena. È importante evitare il contatto con altri cani e con l'uomo.

Consigli generali

È importante evitare il contatto con altri cani e con l'uomo. È importante evitare il contatto con altri cani e con l'uomo.

Per ulteriori informazioni

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SINDROME DELLA LEPRE BRUNA EUROPEA

European Brown Hare Syndrome (EBHS)



Federazione Italiana della Caccia

Settore Provinciale di Arezzo

IN COLLABORAZIONE CON

ISTITUTO ZOOPIROLOGICO SPERIMENTALE DELLA SANITA' PUBBLICA "ROSSI" DI ROMA

Redazione e impaginazione: ANTONIO DI MARCO, Settore Dipartimentale di Arezzo, Via S. Maria Maddalena, 100, 00187 Roma, Tel. 06/49811111, Fax 06/49811112, E-mail: info@medvet.it

EBHS

Causa

La sindrome della lepre bruna europea è causata dal virus della sindrome della lepre bruna europea.

Specie colpite

Le specie colpite sono i cani e i gatti, in particolare i cuccioli.

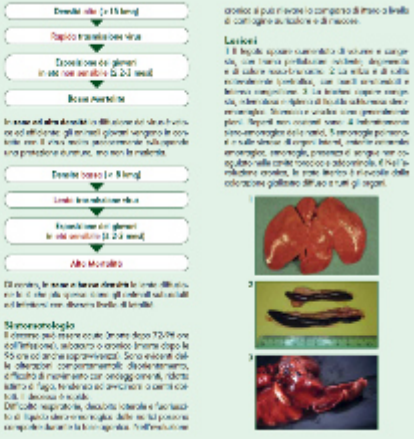
Ciclo biologico

Il ciclo biologico della sindrome della lepre bruna europea è molto breve, con un periodo di incubazione di pochi giorni.

Per ulteriori informazioni

Dr. Paolo Di Marco, Veterinario di Medicina Veterinaria, Via S. Maria Maddalena, 100, 00187 Roma, Tel. 06/49811111, Fax 06/49811112, E-mail: info@medvet.it

EBHS



Lesioni

Le lesioni causate dalla sindrome della lepre bruna europea sono localizzate principalmente sulle zone colpite.

Per ulteriori informazioni

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Salvatici
Le malattie sono prevalentemente in modo degli animali salvatici e si diffondono in modo diverso a seconda della specie.

Salmonellosi
In tutti gli animali le malattie possono dipendere da forme enteriche e dalla regione (funzione) del tratto gastroenterico. La presenza della malattia dipende dalla presenza di batteri, virus, protozoi e funghi.

Trasmissione di focolai
Le malattie possono essere trasmesse per contatto diretto, attraverso il contatto di carne cruda o poco cotta, carne di latte non pastorizzata, ecc.

Prevenzione
Le malattie possono essere prevenute con vaccini, con antibiotici, con farmaci antiparassitari, ecc.

COME INTERVENIRE

Controlli preventivi
È necessario monitorare l'attività più comune, come il controllo delle carni, la presenza di animali morti, ecc.

Trasmissione di focolai
Le malattie possono essere trasmesse per contatto diretto, attraverso il contatto di carne cruda o poco cotta, carne di latte non pastorizzata, ecc.

Prevenzione
Le malattie possono essere prevenute con vaccini, con antibiotici, con farmaci antiparassitari, ecc.

LA TRICHINELLA NEI SELVATICI

Federazione Italiana della Caccia
Settore Provinciale di Brescia
in collaborazione con
ISTITUTO EUROPEO DI STUDI SULLA SALUTE E SULLA SICUREZZA ALIMENTARE

Salvatici
Le malattie sono prevalentemente in modo degli animali salvatici e si diffondono in modo diverso a seconda della specie.

Salmonellosi
In tutti gli animali le malattie possono dipendere da forme enteriche e dalla regione (funzione) del tratto gastroenterico. La presenza della malattia dipende dalla presenza di batteri, virus, protozoi e funghi.

Trasmissione di focolai
Le malattie possono essere trasmesse per contatto diretto, attraverso il contatto di carne cruda o poco cotta, carne di latte non pastorizzata, ecc.

Prevenzione
Le malattie possono essere prevenute con vaccini, con antibiotici, con farmaci antiparassitari, ecc.

LA TRICHINELLA

Ciclo
Il ciclo di vita di Trichinella spiralis è molto complesso, che coinvolge la muscolatura di alcuni animali salvatici. Con la sua diffusione, questa parassita si trasmette da un animale all'altro.

Spazio ospite
Le forme adulte di questa parassita sono le più comuni nei piccoli animali salvatici, come il foino, il capriolo, il cervo, il cinghiale, il capreolus, il capriolo, il capreolus, ecc.

Ciclo di parassitismo
1. Il ciclo di vita di Trichinella spiralis è molto complesso, che coinvolge la muscolatura di alcuni animali salvatici. Con la sua diffusione, questa parassita si trasmette da un animale all'altro.

Ciclo biologico
1. Il ciclo di vita di Trichinella spiralis è molto complesso, che coinvolge la muscolatura di alcuni animali salvatici. Con la sua diffusione, questa parassita si trasmette da un animale all'altro.

IL MONITORAGGIO SANITARIO DELLA FAUNA SELVATICA

Federazione Italiana della Caccia
Settore Provinciale di Brescia
in collaborazione con
ISTITUTO EUROPEO DI STUDI SULLA SALUTE E SULLA SICUREZZA ALIMENTARE

Come deve essere fatto il campionamento?
Ciascun campione deve essere raccolto in modo da essere rappresentativo della popolazione.

Chi può effettuare il campionamento?
Il campionamento deve essere effettuato da personale qualificato e autorizzato.

Quali sono gli obiettivi del campionamento?
L'obiettivo è di individuare la presenza di malattie e di valutare l'incidenza delle stesse.

IL MONITORAGGIO SANITARIO DELLA FAUNA SELVATICA

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ISTITUTO EUROPEO DI STUDI SULLA SALUTE E SULLA SICUREZZA ALIMENTARE

Strategie preventive (prevalenti)
Semplificazione di TUTTI i casi sospetti di malattia.

Strategie di sorveglianza
La sorveglianza di tutti gli animali salvatici in modo da individuare la presenza di malattie.

Strategie di controllo
L'obiettivo è di individuare la presenza di malattie e di valutare l'incidenza delle stesse.

Figure 8. The five brochures made in order to share knowledge on both wildlife diseases and the regional monitoring program created with the collaboration of the Brescia division of the Federazione Italiana Della Caccia (F.I.D.C.)

“Between animal and human medicine there is no dividing line-nor should there be. The object is different but the experience obtained constitutes the basis of all medicine.” Rudolf Virchow (1821-1902).

Virchow's statement is as wise today as it was over a century ago. That all animal species, including *Homo sapiens*, are related and that knowledge gained in one species benefits all lead to the concept of “One Medicine”. The One Medicine approach takes advantage of commonalities between species; few diseases affect exclusively one group of animals (wildlife, domestic animals, or humans). On the basis of that view, we have the opportunity to organize a conference (Figure

9) with the collaboration of EXA (Mostra internazionale delle armi sportive e dell'outdoor. Brixia Expo - Fiera di Brescia spa) and Brescia Province. At this conference took part the Minister of Health, the National Reference Laboratory for diseases of wildlife, regional and provincial administration of both veterinarian and wildlife management units and hunting associations.

<p>Modalità di partecipazione</p> <p>La partecipazione al workshop è gratuita. Sarà possibile effettuare la preregistrazione online sul sito www.fieradibrescia.it, compilando l'apposita scheda.</p>	<p>Informazioni</p> <p>Segreteria organizzativa: Fiera di Brescia Tel 030 3463484 Fax 030 3463480 e-mail: info@exa.it</p> <p>Segreteria scientifica Dr. Lavazza Antonio (I.Z.S.L.E.R.) e-mail: antonio.lavazza@iszler.it Dr. Chiari Mario (I.Z.S.L.E.R.) e-mail: mario.chiari@iszler.it</p> <p>Sede Svolgimento Fiera di Brescia via Caprera 5, 25125 Brescia Sala Conferenze, Il piano</p> <p>Come arrivare Tangenziale Sud: uscita 5 Autostrada A4 Milano-Venezia: uscita casello Brescia Ovest Proseguire dritto per circa 500 mt seguendo le indicazioni Fiera</p>	<p>Convegno</p> <p>CHECK UP SANITARI DELLA FAUNA SELVATICA:</p> <p>approccio interdisciplinare alla salute umana ed animale</p> <p>22 marzo 2014</p> <p>Fiera di Brescia Sala convegni - 2° piano via Caprera, 5</p>
<p>Organizzato da:</p> <p>FIERA DI BRESCIA</p> <p>In collaborazione con:</p>  		 <p>Fotografie di Andrea Zampatti</p>

Check up sanitari della fauna selvatica

Presentazione

Nell'ultimo decennio in tutta Italia si è assistito ad un continuo ed esponenziale aumento delle popolazioni di animali selvatici, sia per consistenza numerica sia per distribuzione geografica, raggiungendo livelli tali da rappresentare un'entità non più trascurabile e diventare potenziali fattori di rischio sanitario per gli animali domestici e per l'uomo.

Nel corso degli ultimi anni le politiche comunitarie, recepite a livello Nazionale e Regionale, hanno attivato piani sanitari che includono a pieno titolo la componente faunistica a riconoscimento della sua importanza per tutelare la sanità pubblica e animale e la biodiversità.

Di fatto attività di monitoraggio e controllo sanitario degli animali selvatici hanno scopo di raccogliere informazioni utili ad una valutazione del rischio per le popolazioni domestiche di animali da reddito, per l'uomo e per gli stessi animali selvatici, siano mammiferi che volatili.

"Non esiste linea di demarcazione tra la medicina animale e quella umana, l'obiettivo è differente, ma l'esperienza ottenuta costituisce la base di tutta la medicina" Rudolf Virchow (1821-1902). Questa concezione che vede come attori protagonisti le popolazioni animali, l'uomo ed ambiente, compresi i vettori, comporta sempre nuove sfide non solo per i gestori della fauna, ma anche per il mondo veterinario.

Su questo tema si è ritenuto opportuno promuovere un momento d'incontro per esaminare e discutere il ruolo degli animali selvatici nei cicli epidemiologici delle principali patologie zoonosiche e non la conseguente situazione circa le attività di monitoraggio sanitario della fauna selvatica in essere in diversi contesti Nazionali.

Chairman

Dr. Ettore Zanon – Giornalista

Relatori

Prof. Ezio FERROGLIO – Università degli studi di Torino

Dr. Vittorio GUBERTI - ISPRA

Dr. Michele DOTTORI – IZSLER

Dr.ssa Gaetana FERRI – Dir. Gen. della sanità animale e del farmaco veterinario - ufficio II

Dr. Riccardo ORUSA - Centro di Referenza Nazionale per le Malattie degli Animali Selvatici

Dr. Marco FARIOLI – Regione Lombardia U.O. Veterinaria

Dr. Mauro FERRI - Servizio Veterinario Azienda USL Modena (Referente sanità selvatici, Azienda USL Modena)

Dr. Enrico MERLI – Provincia di Piacenza

Dr.ssa Emanuela GIOIA – Libero professionista, Piacenza

Programma

- 09.00 Registrazione partecipanti
- 09.15 Saluti di benvenuto e Introduzione ai lavori
Prof. Lanfranchi - Dr. Zanon
- 09.30 Monitoraggi e patologie: i mammiferi
Dr. Ezio FERROGLIO
- 10.00 Monitoraggi e patologie: l'avifauna
Dr. Vittorio GUBERTI
- 10.30 Monitoraggi e patologie: i vettori
Dr. Michele DOTTORI
- 11.00 *Coffe break*
- 11.15 Attività e proposte del Ministero della Salute
Dr.ssa Gaetana FERRI
- 11.30 Attività in Regione Piemonte e attività del CdR
Dr. Riccardo ORUSA
- 11.45 Attività in Regione Emilia Romagna a livello locale - Dr. Mauro Ferri
- 12.00 Attività in Regione Lombardia
Dr. Marco FARIOLI
- 12.15 Attività in Provincia di Piacenza
Dr. Enrico MERLI
- 12.30 Ruolo e attività dell'esperto Faunistico
Dr.ssa Emanuela GIOIA
- 12.45 Interventi liberi
- 13.00 *Chiusura Lavori*

Figure 9. Program and details of the organized conference.

CONCLUSION

In a fast changing world with an increasing number of emerging diseases affecting wildlife, domestic animals and humans, the need for effective wildlife health investigations including both surveillance and research, is now widely recognized. The lack of surveillance schemes is often mentioned as a cause of emerging diseases. In contrast, wildlife health surveillance produces knowledge that benefits at least three different agencies, namely animal health, public health and conservation (Boadella et al., 2011).

The approach of the present study on surveillance is in agreement with the recommendations described by Boadella et al. (2011) for monitoring of wildlife diseases, as underlined also by Ryser-Degiorgis (2013): (1) communication and collaboration (human dimension, networking and publication); (2) use of synergies and triangulation approaches; (3) investments for the long term; (4) systematic collection of metadata, i.e., information on the sampled animals such as age, sex and geographical origin; (5) harmonization of definitions and methods.

Participatory approaches, networking and trans-disciplinary communication are key factors in efficient wildlife health surveillance. Firstly, reports of outbreaks of diseases and the submission of carcasses largely depend on disease awareness, personal interests and the involvement of the public and of field professionals. Secondly, target surveillance (dealing with infectious pathogens) generally require an effective collaboration with hunters, as the carcasses of hunted animals are an irreplaceable source of samples. In this context, direct human contacts are essential for a sustainable surveillance system. Close interactions with field partners and regular feedback should be an integral part of any project requiring wildlife samples. Sharing knowledge is a bilateral process in which all involved partners give and receive. The involvement of hunters association, hunters, wildlife managers let us to collect not only biological samples, but also essential information from the field. In particular, complete data that

including specific species affected, number of dead animals, precise geo-location, and any pertinent epidemiological information such as habitat, proximity to domestic farms, historical sanitary data on domestic animals, provide necessary information that enables appropriate disease outbreak investigations. These informations are basilar for planning disease prevention and mitigation strategies making wildlife diseases surveillance a real and useful tool in one health perspective.

The application of the recommended approach (Boadella et al. 2011; Ryser-Degiorgis 2013) let to identify zoonotic disease as *M. caprae* in red deer, evaluate the natural prevalence of disease with high value for domestic as Aujeszky disease in wild boar, define through modelling the epidemiological trends of wildlife specific disease as EBHS in hare and standardize a new way of sampling and analyzing blood for EBHS. From the present experiences, it's more evident that effective wildlife disease surveillance depends on knowledge of the biological characteristics of the target populations, as well as changes in population sizes and in geographical distribution over time. Such knowledge is required in order to design appropriate sampling protocols for disease surveys, to develop disease contingency plans, to assess the risk of disease transmission to other species, and to guide wildlife management strategies in general. For these reason, three actions can help to improve knowledge on wildlife diseases and capacity to deal with their consequences on animal and human health as well as conservation: (1) extend surveillance schemes to the not yet included regions and taxa, (2) improve coordination between surveillance schemes and other wildlife monitoring and (3) promote multidisciplinary research on the relevant wildlife diseases linked with local health situation.

The present study was based on active surveillance, since targeted (or active) surveillance is essential to known the status of diseases of economic or public importance amongst wildlife, such as the selected diseases in the present study. In any case, the activities performed with stakeholder were addressed in the retrieval of dead/sick animals too, since they are the target of

wildlife general (or passive) surveillance. In fact, there is a clear positive correlation between the case fatality rate of an infection and the probability that the passive surveillance has to detect it early.

Surveillance and monitoring programs are the first steps toward providing an appropriate awareness of the health status of wildlife populations. Justification for developing and maintaining such a capability includes the need for knowledge to: manage wildlife populations and biodiversity, limit risks related to animal export trade and translocation of animals and protect public health. Wildlife disease monitoring programs, as the present study, integrated within existing national animal health surveillance infrastructures are essential to adequately respond to wildlife diseases. Such systems should be developed at a larger scale. Each Province, Region and, at European scale, State should be able to provide relevant information on the health status of wild populations. To help other provinces and regions developing surveillance systems, it may be interesting to provide guidelines with different modalities in function of the specific epidemiological situation. Standardization of protocols between the different areas would permit a better global and harmonized evaluation of diseases status, and would allow the implementation of an efficient surveillance system at a large scale. A better surveillance of wildlife diseases implemented in an integrated system involving international, national and local actors would be of major relevance to understand the epidemiology of diseases and subsequently their control.

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