Sarcoptic mange and other ectoparasitic infections in a red fox (*Vulpes vulpes*) population from central Italy

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Abstract

Fifty red foxes (*Vulpes vulpes*) from the district of Pisa (central Italy) were examined for ectoparasites. Sarcoptic mange was diagnosed on the presence of clearly visible skin lesions with confirmatory demonstration of *Sarcoptes scabiei* at parasitological and histopathological analysis. Ticks and fleas were collected directly from the carcases during *post mortem* examination, fixed and identified by morphological examination. For the detection of ear *Malassezia* and mite infections, cytological and parasitological examinations of ear wax samples collected by means of cotton swabs were performed. Data were statistically analysed. An overall prevalence of 84% for ectoparasitic infections was found in examined red foxes. In regard to isolated ectoparasites, 38%, 8%, 82%, 6% and 8% of foxes resulted positive for *S. scabiei*, *Otodectes cynotis*, *Malassezia* spp., fleas (*Archaeopsylla erinacei erinacei*, *Pulex irritans*, *Ctenocephalides canis*) and ticks (*Ixodes ricinus* and *Rhipicephalus sanguineus*), respectively. *Malassezia* ear infection was significantly more prevalent in animals older than 1 year ($P<0.01$). Prevalence (38%), severity of lesions and poor body conditions observed in most *Sarcoptes*-infected animals indicate that sarcoptic mange should be considered the most important ectoparasitic infection of red foxes in the examined area.

Key words: red fox (*Vulpes vulpes*); ectoparasites; prevalence; sarcoptic mange; central Italy.

1. Introduction

Red foxes are the most widespread wild carnivores in Italy and the rest of Europe [1] and represent a possible reservoir of zoonotic, antropophilic and domestic animal ectoparasites, such as *Ixodes ricinus*, *Sarcoptes scabiei* and *Pulex irritans* [2, 3]. In the same way, red foxes may act as reservoirs for vector-borne infections in domestic animals and humans [2, 4, 5]. Among ectoparasitic infections, sarcoptic mange is a well- known cause of severe disease and high mortality rates in red foxes and it is often responsible for rapid decline in population densities [3, 6, 7, 8]. Available data on ectoparasitic infections of European red fox populations in Italy are very limited since, with the exception of a single report on flea species identified in red foxes from southern Italy [5] and on sarcoptic mange in red foxes from the western Italian Alps [9], information is almost absent in Italy. The aim of the present study was to investigate ectoparasitic species and prevalence of ectoparasitic infections in a red fox population living in central Italy (Pisa, Tuscany).

2. Study area and population

From January to December 2010, 50 red foxes of both genders (20 males and 30 females) and different ages were examined. All examined subjects were shot during the regular hunting season in the Province of Pisa, central Italy (43°N, 10-11°E), and chilled at 4°C until examination, that was carried out within 24 hours. The sampling area is characterized by large woodlands, farmland and hills with an elevation ranging from 4 m to 576 m above sea level. With an area of 2,448 square kilometres and a total population of 421,642 inhabitants, the area is densely populated (from about 8 to 865 inhabitants/km^2) (http://www.tuttitalia.it/toscana/provincia-di-pisa/42-comuni/densita/). In this area, redfox population density is of approximately 1.2-1.6 red foxes /km^2 [10]. Higher fox density (up to 2,6) data are found in hilly and farmland areas often near urban and periurban settings [11]. Fox age determination was achieved based on the weight of dried eye lenses as previously reported [12].

3. Materials and methods

All foxes underwent a complete *post mortem* examination during which body score was assessed based on the amount of retrobulbar and perirenal fat and the presence or absence of muscular atrophy. More precisely, fox body condition was scored as 1) excellent- excellent amount of fat and no muscle atrophy, 2) average- medium to small amount of fat and no muscle atrophy, 3) poor- no retrobulbar and perirenal fat and no muscle atrophy, and 4) cachectic- no retrobulbar and perirenal fat with muscle atrophy. Ticks and fleas were collected directly from the carcases at *post mortem* examination. Collected arthropods were fixed in 70% ethanol and identified microscopically with or without Hoyer's solution. For their identification, keys and description reported by Manilla [13] and Cringoli et al. [14] for ticks and by Berlinguer [15] for fleas, were used. Cytological and parasitological examination of earwax samples collected by means of cotton swabs were performed in order to detect *Malassezia* and ear mite (*Demodex* spp. and *Otodectes cynotis*) infections, respectively. Diagnosis of *Malassezia* otitis was achieved when more than 10 blastospores/microscopic field (mean number of 10 fields) at 400X were observed in Diff Quick® (Medion Diagnostics AG, Düdingen, Switzerland) stained smears. Sarcoptic mange was diagnosed based on the presence of clearly visible lesions on the skin with confirmatory demonstration of *S. scabiei* by means of parasitological and histopathological analysis. In order to rule out any concomitant diseases that could have affected the body condition score, for histopathology skin samples but also

other organ samples (liver, spleen, kidneys, lungs and heart) were collected during the *post mortem* examination, fixed in 10% buffered formalin, processed and paraffin embedded for routine histopathology. Four-µm serial sections were cut and stained with haematoxylin-eosin for general examination. A scoring system of distribution and severity of sarcoptic mange associated lesions was obtained based on a previous published classification [16]. At histopathological examination of skin samples from subjects with lesions consistent with sarcoptic mange, the presence of bacteria and yeasts was also evaluated. For parasitological analysis of mange infections, microscopic examination of skin scrapings and ear wax samples at 40X under a dissecting microscope and at 100X and 400X under an optical microscope was performed for the presence of mites and eggs, then digested in boiling 10% NaOH, centrifuged and microscopically examined (at 100X and 400X) according to the method described by Sréter et al. [3]. *Malassezia* yeasts found in ears and in skin histological sections were identified by their morphology [17, 18], while *Otodectes* and *Sarcoptes* mites were identified according to Sweatman [19] and Fain [20], respectively.

4. Statistical analysis

Data of different parasitic species isolated and biological data of red foxes sampled were statistically analysed using the statistical package SPSS Advanced Statistics 13.0 (SPSS Inc., Chicago, IL, USA). A $\chi 2$ test with the Yates correction was chosen as reference test. Statistical significance was considered when $p < 0.05$.

Results

According to age determination, 27 out of 50 red foxes examined in this study were classified as juveniles (<1 year), while the remaining 23 foxes were adults (> 1 year). Overall, 84% (42/50) of examined red foxes were found to be positive for ectoparasites. In particular, 82% (41/50) of animals resulted positive for *Malassezia* spp., compatible with *M. pachydermatis* by morphology, from ears and 16% (8/50) from skin lesions, 8% (4/50) of foxes resulted positive for *Otodectes cynotis*, 6% (3/50) were positive for fleas (*Archaeopsylla erinacei erinacei* 2%, *Pulex irritans* 2% and *Ctenocephalides canis* 2%), 8% (4/50) resulted positive for ticks (*Ixodes ricinus* 6% and *Rhipicephalus sanguineus* 2%), while 38% (19/50) of foxes resulted positive for *S. scabiei* (Table 1). Lice and *Demodex* mites were not isolated. Except for *S. scabiei*, a low number (≤ 10) of parasites per infected fox was counted in regard to all other isolated arthropod species. A significant positive correlation was found between the age of foxes and *Malassezia* in ears (P<0.01), with *Malassezia* ear infections more prevalent in animals older than 1 year old. In the *S. scabiei* infected red foxes, gross lesions were distributed mainly on the tail and focally on the distal legs (pattern 1), on the back and neck as well as the previously described regions (pattern 2) or diffusely including the head and ears (pattern 3) (Table 1). Sarcoptic lesions ranged from focally extensive moderately hyperkeratotic, proliferative and scaling dermatitis (type A), associate with few mites at histopathology, in 31% (6/19) of the infected subjects, to generalized severe hyperkeratotic lesions with thick crusts and numerous mites (type B) in 47% (9/19) of infected foxes or focal alopecic lesions without crusting and very rare mites observed (type C), in almost 21% (4/19) of the *S. scabiei* positive animals (Table 1; Figure 1.1, 2). Histopathological lesions were characterized by different degrees of epidermal edema, degeneration and necrosis, and moderate to severe areas of spongiosis, hyperkeratosis, multifocal parakeratosis and sero-cellular crusts (Figure1.3). Dermal changes included edema, superficial, often perivascular to interstitial infiltration of eosinophils and mast cells, lymphocytes and macrophages. Focally, cutaneous ulcerations could be observed with increased number of neutrophils and plasma cells around dense bacterial colonies of bacillary elements that were often associated, on the

skin, with numerous bottle-shaped yeasts showing broad based budding, consistent with *Malassezia pachydermatis* by morphology. Yeasts were constantly associated with hyperkeratotic lesions and the presence of a large number of *S. scabiei* mites (type B lesions). Large numbers of mites were seen multifocally and mainly associated with more severe hyperkeratotic and proliferative lesions (type B lesions). Mites were found embedded in the epidermal layer or free in the surface of the epidermis (Figure1.4). Focally, inside adult female mites, numerous eggs were observed.

In a *Sarcoptes* infected animal, diffusely in the renal glomeruli a focally extensive deposit of acellular, finely fibrillar to waxy, pale eosinophilic material, Congo red positive and consistent with amyloid was also observed. The amyloid was primarily present in the mesangial areas and in the subendothelium of glomerular capillaries, eventually expanding to the interstitium dissecting through surrounding tubules and vessels.

Discussion

In Europe, red fox density is highly variable [21]. In the UK, density varies between 0.98 and 4.7/km2 with an average of 2.04/km2 [22], but can be as high as 30-58 foxes per km² in some urban areas where food is superabundant [6, 21] . Fox density in Switzerland is about 1-3 fox/km² or 0.3-0.4 family/km2 [21, 23]. In other European countries, red fox density range between 0.1 and 3.2/ km^2 [24]. Density of fox population in the studied area is about 1.2-1.6 red foxes /km^2 [10], but higher densities are found in hilly and farmland areas near urban and periurban settings [11]. Thus, red fox density in the examined area should be considered similar or higher than in other European areas.

A high prevalence of ectoparasitic infections was found in the examined red fox population, and most of the isolated species have zoonotic potential [3]. However, in examined red foxes intensity and prevalence of tick, flea and *O. cynotis* infestations were low. *I. ricinus* was the most prevalent (6%) and common tick species encountered among examined animals, while only a single fox was found infected by *R. sanguineus*. Indeed, the red fox is considered a particularly important host for the maintenance and the geographical distribution of *I. ricinus*, due to foxes being able to host all three different developmental stages of *I. ricinus* [25]. Foxes can also carry a large number of ticks, may migrate over long distances, and are often attracted by periurban and urban areas [25], as in the area examined in this study. Nevertheless, prevalence of *I. ricinus* found in the examined fox population was lower than that found in other European countries, such as Spain, France, Germany and Hungary [3, 26]. Although reported, *R. sanguineus* has not been frequently observed within European wild fox populations during previous studies [26]. All the flea species isolated in this study, i.e. the human flea *P. irritans*, the dog flea *C. canis* and the hedgehog flea *A. erinacei erinacei*, have been previously reported in foxes from other European countries [3, 26], while of these species only *C. canis* has been previously reported in red foxes from Italy [5]. According to Sréter et al. [3], the isolation of *P. irritans* and *C. canis* may be indicative of the urbanisation of foxes and of closer contact with animals living in the rural and suburban areas. However, the prevalence (6%) of flea infections found in this study was low when compared with previous data [3, 26]. This finding could be explained by the variable time frame between the death of the animals and the *post mortem* examination in which the foxes were stored refrigerated. On the contrary, the prevalence (8%) of *O. cynotis*, the ear canker mite of many domestic and wild carnivores [27], found in free-ranging red foxes in this study is consistent with previous data reported for other European red fox populations [3], showing low to medium (2−17%) prevalence rates. The highly contagious mite *S. scabiei* is the etiological agent of sarcoptic mange in wild and domestic mammals and of scabies in humans and it is considered as a single species divided into several varieties which show a certain degree of host specificity [28, 29]. Epidemiological studies have showed the occurrence of cross-transmission episodes between wild and domestic animal species and zoonotic infections of *S. scabiei* in humans [30, 31, 32]. Evidence of

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sarcoptic mange transmission from foxes to dogs has been documented [6]. Sarcoptic mange is one of the most frequently diagnosed diseases in wild red fox populations in Europe [3, 33]. Data from epidemiological studies [6] suggest either that juveniles are more prone to infection due to less effective immune systems and increased nutritional stress from independent foraging and that they possibly encounter infected individuals more often than adults for their specific movement pattern. The prevalence of sarcoptic mange observed in the present study (38%) is higher if compared with previous data reported in free ranging red foxes in most European countries (14-25%) [3, 6] and in northern Italy (25.3%) [9] although in some other countries considerably higher prevalence rates (about 45−67%) have been reported [33, 34]. Considering that density of red fox population examined in the present study is higher in areas near urban and periurban settings, the possibility of direct contact and of sarcoptic mange transmission from these foxes to other animal species, mainly dogs, should be likely. Despite this high prevalence, in the area studied no reduction in the population of foxes was observed [11]. Approximately one month after exposure, infected foxes commonly develop skin lesions characterised by hyperkeratosis [35]. Severe loss of hair and progressive deterioration of body condition eventually follows and infected foxes may die within two to three months due to starvation [16, 36]. In the present study, a body score of 3 and 4, with absence of retrobulbar and perirenal fat without or with muscle atrophy respectively, was mainly associated with severe crusting and the presence of more than 3 mites/high power field at histopathology (type B lesions), suggesting that *S. scabiei* was primarily involved in the pathogenesis of the generalised wasting observed in examined red foxes. The gross lesions and the histopathological findings described in the examined population are consistent with the previous study from Nimmervoll et al. [16] in which three main presentations, i.e. focally extensive thin crusting, diffuse thick crusting and focal alopecia without crusts, were observed and associated with different degrees of *S. scabiei* infection intensity. Glomerular amyloidosis observed at histholopathology in a severely *S. scabiei* infected animal included in this study, is a further finding confirming previous data [37, 38] regarding the frequent observation of amyloid deposits in internal organs of chronically *S. scabiei*-infected subjects. Another data from this study that is consistent with previous studies [16, 39], is the almost constant association of skin yeasts with the presence of thick crusting and numerous mites, while the finding of bacteria on skin lesions was associated only with the presence of ulcers and crusting on the skin and not with the presence of mites. This observation further reinforces the hypothesis [40] according to which mites might act as a carrier for yeasts through the exoskeleton, and further suggests a possible secondary pathogenic effect of yeasts contributing to the progression of sarcoptic lesions. *Malassezia* yeasts are lipophilic organisms which are recognised members of the normal skin flora [40, 41]. On the other hand, *Malassezia* overgrowth is a very well known cause of several skin diseases of humans as well as a wide range of warm-blooded animals, including domestic and wild canids [40]. With regard to *Malassezia*, a very high percentage (82%) of foxes examined in the present study was found also positive for *Malassezia* overgrowth at cytological analysis of ear cerumen.

In conclusion, the high prevalence of ectoparasitic infections found in the red fox population living in the province of Pisa (Tuscany, central Italy) may suggest that in this area the red fox may act as a reservoir host for several ectoparasites, among which are included potentially zoonotic species and arthropod species that are reservoirs of vector-borne infections. In addition, as previously reported for other European red fox populations [3], based on prevalence (38%), severity of lesions and poor body conditions of most examined *S. scabiei*-infected animals, sarcoptic mange should be considered as the most important ectoparasitic infection of the red fox also in the area considered in this study.

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Figure 1 legend

Examples of gross and histopathological lesions observed in *Sarcoptes*- positive (19/50) red foxes (*Vulpes vulpes*) from central Italy examined for ectoparasitic infections.

1. Red fox (*Vulpes vulpes*) No 9. Example of pattern 3 distribution of gross lesions associated with sarcoptic mange infection distributed throughout the entire body surface and characterized by diffuse severe hyperkeratotic, proliferative and exudative dermatitis;

2. Red Fox (*Vulpes vulpes*) No 46. Example of pattern 1 distribution of gross lesions associated with sarcoptic mange infection, confined to the tail and hind limbs characterised by moderate to severe multifocal to coalescing areas of ulceration, alopecia and crusting;

3. Red Fox (*Vulpes vulpes*) No 38. Section of haired skin including epidermis and superficial dermis with diffuse severe orthokeratotic hyperkeratosis, hyperplasia and multifocal areas of parakeratosis associated with several cross sections of mites embedded in the epidermis or free on the epidermal layer along with numerous bacteria. Dermal changes include moderate multifocal perivascular to interstitial mixed inflammatory infiltrates. (H&E; Ob. 20X);

4. Red Fox (*Vulpes vulpes*) No 38. Haired skin, epidermis showing severe diffuse orthokeratotic hyperkeratosis, parakeratosis, hyperplasia, degeneration, spongiosis and one embedded cross section of an adult mite. (H&E; Ob. 40X)