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Research Article

Exploring the use of red fox (*Vulpes vulpes*) counts during deer censuses as a tool to evaluate the fox population trend in the framework of disease surveillance

Federica Obber[1], Katia Capello[2], Paolo Mulatti[2], Monica Lorenzetto[2], Stefano Vendrami[3], Carlo Vittorio Citterio[1,*]

[1] *Istituto Zooprofilattico Sperimentale delle Venezie - SCT2 Belluno, U.O. Eco-pathology. Via Cappellari 44/a – 32100 Belluno – Italy*
[2] *Istituto Zooprofilattico Sperimentale delle Venezie – SCS4 Veterinary Epidemiology. Viale dell'Università 10 – 35020 Legnaro (PD) – Italy*
[3] *Provincia di Belluno – Wildlife Management Office – via S. Andrea 5 – 32100 Belluno – Italy*

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Abstract

Improving the knowledge on the distribution and status of the wild populations is desirable to inform strategies for disease surveillance, early detection and control. This is the case for the red fox in North-Eastern Italy, where rabies has been eradicated after a recent epizootic but the risk of its re-introduction through the eastern border is still acknowledged. Nevertheless, the systematic collection of data concerning fox distribution and dynamics remains difficult for many reasons, among which the very limited interest for this species and the land use changes. Since specific research performed by trained personnel, needed for providing accurate estimates of the population size and density, implies high expenses and can be maintained only for limited periods of time in small sample areas, a possible option is to exploit methods as cost-effective as possible, with the aim of a continuous monitoring at least of the fox population trend in time. For this aim, in this paper we explore the use of the spring night censuses for the red deer as a tool for the systematic collection of fox counts. This five-year study was carried out in the alpine province of Belluno, along the course of two subsequent epizootics in the fox population: the first caused by rabies and the second by canine distemper. The fox relative abundance (in terms of index ok kilometric abundance – IKA) was examined in the light of the consecutive presence of these two viral diseases by the passive disease surveillance data, in order to approach the use of spring night counts in detecting significant variations in the fox population size. The method herein proposed appears promising in the detection of these variations, such as those caused by severe epizootics, and further investigation aimed at its validation is worthwhile for both informing passive surveillance on disease and research in fox ecology.

Introduction

Improving the knowledge on the distribution and status of wild populations is desirable to inform strategies for disease surveillance, early detection and control. In recent years, wildlife populations have been increasingly involved in both livestock diseases and zoonoses (Jones et al., 2008), with well documented examples all over the world (Jakob-Hoff et al., 2014b). Focusing on Europe (see Gortazar et al., 2007), the list of pathogens shared between wildlife and livestock includes a consistent number of important zoonoses (e.g. rabies, tuberculosis, brucellosis), diseases with a heavy economic impact (e.g., besides the aforementioned diseases, classical and African swine fever, HP avian influenza, Newcastle disease) and diseases with important conservation concerns (e.g. canine distemper virus and sarcoptic mange). Nevertheless, a substantial gap remains between wildlife disease surveillance and surveillance in livestock, which is mainly due to a lack of knowledge of the demographic parameters of wildlife populations. For some species, basic data such as geographical distribution, population size and natural mortality are very few, so that relevant and original information could be found not only in specific ecological studies, but also in the field knowledge and files of various stakeholders, as wildlife managers, hunters and various associations/organizations dedicated to wildlife protection, conservation and management (Jakob-Hoff et al., 2014a).

Focusing on the main subject of the present paper, the red fox (*Vulpes vulpes*) represents the main reservoir of wild rabies in Europe (EFSA AHAW, 2015), also playing a basic role in the epidemiology of other zoonoses as *Trichinella* (Pozio and Zarlenga, 2013) and *Echinococcus multilocularis* (Ito et al., 2003; Rataj et al., 2010; Mackenstedt et al., 2015) and being involved in diseases with a considerable conservation impact (as canine distemper - Monne et al., 2011; Origgi et al., 2012; Di Sabatino et al., 2014). Nonetheless, basic data on its distribution and dynamics are incomplete in Europe, so that "collaboration between epidemiologists and wildlife experts is recommended to facilitate data collection and estimation of population parameters" (EFSA, 2014). The reasons determining this lack of knowledge may be different but, in the specific context of North-Eastern Italy, this is mainly due to both very limited interests (including a hunting interest) in the red fox in the area (see for example the URL http://www.cacciatoritrentini.it/lo-status-della-volpe/32-76/ - in Italian), and to the considerable variability from year to year also in the fox population control activities to prevent damage to rural farming. Actually, the use of hunting statistics, although suggested by the European Commission (2002) as acceptable for estimating fox trends at a regional level and being probably the most cost-effective method, appears in North-Eastern Italy as not reliable. Notwithstanding, in this area the availability of basic information on the red fox population would be crucial, also considering its geographical location, for the surveillance and control of emerging or re-emerging diseases. The reappearance of rabies in Italy, in October 2008, represents a recent example of the situation described above. The last rabies outbreak in North-Eastern Italy, in 2008-2011, was linked to

*Corresponding author
Email address: ccitterio@izsvenezie.it (Carlo Vittorio Citterio)

the epidemiological situation in the adjacent Balkan region (De Benedictis et al., 2008): the first case of the epizootic occurred in 2008 in the Friuli Venezia Giulia region close to the Slovenian border (De Benedictis et al., 2008), then followed by a wide and fast westward spread of the disease. Relying on previous experiences in the same area (Balbo and Rossi, 1988; Mutinelli et al., 2004) was not an adequate approach to control and eradicate the disease (Mulatti et al., 2011a). In fact, the use of the sole manual vaccination failed in stopping rabies, while eradication was achieved only after a series of aerial vaccination campaigns, starting in 2009 (Capello et al., 2010) and lasting four years, ultimately leading to the reacquisition of the rabies-free status in 2013 (Mulatti et al., 2011b, 2013; EFSA AHAW, 2015). The rapid spread of the disease front and the presence of cases at altitudes higher than 1500 metres above sea level, previously considered as unfavorable for foxes (Mulatti et al., 2011a), showed an unexpected temporal and spatial pattern, suggesting that modifications of the ecosystem might have exerted unexpected effects on the population dynamics and distribution, thus influencing the disease patterns. Several sub-lineages of rabies virus were still circulating in the Balkan countries after eradication from Italy (McElhinney et al., 2011; Fusaro et al., 2013) and, although the rabies situation in the Balkan region appears as definitely more favourable in recent years (for an updated picture of the rabies cases distribution in Europe, see http://www.who-rabies-bulletin.org), the risk of a virus re-introduction through the eastern border of Italy is still acknowledged (EFSA AHAW, 2015). Basic information concerning the distribution and status of the red fox population would therefore be desirable to inform strategies for rabies surveillance and early detection. Interestingly, this kind of information would also be helpful for surveillance of other zoonoses, as *Trichinella* and *E. multilocularis*, as well as for pathogens with potential conservation issues as Canine Distemper Virus (CDV), which has been affecting the same areas (Monne et al., 2011; Nouvellet et al., 2013), where it was still circulating in 2017.

Although specific research, such as den use assessing (Keuling et al., 2010), fecal density counts (Webbon et al., 2004), distance sampling (Ruette et al., 2003a; Newsome et al., 2014), capture-marking-recapture and radiotracking (Baker et al., 2000) would allow to obtain high quality data and reliable estimates of the population density, these techniques generally imply high expenses (Delahay et al., 2009; Grilo et al., 2009) and their use is often limited to a restricted period of time and smaller sample areas (Elphick, 2008). Therefore, to maintain a consistent basic surveillance in the field, it would be paramount to explore methods that allow, at least, to evaluate the fox population trend in a way as cost-effective as possible (Vine et al., 2009). Such a method should be sensitive enough to detect major variations in the fox population abundance, such as those caused by important epizootics, in order to evaluate and inform passive surveillance at a local/regional level and, as a positive side effect, promote a continuous training of professional and volunteer field personnel.

The aim of this work is to explore and evaluate one of these possible methods, presenting a five-year study aimed at the evaluation of the trend of the fox population in an area of North-Eastern Italy (Belluno province), by exploiting the spring night censuses of the red deer (*Cervus elaphus*) as a tool for the systematic monitoring of fox relative abundance. Fox abundance results have been compared, by plotting, to passive surveillance data, and in particular to the trends of rabies and CDV, to evaluate their use in a sanitary perspective, under the hypothesis that severe disease in a naïve population would cause a population decrease detectable even by basic and cost-effective methods. In fact, both rabies and CDV heavily affected the Belluno province (e.g., 216 out of the 287 rabies cases in North Eastern Italy during the 2008–2011 epizootic came from there), making this study area very suitable to test this hypothesis.

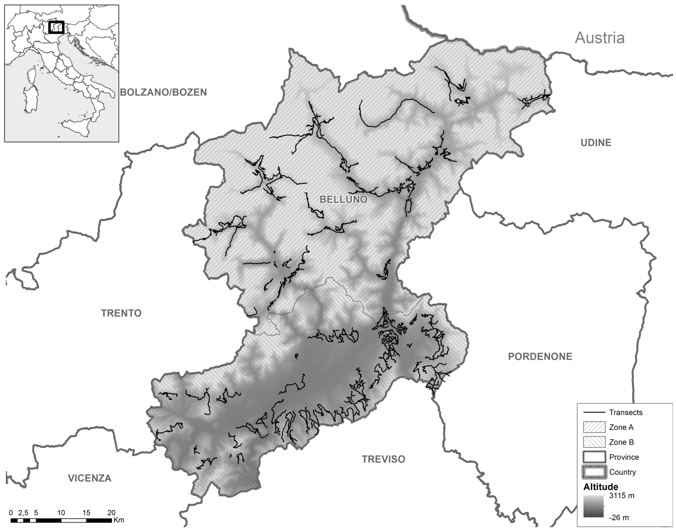

Figure 1 – Distribution of the transects for spring deer census and fox spotlight night counts in Belluno province.

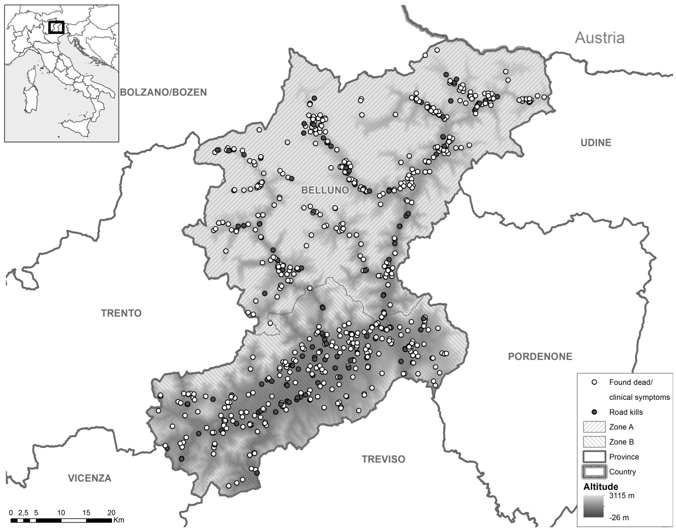

Figure 2 – Distribution by finding description of the foxes collected under passive surveillance in 2010–2014 in Belluno province (foxes culled due to clinical symptoms have been merged with found dead for a better readability of the map).

Materials and methods

The study area is represented by the territory of the Belluno province, in the North-Eastern Italian Alps (lat. min, lon. min: 45°52′55.56″ N, 11°40′0.84″ E; lat. max, lon max: 46°40′55.20″ N, 12°45′1.44″ E; CRS: WGS84). This area covers about 3,600 km^2 and is mainly mountainous, with altitudes spanning from less than 500 metres to over 3000 metres above sea level (Ramanzin and Sommavilla, 2004). The southern area comprises mainly agricultural crops and deciduous forests. Temperatures are mild (yearly average 13–14°C) with abundant precipitation (yearly average 1400–1500 mm). Further north, from 800 to 1600 m a.s.l., the province comprises mixed wood forests: climate is intermediate, with colder temperatures (yearly average 6–7°C) and less precipitation (yearly average 1300–1400 mm) than in the south. The forest in the most northern part of the province at high altitudes is coniferous and climate is mountainous (yearly average temperature 4–5°C; yearly average precipitation 1100 mm). According to Zannèse et al. (2006), spatial analysis of environmental variables (vegetation cover, topography and climate) divided the whole area into two main biogeographical regions (Fig. 1 and 2), corresponding to Zone A (northern Belluno province) and Zone B (southern Belluno province). The present study is based on data collected from 2010 to 2014 in the context of disease surveillance on foxes, namely for rabies and CDV, and on fox spotlight counts carried out during the spring night censuses for red deer. It must be noticed that in this paper the generic word "census" has been avoided for foxes, but it has been maintained for deer for a better readability, although also for this species the activities consisted in relative abundance estimates.

Surveillance data

Only foxes collected in the framework of passive surveillance activities were considered for this work, assumed as a proxy of the natural mortality of foxes for different causes, including diseases. In the passive surveillance, we also included road kills and foxes culled because they showed evident clinical symptoms (mainly respiratory and/or neurological signs). Animals actively collected, as foxes culled for monitoring the oral vaccination efficacy (according to European Commission, 2002) or legally hunted for sport or population control purposes, were excluded, basically for the following reasons: i) no coordinated sport hunting of foxes is present in the study area, while the interest in this species is very limited in both time (winter) and space (only in some municipalities in the southern part of the province); ii) fox population control to prevent damage to rural farming is performed in summer, but this activity is not consistent in time and space and is not performed in the whole area; iii) from 2010 to early 2013, in the whole study area fox hunting or population control largely coincided with activities of culling for bait uptake monitoring. According to different Authors, uncoordinated control programs are not able to significantly reduce fox density (Gentle et al., 2007; McLeod et al., 2010; Saunders et al., 2010; McLeod et al., 2011; Towerton et al., 2011; Newsome et al., 2014): therefore, considering the poor interest in foxes and the lack of coordinated and diffused sport hunting or population control activities, we assumed that neither sport hunting nor population control could have exerted a significant impact on the fox population size and density in the study area. The strict and continuous collaboration between the IZSVe and the Operators for passive surveillance in the field, in particular the Belluno Provincial Police in cooperation with Carabinieri Forestali (former Corpo Forestale dello Stato) of Belluno, allowed to assume a homogeneous intensity of surveillance in the study area through the years.

Data consistently available for each collected fox included the geographical location, the date of finding and the results of laboratory tests for both rabies and CDV (as described by De Benedictis et al., 2011 and Monne et al., 2011). In case geographical coordinates were not available, information on the location was based on administrative units (e.g. municipality) where the foxes were found and other known geographical features (i.e. rivers, roads, forests, valleys, etc.), including their approximate distance to the place the animals had been found. This information was reported on a detailed map of the territory, allowing localization of the finding locations with sufficient accuracy. Finding locations were georeferenced using QGis 2.2.0 Valmiera (QGIS Development Team, 2014).

Each fox was then assigned to one out of six temporal classes of the biological cycle of the species, according to Boitani and Vinditti (1988), on the basis of the finding time:

1) January-February: mating period;
2) March-April: cubs birth period and early denning period, when cubs remain in the dens or in their immediate surroundings;
3) May-June: cubs spend time and become visible also outside the dens, seeking for food;
4) July-August: cubs begin to be more independent;
5) September-October: cubs begin to move out of their family group;
6) November-December: dispersal period, when cubs move to find, establish and mark a new territory.

This schematic subdivision was used in the graphical representations, as a proxy of the mortality trend in relation to the fox ecology, since both surveillance intensity and the status of carcases submitted for the analyses allowed us to assume the finding date in the field as an acceptable proxy of the actual date of the death when framed into bimestrial temporal classes (Belluno Provincial Police, personal communication).

Spotlight counts

Spotlight fox counts were carried out by local hunters and game wardens of the Belluno Provincial Police during the yearly census scheduled for red deer, in 75 transects (Fig. 1) designed by wildlife managers to be as representative as possible of the study area (Ramanzin and Sommavilla, 2004). The distribution of the sampling transects was planned to include main and minor public roads and aimed at minimizing the flushing of animals from one transect to another. To further reduce possible flushing, all sessions of the whole area were scheduled and performed simultaneously (once a week). Moreover, a check of possible double counts was performed at the end of each single day of survey. Therefore, the main limitation of the sampling method was its strict correlation with the presence of roads, which are often absent at the highest altitudes (Fig. 1). In the study area, deer census is scheduled in April, when red deer are detectable at night in the pastures, browsing on the spring grass shoots. The censuses exploited for fox counts in this study were performed from 2010 to 2014. All spotlight counts were done from 10 p.m. to midnight, also coinciding with the peak of fox activity. For each transect, the team consisted of two people: a driver and an observer. Each team drove at a speed of 10–15 km/h along each transect, and portable spotlights were used to illuminate the surrounding open area. Each transect was covered three to five times per year. For each transect, the index of kilometric abundance (IKA=number of foxes seen/km), an index frequently used by wildlife managers in the field, was calculated in each session. In case of no foxes detected during the session/s, IKA was calculated by adding a value of 0.5 according to Ruette et al. (2002). Considering that the period in which fox counts are performed is short (one month) and ecologically homogeneous for the species (Sharp et al., 2001), for each transect only the highest count of foxes was taken into consideration for IKA calculation and statistical analyses, as an expression of the minimum ascertained fox number along the transect itself.

Table 1 – Yearly distribution of fox findings in Belluno province from 2010 to 2014.

Year	Culled with clinical symptoms	Road kills	Found dead	Total
2010	13	29	282	324
2011	3	36	96	135
2012	12	26	107	145
2013	17	14	67	98
2014	4	13	17	34
Total	**49**	**118**	**569**	**736**

Table 2 – Results of LMM analysis. Ls-means and standard errors of IKA index, stratified by year and biogeographical area (Zone A and B). Ls-means and standard errors are reported in the original scale of the variable, while statistical test are referred to the transformed variable.

Effect		IKA Ls-Mean	Std. Err.	F value	*p* value
Year	2010	0.107	0.011	5.09	<0.001
	2011	0.119	0.013		
	2012	0.168	0.018		
	2013	0.133	0.014		
	2014	0.111	0.012		
Zone	A	0.134	0.017	0.64	0.426
	B	0.118	0.013		

Data analysis

The evaluation of IKA index was performed by means of a Linear Mixed Model (LMM), after log-transforming the variable to allow a better fit. Fixed effects of the model were transect location according to Zone A or B, year and their interaction. Notice that information about Zone A and B was included in the model in order to take into account the environmental variability, including possible ecological and anthropic factors, between the northern and southern province, thus increasing the compliance of the method to the field situation. In fact, our purpose was outlining the general fox population trend across the years, also evaluating similarities or differences in its shape between different areas, while not pretending to estimate fox abundance/density or to compare these between different areas. To model multiple meas-

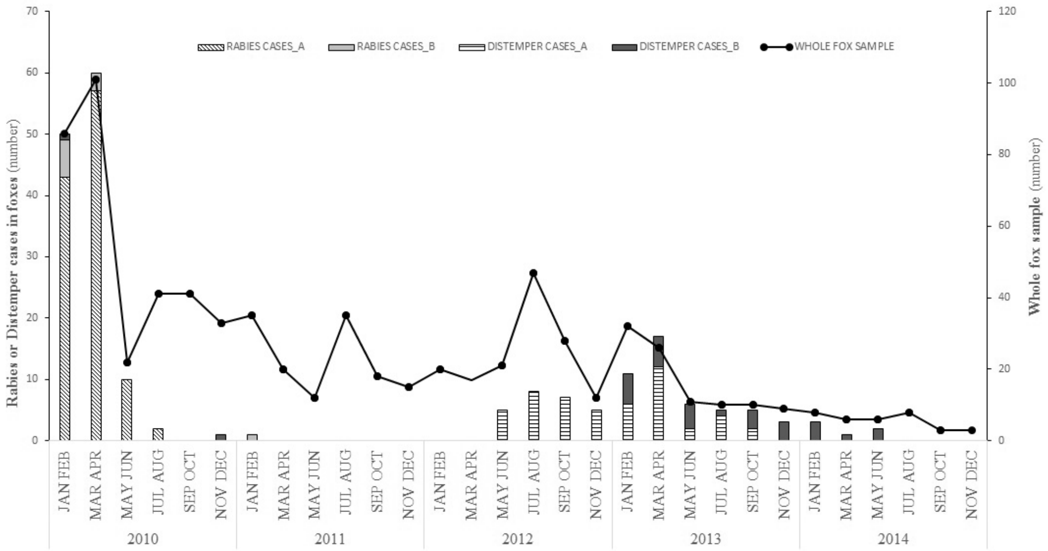

Figure 3 – Distribution by year of the study, bimestrial temporal class and biogeographical area of rabies and distemper cases in foxes collected under passive surveillance in Belluno province, plotted in comparison to the total fox sample distribution.

ures made on the same transects, the year was also included in the random part of the model as a repeated effect. The results were expressed as Least-Squares means (LS-means) and standard errors. Statistical analyses were performed using the software SAS v.9.3.

Concerning passive surveillance, since most of this activity was performed by the same personnel, a consistent intensity was assumed through the study period. As quoted above, the current knowledge of the fox population status and ecology in the study area did not allow a proper statistical comparison between IKA and disease surveillance data: therefore, a descriptive analysis was performed by plotting their trends and evaluating their biological compliance.

Results

Passive Surveillance

A total of 736 records were submitted for analysis in the frame of the passive surveillance (Fig. 2). The altitude range of fox findings was 191–2,289 m a.s.l. The yearly distribution of fox findings is reported in Tab. 1, while Fig. 3 focuses on the distribution of the rabies and distemper cases in each biogeographical area across years and bimestrial temporal classes.

The peak of rabies occurred in early 2010, with a vast majority of cases in Zone A, a lower number of cases in Zone B and concurrent sporadic (n=2) CDV cases in Zone B too. In early 2011, the last rabies case occurred in Zone B. Then, after rabies eradication and about a year with no rabies or distemper, CDV re-emerged in the fox population, although not concurrently in the two biogeographical areas: Zone B was affected by this second distemper wave about 8 months after Zone A.

Spotlight counts

The results of the statistical model applied to IKA are reported in Tab. 2 and Fig. 4. A significant association with the year was observed (p<0.001). In both Zone A and B, IKA peaked in 2012, and subsequently decreased in 2014. IKA showed in 2012 an average increase of 0.06 foxes per kilometre (about 60%) compared to 2010 (p<0.001), while a specular decrease was then observed until 2014 (p<0.001). Regarding the area, no overall statistical differences were observed (p=0.426), given the other effects in the model. Notwithstanding, the interaction between area and year resulted as statistically significant (p=0.033), principally due to a different trend observed between 2012 and 2014 (Fig. 4). In fact, in Zone A IKA sharply decreased between 2012 and 2013, while in Zone B a similar decrease was observed one year later, between 2013 and 2014.

Discussion

To study red fox populations, two main approaches are available: censuses aimed at approximating the effective abundance and density, or methods aimed at obtaining relative abundance indices (Beltràn et al., 1991; Wilson and Delahay, 2001; Sadlier et al., 2004; Keuling et al., 2010). Concerning the former approach, although techniques to extrapolate and model the results from smaller sample areas to larger territories are available (Elphick 2008), the main drawbacks derive from the consideration that the evolution of the environment would require a continuous monitoring. Maintaining a research project dedicated to the fox, carried out by researchers and field personnel in large areas and in a time continuum, seems unrealistic for both logistic and economic reasons (Lancia et al., 1994; Gaidet-Drapier et al., 2006). Therefore, a more cost-effective system should be pursued to get consistent fox abundance estimates through the years, and relative abundance could fit with this purpose (Wilson and Delahay, 2001; Acevedo et al., 2008).

Concerning in particular spotlight counts, many examples of their use to estimate relative abundances of the fox and other terrestrial mammals are present in literature, encompassing a long period of time (e.g. Stahl, 1990; Vincent et al., 1991; Maillard et al., 2001; Ruette et al., 2002; Schauster et al., 2002; Reynolds and Short, 2003; Ruette et al., 2003b; Sadlier et al., 2004; Marchandeau et al., 2006; Mitchell and Balogh, 2007; Preatoni et al., 2012). Heydon et al. (2000) argued that the use of roads seems the only way to grant a sufficient length of the transects and a low degree of disturbance, since a vehicle travelling along roads may cause fewer disturbances than an observer walking across fields. On the other hand, the use of roads as transects has been opposed by some authors (Buckland et al., 1993), since the area adjacent to a road may be unrepresentative of the actual habitat of the species and foxes could behave differently in their proximity. By contrast, other examples from literature show that areas adjacent to roads may be even more suitable than expected for foxes, according to different environmental features. As an example, Mahon et al. (1998) showed a fox preference for roads over other habitats in a sand-dune desert where roads constituted natural runways, and Ruette et al. (2003a) hypothesized that in the rural areas of Europe, due to the high road density and foxes being long accustomed to human presence, foxes avoidance of road proximity would be unlikely.

Concerning the sensitivity of spotlight counts in detecting fluctuations in the fox abundance, Stahl and Migot (1990) evidenced a good correlation between spotlight counts and changes in population size. However various factors could limit spotlight count sensitivity. As an example, according to Sharp et al. (2001), spotlight counts could not detect small changes in population abundance. Another limitation of

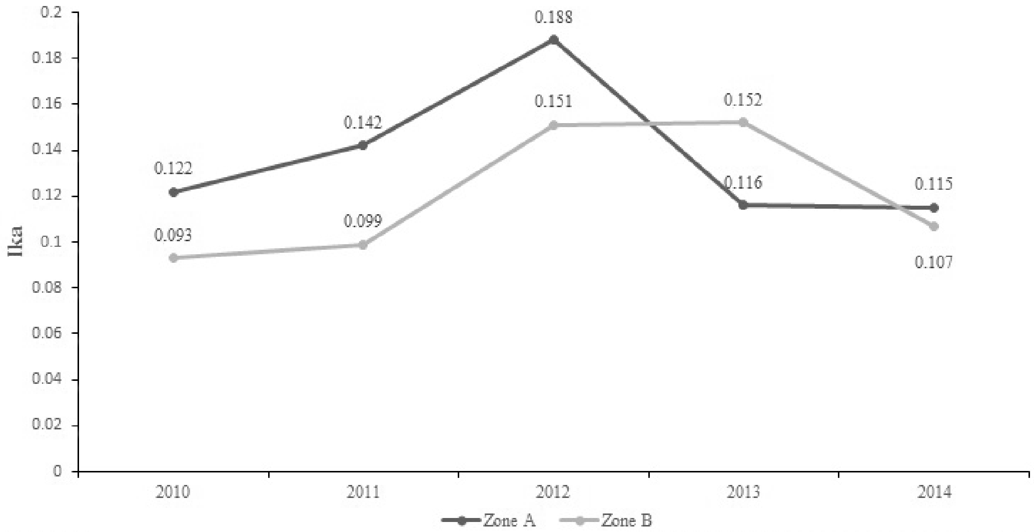

Figure 4 – Trend of IKA during the study period, stratified by biogeographical area. The values in the graph are the LS-means obtained by LMM analysis.

this method is related to the number of animals escaping before being detected, the magnitude of which is difficult to evaluate in foxes (Ruette et al., 2003a). Finally, since this method does not take into account variations among different habitats that greatly influence fox home range and detectability, comparisons are difficult and questionable. Undoubtedly, all the issues above would be critical when comparing relative abundance estimates between different areas, different ecological phases or periods definitely far apart in time. By contrast, as in the present work, the same issues would not affect the results when comparing repeated measures, taken in the same transects and in a single ecological phase (Ruette et al. 2002), thus making the estimates herein acceptably consistent along a time span of five years.

Coming to the results of the present work, the value of about 60% more foxes per transect observed in 2012 compared to 2010 and 2014 is not negligible, also considering that a few factors, as extreme weather conditions, appear to have a significant effect on fox counts (Stahl, 1990), thus accounting for an acceptable consistency through time. Concerning the red fox ecology, territoriality is still considered as a key factor in the social structure and spatial distribution of fox populations, and therefore in disease transmission, maintenance and control (Vos, 2003; Thulke et al., 2004; EFSA AHAW, 2015). According to this view in the present study, comprising of repeated measures in a single ecological period, we assumed a consistent stability in the fox spatial distribution, where the number of foxes observable along a transect depends on the number of territories (each surrounding a fox den) the transect passes through. In general, fox populations show an increase in abundance from late spring through to autumn (independence and dispersal of juveniles), then declining during winter and early spring (Sharp et al., 2001). Regarding our study, the number of foxes observable in April (cubs birth period and early denning period), when red deer censuses are scheduled and fox counts are performed, is considered as medium-low (Boitani and Vinditti, 1988; Stahl, 1990; Ralls and Eberhardt, 1997; Sharp et al., 2001). Although there is no doubt that such a situation represents one of the main limitations of our approach, at the same time it may warrant a consistency of the home ranges, improving the significance when comparing the data between the different years (Weber et al., 1991). This is also supported by the results of other Authors (Ralls and Eberhardt, 1997), who suggested that fox spotlight counts should not exceed the span of one month to obtain a better statistical power.

Relative abundance of foxes resulted as associated to the year, interestingly showing a pattern biologically consistent with the trend in fox diseases derived from passive surveillance. In both Zone A and B fox count activities started in spring 2010, concurrently with the rabies epizootic, showing their lower values and then slightly increasing in spring 2011, just a few weeks after the last rabies case (in Feb 2011), until peaking in April 2012, after about 14 months with no rabies or CDV cases observed. New CDV cases were detected just a few weeks after the fox count campaign in 2012 in Zone A, while in Zone B distemper re-appeared only in 2013. In the same way, CDV lasted until September/October 2013 in Zone A, while Zone B remained affected by distemper until the first half of 2014. In spring 2013, in Zone A relative abundance of foxes sharply decreased compared to 2012, while it remained stable in Zone B. An opposite trend was observed between 2013 and 2014, when relative abundance decreased in Zone B, while remaining stable in Zone A. Finally, results of 2014 showed that both Zone A and B came back to values similar to those observed in 2010. Considering that in a naïve fox population rabies can affect 60–80% of individuals (Grimm et al., 1996), it is assumable that the increasing relative abundance from 2010 to 2012 reflected a true increase in the population size due to the eradication of this disease. According to Boitani and Vinditti (1988), the gap due to a population decrease induced by a rabies epizootic may be filled in 2–3 years if human pressure remains low to moderate. This might have been the case in our study area where, after the first rabies case, oral vaccination was immediately started, vaccination campaigns lasted from January 2010 to December 2012 (Mulatti et al., 2013) and hunting pressure was consistently low, thus allowing the fox population to quickly recover by both new births and immigration of foxes from neighbouring areas (Gentle et al., 2007). In the same way, the re-emergence of canine distemper could have been involved in the subsequent population decrease after 2012, all the more reason considering the compliance observed between the period of disease presence and the fox relative abundance decrease in each biogeographical area. However, since the dynamics of CDV are still unclear in the European wild carnivore populations, further work is needed on this hypothesis and, in general, to assess the magnitude in which a population variation can be considered as "significant" in a disease surveillance perspective. In the case of CDV, since this disease has been apparently affecting the wild carnivore population of North-Eastern Italy by subsequent epidemic waves and the virus is still circulating in the area, future data collection in parallel with passive surveillance may provide relevant information.

Of course, we are aware that fox ecology is definitely more complex than briefly outlined herein. Territories appear as not static for this species: drifting can occur anytime (Vos, 2003) and recent research, mainly in urban foxes, has shed a new light on the actual degree of territoriality of the fox and its home range stability, showing possible spatial overlap mainly in relation to landscape composition and food resources (Newsome et al., 2014). Even if the proposed method, aimed at a field disease surveillance purpose, cannot take into account such a dynamicity, nevertheless it could represent a basis for the "collaborative exercise" (Jakob-Hoff et al., 2014a) needed for a step forward in the knowledge of fox demography, also as basic information to be exploited in wildlife disease risk analysis and contingency plans (European Commission, 2015). In this sense, although our work had neither the purpose of estimating nor comparing fox abundance and density between different areas, it should be noticed that our results did not show any effect of the biogeographical location on IKA. Even though we did not take into account possible environmental factors influencing fox detectability, we cannot exclude that harsher areas (as the northern Belluno province) are nowadays more favorable for foxes, hosting a number of individuals similar to milder areas (as the southern Belluno province) if not even higher. The higher number of rabies cases in northern compared to southern Belluno province further supports the hypothesis of a significant variation in the population dynamics of the red fox with respect to the past, that could be due to factors as land use changes (Cagnacci et al., 2014), depopulation of mountain areas and dramatic increase of wild ungulates.

In conclusion, the method herein proposed appears as promising in detecting major variations in the fox population, such as those caused by severe disease epizootics. In this perspective, it may already provide relevant information to evaluate the efficacy of passive surveillance: as an example, an "*a posteriori*" comparison between the number of dead/diseased foxes found in the field in large areas and their observed population trend would allow to detect possible "low surveillance situations", whenever the number of suspect cases delivered to the lab appears too low if compared to major decreases observed in fox relative abundance. Moreover, it could provide a simple and cost-effective basis for a continuous training of professional and volunteer field personnel, to keep a constant monitoring of the fox population even in "peace time". Further action seems, therefore, worthwhile in order to validate this approach, in particular i) by enhancing collaboration and increasing the value of the specific knowledge typical of local hunters and game wardens (Gaidet-Drapier et al., 2006) and, ii) by promoting specific experimental studies (e.g. distance sampling, radio-tracking, home range and density estimates - Ruette et al., 2003a; Sadlier et al., 2004; EFSA AHAW, 2015) that would provide the knowledge needed for a proper statistical comparison between population and disease data. Interestingly, the same information would be helpful for the study and surveillance on other pathogens important for public health (as *Trichinella* spp. – Pozio and Zarlenga, 2013; and *Echinococcus multilocularis* - Rataj et al., 2010) and strictly related to the ecology of the host within the biocenoses.

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Supplemental information

Additional Supplemental Information may be found in the online version of this article:

Table S1 Ls-means and standard errors obtained applying GLMM analyses on fox counts.

Table S2 Pairwise differences of Ls-Means of fox counts between years in 2010–2014.