J Vet Res 68, 551-562, 2024
DOI:10.2478/jvetres-2024-0068

Fatal *Sarcoptes scabiei* and *Demodex* sp. co-infestation in wolves (*Canis lupus*) at the Białowieża National Park, Poland – is it a consequence of climate change?

Michał K. Krzysiak[1], Agnieszka Świątalska[2], Elwira Plis-Kuprianowicz[3], Andrzej Konieczny[4], Sławomir Bakier[5], Krzysztof Tomczuk[1], Magdalena Larska[6]✉

[1]Department of Parasitology and Invasive Diseases, University of Life Sciences in Lublin, 20-950 Lublin, Poland
[2]Veterinary Hygiene Laboratory, 80-316 Gdańsk, Poland
[3]Białowieża National Park, 17-230 Białowieża, Poland
[4]Faculty of Agrobioengineering, University of Live Sciences, Lublin 20-950, Poland
[5]Faculty of Civil Engineering and Environmental Sciences, Institute of Forest Sciences, Białystok University of Technology, 15-351 Białystok, Poland
[6]Department of Virology, National Veterinary Research Institute, 24-100 Puławy, Poland
m.larska@piwet.pulawy.pl

Received: May 21, 2024 *Accepted: December 12, 2024*

Abstract

Introduction: In winter 2021/2022, a wolf population in the primeval Białowieża Forest in Poland was struck by an outbreak of severe mange caused by mixed infestations of *Sarcoptes* and *Demodex* mites. We present an epidemiological analysis of this mange which caused significant morbidity and mortality. **Material and Methods:** Ten sites known for wolf activity were monitored by camera trapping. A diagnostic necropsy and testing of a young wolf was performed to determine the causes of death. **Results:** Five young wolves with severe alopecia of the entire body and some other individuals with minor to medium mange lesions were identified by the camera surveillance. The necropsy of the carcass revealed emaciation, dehydration and anaemia with starvation as the cause of death, likely attributable to severe infestation with *Sarcoptes scabiei* and *Demodex* sp. mites. Rabies and infections with *Borreliella* sp., *Anaplasma* sp., *Ehrlichia* sp., *Francisella tularensis*, *Babesia* sp. and tick-borne encephalitis virus were excluded by specific tests. **Conclusion:** The described analysis is the first documented co-infestation of this kind in wolves. The outbreak coincided with very mild winter conditions with a high average minimum temperature, which may have favoured mite survival outside the host, and light snowfall, which may have influenced the wolves' ability to hunt. Other potential drivers of the outbreak could be the large proportion of wetland terrain, increasing number of wolves in the area and anthropogenic pressure on their habitats including the migration crisis at the Polish–Belarusian border and the increased presence of military and border forces, even despite the relief from the anthropogenic pressure from tourism due to the COVID-19 lockdown.

Keywords: wolf, mange, *Sarcoptes, Demodex,* Poland.

Introduction

The grey wolf (*Canis lupus*) is the largest carnivorous mammal of the canid family, the range of which is concentrated in the northern hemisphere, and the favoured habitats of which are the forests, plains, wetlands and mountains of North America and Eurasia. At the beginning of the last century, this species was common in Poland. In 1927, its high abundance caused it to be declared a game animal, which started the process of its culling, culminating in a resolution of the Presidium of the Government of 29 January 1955 "on the extermination of wolves". These measures resulted in the wolf's numbers falling to below 60 individuals in 1972 (52). In 1998, the wolf was placed on the list of most-protected species, taking into account their role as predators in maintaining the ecological balance. Currently, the wolf population in Poland is estimated at 2–2.5 thousand individuals (15), although the Polish Hunting Association (PZŁ) suggests that this number is a gross underestimation. Nevertheless, the Polish wolf population is among the largest in Europe (19). Three genetic lines of the species are observed in the country: the Central European Lowland, Baltic and Carpathian populations (30). Wolves are present all over the country; however, the distribution is patchy and its density varies significantly between the largest forest complexes. In addition, wolves have been increasingly observed migrating to new areas and forming new packs (64). The highest density, one of over nine wolves per 100 km^2, was noted in the Bieszczady mountains, while the lowest was in Żywiec and the Silesian Beskids and was approximately one individual per 100 km^2. The density of the wolf population in the studied area of the Białowieża Forest (BF) fluctuates around three wolves per 100 km^2, and the population comprises at least four main packs (70). Since anthropopressure and poaching of wolves on the Polish side of the BF is not as frequent as on the Belarusian side (34), the wolves' habitat there had not been disturbed as heavily as habitats had in other areas of Poland until the recent migration crisis on the Belarusian border because of the Russian invasion of Ukraine. Despite the high protection of the species in the country, the situation of the wolf is complicated for several reasons. While it may not threaten the conservation of the species, poaching or illegal shooting remains a problem. The increasing number of wolves and the human pressure on the environment force them to migrate, leading to increasingly frequent traffic accidents (63). Post-mortem examinations of wolf carcasses reveal lesions indicative of multiple injuries: not only the fatal ones, but others associated with hunting large animals, *i.e.* red deer (*Cervus elaphus*), which is the main species wolf packs prey in Poland (36), or European bison (*Bison bonasus*) which are occasional wolf prey in the BF (35). The epidemiological risks for wolf health and conservation in Poland have not yet been investigated. So far, studies on wolves have focused on the characterisation of their endoparasites, and have not explored the perspective that the wolf is a species with its conservation endangered by these parasites, but rather have regarded the wolf as a potential reservoir (5, 12, 13, 69) or indicatory species (39) with a role in the transmission of pathogens threatening humans or other carnivores, including domestic dogs (28, 62).

The current lifestyle of wolves is the result of adaptation, resilience and innate behaviour in an almost completely urbanised environment. This increasing interpenetration of the synanthropic and sylvatic environments raises the potential for pathogen transmission, many of which are zoonotic. An additional driver for the emergence of diseases that threaten protected species in particular is climate change, which may directly affect the ecology of microorganisms, their vectors such as arthropods and their reservoirs such as rodents and bats (16). An indirect effect of climate change is heat stress in animals, which affects their immune and endocrine functions, reproduction and metabolism and increases their susceptibility to infection/invasion by pathogens which are often opportunistic. Furthermore, climate change may reduce access to food and water, force migration and increase local animal density, and therefore increase interspecies and intraspecies encounters. Sudden climate events are particularly dangerous and do not allow for the adaptation which takes place when the changes gradually progress (3). Sarcoptic mange is considered a neglected disease, whereas *Demodex* is a frequent skin commensal; however, their re-emergence as pathogens may be a consequence of the recent changes in the ecosystem. The outcomes of skin parasite infestations may serve as an indicator of the health of the environment and inform the investigations of adherents to the common concept of One Health. The European wildlife species for which scabies is most frequently fatal are usually smaller predators like red foxes (*Vulpes vulpes*), raccoon dogs (*Nyctereutes procyonoides*) and badgers (*Meles meles*). In contrast, grey wolves which have scabies usually display alopecic, pruritic skin lesions with crusting which are limited to some areas on the head, hind limbs, flanks and tail and are rarely fatal.

This is the first clinical description of *Sarcoptes* mange in wolves in Poland and the first identification of *Demodex* mites in the species. Climate conditions, the geopolitical situation and wolf population dynamics are considered as possible factors associated with the progression of these infestations, which are usually non-fatal in wolves, and are discussed at the microscale of the last remaining primeval forest in Europe.

Material and Methods

Study area. The Białowieża National Park (BNP) (52°42'11"–52°48'58" N, 23°55'30"–23°56'22" E) covers an area of 10,517.27 ha, which constitutes one sixth of the Polish part of the primeval Białowieża Forest (BF). Strict protection covers 6,059.27 ha (57.6% of the Park's area), active protection 4,104.63 ha (39%) and landscape protection 353.37 ha (3.4%). Around the Park, a protection zone (buffer zone) has been created, which includes managed forests of 3,224.26 ha. The buffer zone is a game protection zone, where hunting is not practiced (26). The BNP protects the best-preserved fragment of the BF – Europe's last lowland natural forest of primeval character. The relief of this area shaped by a glacier is not very varied, ranging in the BNP from 145.5 m above sea level (at the confluence of the Braszcza and Narewka rivers) to 175.3 m above sea level (at Babia Góra near Masiewo), which corresponds to only an approximate 30 m difference in absolute height. The whole BNP lies in the basin of the Narewka river, a left tributary of the Narew. The BF is characterised by a high proportion of wetland forested areas, meadows and river valleys, which are periodically flooded. The climate is classified as moderate continental and cool with influences of the Atlantic. The average annual air temperature between 1991 and 2020 was 7.4°C, and the average annual precipitation is 638.3 mm. Most of the precipitation falls in the vegetation season (53). The BNP is characterised by high biodiversity. More than 800 species of vascular plant, more than 3,000 species of spore plant and fungus, almost 200 species of moss and approximately 300 species of lichen can be found here. More than 8,000 species of invertebrate, about 120 species of breeding bird and 52 species of mammal are found in the Park area. Besides the protected grey wolf and lynx, the BF is inhabited by the world's largest population of free roaming European bison (*Bison bonasus*) – approximately 900 animals. Other ungulates include wild boar (*Sus scrofa*), moose (*Alces alces*), roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*), the latter being the most frequent wolf prey (52). The population of wolves consists of four packs: the BNP pack, the Leśna I and Leśna II packs and the South BF pack, which have overlapping territories (52, 70). The packs are usually formed by 7–12 wolves, the number varying annually. The present size of the population in BPF is unknown, as no such monitoring has been carried out for the last decade (33). A few years ago, the number of wolves was estimated at 20–40; however, this is likely to be an underestimation, as supported by frequent sightings of the species by BNP staff, foresters and even visitors posting pictures to social media accounts.

Camera trapping and field observation. In the early spring of 2022, several apparently sick wolves with alopecia were reported by forest rangers and border patrols inside the territory of the BNP. Monitoring was then implemented across the whole Park's 10,517.27 ha. More than half of the BNP remains under strict protection, meaning anthropopressure is kept to a minimum and only educational and research work is allowed there. It is a valuable natural area which has been included in the UNESCO World Heritage List since 1977. The monitoring sites selected for the study were an adequate representation of the species' range in terms of numbers and distribution of animals. The locations for cameras for wolf observation were selected on the basis of direct observations of park rangers and data from the BNP inventory, including data on individuals recorded on photo and video traps giving the exact location and number of individuals. The cameras were placed in different places over the whole territory of the BNP, in strict, active and landscape (buffer zone) protection areas. Apart from photo and video monitoring, foresters observed wolves directly all the time and reported what they saw. Two models of camera were used, the first being a TETRAO SPROMISE S328 trap (IBO PL, Kraków, Poland) for recording from a distance of up to 25 m using a 0.6 s shutter speed and motion sensor. Its 940 nm invisible beam infrared illuminates night shots and is undetectable. The camera operates in temperatures between −20°C and +60°C and takes photographs with resolution 12/30 Mpx or captures video at 30 frames/sec for up to 30 s with audio recording. The second camera was a SnapShot Cloud 4G (DÖRR, Neu-Ulm, Germany). It is equipped with 57 invisible Black Vision LEDs, allowing monitoring up to 20 m. The shutter speed is 0.4 s, and video capture is in HD with sound for up to 59 s.

For the on-site surveillance of possible mange cases, analysis was made of the sampling data recorded between January and March 2022 by ten camera traps located as described in Table 1. In the morning of 17th March 2022, the carcass of a young female wolf was found in forest division No. 339C of the BNP (C1, Table 1). The carcass was frozen at −20°C until it was necropsied by the BNP's supervising veterinarian, at which time samples for further laboratory analysis were collected and a necropsy protocol was prepared.

Table 1. Locations of sampling sites for wolf monitoring by camera traps in the Białowieża National Park

No.	Division No.	GPS coordinates	Presence of alopecic/ sick wolves (number)
C1	339C	52.7418N; 23.7959E	Yes (2)*
C2	159B	52.7991N; 23.8267E	Yes (1)
C3	130C	52.8031N; 23.8280E	No
C4	226A	52.7803N; 23.8770E	Yes (1)
C5	159D	52.7926N; 23.8271E	Yes (1)
C6	374A	52.7282N; 23.9074E	No
C7	135D	52.8041N; 23.9189E	No
C8	287C	52.7537N; 23.8758E	No
C9	284B	52.7570N; 23.8430E	No
C10	259B	52.7683N; 23.9189E	No

* – the same location as where the dead wolf (Figs 2a and 3) was found which was later necropsied; here another alopecic one was recorded (Fig. 2d)

Mite isolation and identification. The nature of the skin lesions suggested a parasitological infection. Scabies invasion was initially diagnosed based on the presence of clearly visible, characteristic lesions on the skin. In order to confirm the primary diagnosis, scrapings were taken from the lesions for parasitological examinations. First, the scraping was examined directly under an optical microscope at 100× and 400× magnifications. Then the sample was placed in a Petri dish and treated with 10% KOH, heated to 40°C and incubated for 2 h. After the epidermis had been dissolved by the KOH, the surface of the preparation was examined under an optical microscope under the magnifications given earlier.

Rabies test. The test was performed at the Veterinary Hygiene Laboratory in Gdańsk. A sample of brain tissue was collected following the opening of the skull in the necropsy room. Tissue was collected from Ammon's horn and the thalamus, cerebral cortex and medulla oblongata and subjected to the direct fluorescent antibody test (FAT) according to the WOAH Terrestrial Manual (68) as used in all diagnostic and reference laboratories (59, 61). After smears were prepared on slides and flame fixed, they were stained with a drop of specific conjugate for 30 min at 37°C and washed with phosphate buffered saline. For the purpose of the FAT, a lyophilised, adsorbed anti-rabies immunofluorescent conjugate (Bio-Rad Anti-Rabies Nucleocapsid Conjugate; Bio-Rad, Marnes-la-Coquette, France) was used, which is a suspension of fluorescein isothiocyanate-labelled monoclonal antibodies. Fluorescent antibody test slides were examined for specific fluorescence and the presence of nucleocapsid protein aggregates of rabies virus using a microscope.

Testing for tick-borne pathogens. Commercial kits were used for the detection of DNA sequences specific for bacteria among the *Borreliella* spp. (formerly the *Borrelia burgdorferi* sensu lato group (7)), *Anaplasma* spp. and *Ehrlichia* spp., *Francisella tularensis*, selected species of *Babesia* protozoa (*B. gibsoni*, *B. canis* and *B. divergens*), and specific RNA sequences of tick-borne encephalitis virus (TBEV). They were the AmpliTest Tick-borne Diseases (Real Time PCR) multiplex kit (Amplicon, Wrocław, Poland). All tests were performed using a whole-blood sample according to the manufacturer's instructions and following the quality assurance procedures of the Veterinary Hygiene Laboratory in Gdańsk. Fluorescent dyes released after hydrolysis of the Taqman probes were detected by the optical system of the real-time PCR device (LightCycler 480 System, Roche Diagnostics, Basel, Switzerland). The sensitivities and specificities of all tests were specified by the manufacturer (57).

Meteorological data accusation and analysis. Climate data for the BF were obtained from the Institute of Meteorology and Water Management and the Polish Geological Institute through the https://dane.imgw.pl/data/dane_pomiarowo_obserwacyjne/dane_meteorologiczne/website in CSV files, and were transferred to an Excel spreadsheet (Microsoft, Redmond, WA, USA). The monthly collected data were analysed and graphically presented using STATA v.13.0 software (StataCorp, College Station, TX, USA). The parameters were absolute and average maximum temperature (°C), absolute and average minimum temperature (°C), average temperature (°C), minimum temperature at ground level (°C), monthly precipitation (mm), maximum daily precipitation (mm), first day of maximum precipitation, last day of maximum precipitation, maximum snow depth (cm), number of days with snow cover, number of days with rainfall and number of days with snowfall.

Results

Field observations. The camera surveillance (Table 1, Fig. 1) tracked five wolves with severe alopecia of the entire body (Fig. 2). In addition, border guards (Fig. 2a) and forest rangers (Fig. 2b) also observed alopecic wolves, some of which may have been the same individuals. All the mangy wolves were young, 1–2 years old, and were mostly wandering alone (Supplementary Video S1). Two of them had used haystacks in mid-forest meadows left for European bison and cervids as their shelters instead of the burrows used by other pack members. Skin lesions indicative of S*arcoptes* infestation of low to moderate severity were also observed on several other wolves in the BNP (Supplementary Video S2).

Fig. 1. The distribution of camera traps (C1–C10, Table 1) installed for the monitoring of mange cases in wolves in the Białowieża National Park in winter 2021/2022. Red tags – locations where severely alopecic wolves were observed. Red square in the bottom left corner map – the location of the BNP in Europe

Fig. 2. Observations of severely alopecic wolves in Białowieża National Park: a – camera trap image of the wolf found dead a day later (Table 1, C1 and Fig. 3); b – camera trap image from the camera positioned on a European bison carcass (Table 1, C4); c – photo taken by a Polish border guard on the border with Belarus on the bridge over the Narewka river (Kosy Most; 52.7996N, 23.8284E) near location of trap C2 (Table 1); d – photo taken by a forest ranger in the same midforest meadow as trap C1 (Table 1)

Fig. 3. External inspection of a wolf carcass showing: a – advanced alopecia; hyperkeratosis; emaciation and severe dehydration and anaemia; b – presence of soil and fragments of vegetation in the mouth suggestive of some neurological symptoms, which were nevertheless ruled out by a negative rabies test result

Necropsy findings. The observed clinical signs in the female wolf found dead, which was estimated to be 2 years old (born in 2020), included alopecia of the entire body, hyperkeratosis, emaciation and dehydration, anaemia of the mucous membranes (Fig. 3a), presence of soil and fragments of vegetation in the mouth (Fig. 3b), a stomach with little digestive content, empty intestines, a considerably enlarged gallbladder, precipitation spots in the left lung, foci of emphysema and heart chambers filled with clotted blood. Because foreign bodies were in the mouth and it was unverifiable whether they were taken in during the agonal state, ante or post mortem and the digestive tract was empty, the wolf's brain was first referred for virological examination for rabies.

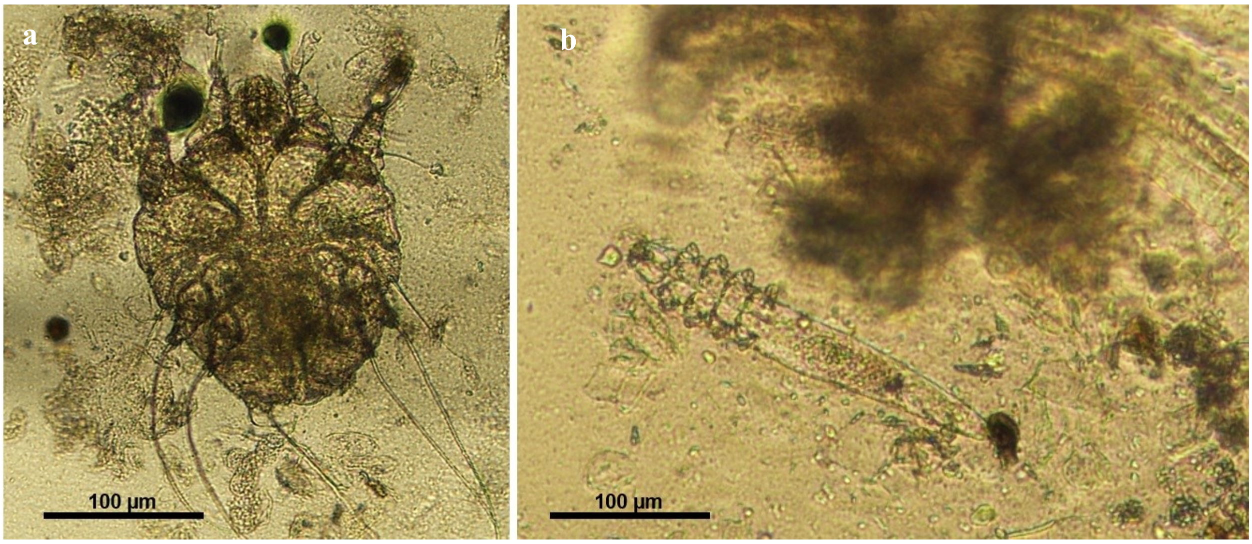

Fig. 4. Ventral views of a *Sarcoptes scabiei* male (a) and *Demodex* sp. (b) isolated post mortem from the skin of an affected grey wolf from Białowieża National Park

Laboratory testing results. Infections with rabies and tick-borne pathogens in the necropsied wolf were excluded. In the skin scrapings, a low number of mature forms of *Sarcoptes scabiei* (Fig. 4a), their eggs (Supplementary Fig. S2c) and *Demodex* sp. (Fig. 4b) were observed. The parasite loads were rather low: 0–10 male and female *Sarcoptes* mites, 0–5 *Sarcoptes* eggs and 0–5 *Demodex* mites were noted in microscopy. The mite dimensions were approximately 160–200 µm × 240–320 µm for sex-dimorphic *Sarcoptes* (example in Supplementary Fig. S2a) and 28–40 µm × 130–200 µm for *Demodex* (Supplementary Fig. S2b), females and males of which did not differ so much in size. All tests for rabies and tick-borne pathogens including *Borreliella* sp., *Anaplasma* sp., *Ehrlichia* sp., *Francisella tularensis*, *Babesia* sp. and TBEV gave negative results.

Meteorological data analysis. The average monthly absolute and average maximum temperature, minimum temperature, average temperature and minimum temperature at ground level for BNP trended upwards between 1951 and 2021, and the increase in temperature has been particularly dynamic in the last 10 years, with an average rise of 2°C (Supplementary Fig. S1 a–c). Interestingly, cyclical changes in the temperature at ground level were observed, with values decreasing from the early 1960s to the 1990s, and then increasing by one degree over the last two decades (Supplementary Fig. S1d). While monthly precipitation has not changed much in years for which data was collected, with the exception of noticeable increases between the 1950s and 1970s (Supplementary Fig. S1e), the annual number of rainy days has been on a steady increase since the 1950s (Supplementary Fig. S1f). However, the maximum snow depth in winter and the annual number of days with snow cover have been decreasing quite substantially. When the data was narrowed down to the winter months only (January–March) in the last two decades, more pronounced changes were observed in the last ten years (Fig. 5). The climate has warmed to such an extent that the average minimum temperature in winter has increased from below −5°C to above −2°C, and the average minimum temperature at ground level has risen by as much as 10°C (Fig. 5a). The winters have become less snowy (Fig. 5b), an example being the 2021/2022 winter when snow cover was almost negligible, as can be seen in the footage from the camera traps (Supplementary Video S1 and S2), despite the trend for snow depth having been rising in recent years (Fig. 5b).

Discussion

Scabies is considered a neglected tropical disease, and its re-emergence in animals and humans is becoming a challenge in many parts of the world. The exacerbation of parasite invasions can be seen as a symptom of environmental changes that affect general health and susceptibility to endemic and conditionally pathogenic commensals. For example, an association between human scabies caused by *Sarcoptes scabiei* varietas *hominis* and cases of COVID-19 was recently observed in developing countries (22). Initially, the treatment with ivermectin of SARS-CoV-2 infections led to a decline in mange cases; however, the prolonged albeit unintentional effect of this antiparasitic therapy may have been the emergence of drug resistance in mites, thus increasing the number of difficult-to-treat skin infestations in humans (27). It has also been observed that the recent increase in cases of mange in humans in Europe is connected to geopolitical changes including massive intercontinental movements and migration (20, 42). However, the zoonotic potential of the *Sarcoptes* mites hosted by animals is low, as it is associated only with occasional cases of pseudoscabies. Most of cases of human scabies transmitted from animals (mostly dogs) were transient and self-limiting, as the parasite has less ability to reproduce and persist in human skin (47). Scabies as a reverse zoonosis (zooanthroponosis) was suspected based on historical data; however, transmission from human to animal was never demonstrated (2, 6).

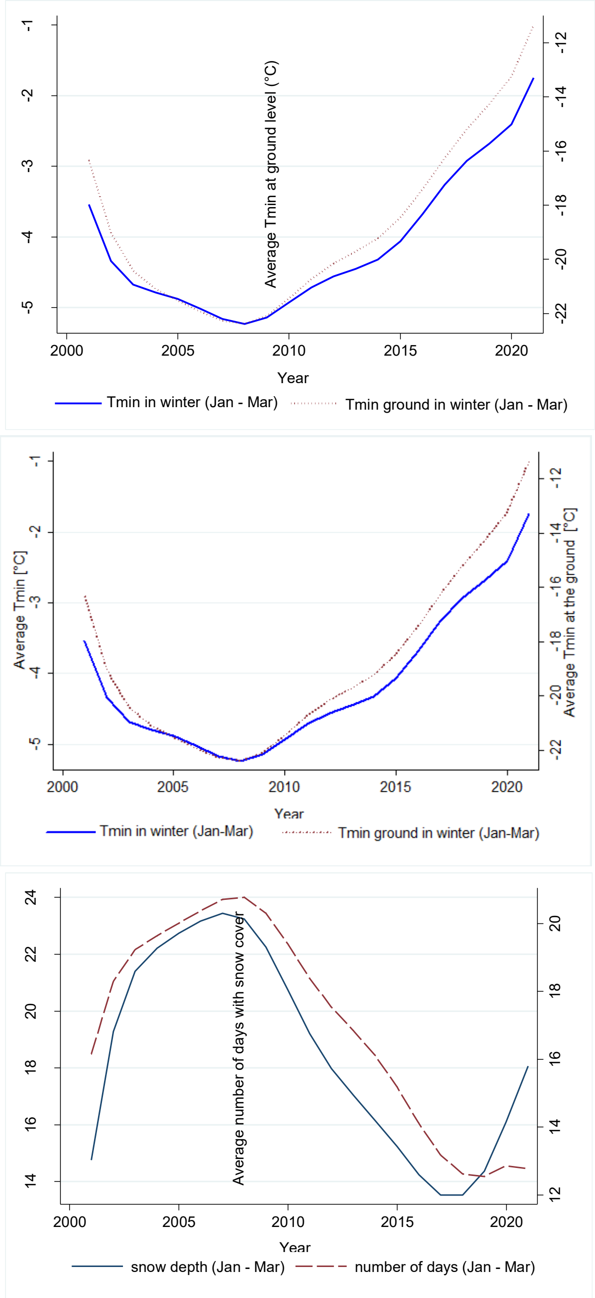

Fig. 5. Local polynomial smoothed lines of the distribution of selected monthly meteorological data for Białowieża Forest, obtained from the Polish Institute of Meteorology and Water Management in the winter months of January–March between 2001 and 2021. a – solid blue line: average minimum temperature (Tmin); dotted maroon line: average minimum temperature at ground level (Tmin ground); b – solid navy-blue line: average maximum snow depth in cm; dashed maroon line: average number of days with snow cover

Scabies and demodicosis are caused by parasitic mites classified as the Acari subclass within the Arthropoda phylum and Arachnida class. They belong to two distinct orders. Mites of the *Demodex* genus represent the Demodicidae family and Trombidiformes order, and include host species–specific parasites, putatively specific to their individual hosts. These mites are genetically highly diverse. Cross-infections are rare; therefore, animal demodicosis does not pose a risk to humans (17, 21, 60). Scabies mites, in contrast, belong to one main species –*Sarcoptes scabiei* – in the *Sarcoptes* genus, Sarcoptidae family and Sarcoptiformes order, and have many host-specific variants. In numerous animal species within the Canidae family, *S. scabiei* varietas *canis* is most commonly found. Genetic studies of scabies isolated from various host species (including humans) have confirmed only minor variations in the genome (8, 46); therefore, epidemiological investigations based on phylogenetic relationship rarely allow the determination of the direction of transmission (6). An additional factor besides genome similarity facilitating interspecies infestation is immunosuppression, which is supported by numerous observations and experimental studies (10, 54). In general, mite isolates have been classified into three main scabies groups of herbivore-hosted, carnivore-hosted and omnivore-hosted on the basis of their genetic homology (2). The exposure to prey-to-predator *Sarcoptes* mite infection has been studied especially for wolves, which prey on large herbivores (54). However, the possibility of cross-infection of other canids such as the rabbit-dog-rabbit system was also demonstrated (10). This supports the observations of possible interspecies transmission of mites between different carnivores and their prey (24, 25, 41). According to this theory, small mammals may therefore be responsible for the mass occurrence of scabies in foxes or badgers, for which rodents and other small mammals are the primary food source. European wolves less frequently prey on small rodents; therefore, they are less likely to be affected by alimentary infestation. However, in the absence of cervids, wolves may prey on alternative species including beavers (51), making a choice which may also be related to weather conditions (45, 48). Therefore, oral contact may be an underestimated route of scabies infestation in carnivores, especially when the primary prey are rodents. The hypothesis could not be confirmed in our case, however, as we did not observe cases of mange in herbivores or other mammal species. Such observations would have been merely incidental, however, because the camera trapping locations were designed to perform a survey of wolves.

Mange in wildlife reflects emerging worldwide challenges and pressure on nature (47). *Sarcoptes scabiei* and *Demodex* sp. are quite common parasites of the skin, and infestations with them are mostly-subclinical and very rarely life-threatening. The infestations may become severe when the host is immunosuppressed because of concurrent factors (56). The widespread use of macrocyclic lactones and acaricides based on active isoxazolinergic substances (fluralaner, sarolaner and afoxolaner) in pets means that both scabies and demodicosis are only marginal problems in domestic animals. Also in humans, clinical mite infestations are extremely rare in highly developed countries because hygiene measures are adequate to prevent them. The situation does not pertain to free-living animals, where these infestations are not countered by prophylaxis or treated. Epidemic mange in wildlife usually heralds an imbalance in nature that negatively influences the affected species. Concern over the disturbance of this balance falls within the concept of One Health, where nature, the environment and humans are one. The imbalance in the ecology of prey animals, access to food, shrinkage of the habitat, overcrowding and competition with other carnivores and humans (hunters) may trigger health and conservation-threatening processes for wolves, as apex predators. On the other hand, the role of predators and hunters may have a positive effect by removing infected animals, contributing to the elimination of carriers or reservoirs of pathogens from the environment. Scabies in Polish wildlife was mostly observed in foxes and badgers, which fared worse and suffered higher mortality when infested (41). The problem became apparent after the eradication of rabies in the main reservoir – foxes – at the beginning of the 21st century. From then, the fox population has grown significantly. In scientific circles, it has been debated whether stopping one plague has unleashed another on foxes (58). The similarities in the mechanisms of occurrence of this type of ecosystem disturbance may have led to the mass occurrence of scabies. Therefore, those mechanisms were considered when studying the scabies epidemic in wolves. Mange in wolves has been observed to be limited to certain parts of their skin (41). The mortality due to mite skin infestation in wolves reported previously was also rather low (23, 41); however, mange epidemics such as the one described here have previously been noted in American grey wolves (18, 38). Mange is a contact disease, in which the survival of the aetiological agent depends on environmental conditions (11, 50). However, since the infestations usually have minor health consequences, the exposure risk will increase with animal density and alterations in animal ecology, health status and behaviour, as it also will because of the anthropogenic factors disturbing the habitat (56). We have concluded that changes in temperature and environmental humidity may allow the pathogen to persist longer in the environment, significantly worsening the threat.

Climate change has been discussed as a driver for scabies in humans (47) and wildlife (31, 66). Optimal humidity and temperature promote efficient reproduction and survival of mites in the environment (66). An indirect influence of climate change is on host health and susceptibility to adverse factors, where it induces heat stress and weakens the resilience of mammals (48). An increasing percentage coverage of water habitats by camera traps was associated with higher probabilities of apparent scabies in foxes (11). Białowieża Forest is characterised by a high percentage of wetlands, which together with milder winter temperatures favouring mite survival may have intensified the exposure of its wolves to mites and fostered mite transmission between them. Any animal infected with *Sarcoptes* or *Demodex* sp. is a potential reservoir of infestation for other hosts. In both infestations, direct contact is the most important route of infection. Therefore, these infestations spread particularly easily in animals living in groups such as packs (14) and are density dependent (4, 31). However, the several-day survival time of these pathogens outside the organism also allows indirect transmission. Animals using burrows abandoned by previously infected individuals even of other species (for example foxes or badgers) are at risk of infection. High humidity and lower temperatures, especially of a few degrees Celsius above zero, are factors that foster the survival of mites outside the host (50). Temperatures around 0°C are not lethal for mites, and only temperatures below −10°C are a limiting factor for their survival, killing them in just a few hours (8). Interestingly, *Sarcoptes scabiei* have been observed to survive outside of the skin inside the fox den longer during the winter (at a mean 12°C) than the summer (mean 29°C) (43). The humidity inside the den did not change throughout the year sufficiently to affect mite survival.

The disease severity and clinical course of both infestations are strictly dependent on the non-specific and specific immune response. This is particularly evident in the course of demodicosis. This invasion is assumed to occur in a significant proportion of the host population in an asymptomatic form. In contrast, its clinical form is closely associated with periodic immune decline or immunomodulatory disorders. For this reason, a *Demodex* species is considered a facultative parasite. These mites reside in hair follicles and sebaceous glands. Their abundance determines the asymptomatic or symptomatic nature of the invasion and is controlled by the reactivity of the immune system. Parasite overabundance leads to inflammatory reactions within the hair follicles, hair loss and infection with pathogenic bacterial flora. In the case of burrowing mites such as *Sarcoptes* sp., the presence of the parasites is frequently associated with skin lesions; therefore, they are considered obligate parasites. The infestation usually spreads from the muzzle area and area peripheral to the eye to cover the entire skin surface in the critical stage (32, 60). During the course of an infestation, numerous immune reactions occur in the host–parasite relationship, which on the one hand facilitate the development of the parasite in the host's skin, and on the other have an inhibitory effect on inflammatory reactions. As a result, at the beginning of an infestation, host defence processes are suppressed (especially in the first four weeks of the infestation), allowing the initial maintenance of the mite population. Later, the increasing parasite loads release more antigenic substances which trigger an inflammatory response stimulated by a cytokine cascade (of interleukin (IL)-1α, IL-1β, IL-6, IL-8 and tumour necrosis factor α) and pro-inflammatory mediators (histamine and leukotrienes) (8, 9), which cause swelling, congestion, pain and pruritus (28, 29). One of the more notable effects of mite infestation is the stimulation of IL-20 release affecting keratinocyte proliferation, which leads to thickening and elasticity loss of skin. However, the inflammatory process is also inhibited by the IL-1 receptor agonist modulating the immune response and inflammatory reactions related to interleukin 1 (8). As a result, in individuals with an efficient immune system, the development of the infestation is somewhat balanced and its intensity and symptoms are usually only locally marked.

It has been observed that scabies in wolves is most often limited to certain parts of the animal's skin (the hind legs, tail and head) (Video S2) and only in extreme cases affects all parts of the body (41, 49, 55). Immune response mechanisms can also explain one species' greater susceptibility to scabies than another's (44). It was recently demonstrated that *S. scabiei* infection induces more inflammatory cell infiltration in the skin of foxes than in that of wolves, which is related to less effective defence permitting severe hyperkeratotic lesions to develop in the smaller carnivores (44). Wolves also engage more macrophages as a result of skin infestation, which more effectively reduce the inflammation at the onset. However, in immunosuppressed animals, the disease can progress dynamically and cover the entire skin surface. The extensive inflammatory process further reduces the body's ability to defend itself against other pathogens, resulting in the death of the animal from disease not necessarily directly caused by the infestation. The cases of *Sarcoptes scabiei* and *Demodex* co-infections in wolves described in this study are a model example of the development of an infestation in a debilitated animal. The season and age of the animals were previously observed to be risk factors for scabies (18, 23, 67). The infestation may be life threatening because of the extensive inflammatory process in afflicted animals, which have a negative energy balance associated with the damage to the protective skin barrier and hypothermia, these resulting in changes in behaviour and an inability to hunt which further exacerbate the energy deficiency. Interestingly, immunosuppression together with adverse climatic conditions may lead also to high mortality of sarcoptic animals in climates at the opposite extreme. Scabies infestations were observed in subadult giraffes in Kenya, which suffered from starvation because of drought, limited access to food and competition for it with the pastoral communities and their livestock (1). The survival of a wolf depends mostly on its ability to hunt and/or remain part of the pack. Solitary wolves have less chance to survive the winter. The most severely alopecic wolves observed in the BNP lived alone, and some used hay stacks as their individual resting place rather than the pack den. In the extreme cases, the sick wolves starved to death, despite seemingly remaining active and even playful until the end (Video 1). However, this impression was misleading and the wolves' activity was due to the skin lesions' extreme itchiness. Our observations suggest that these wolves only hunted small mammals or birds, similarly to foxes, which are the species mostly affected by scabies. This behaviour made their risk of scabies infestation many times higher than that of animals in the pack. The risk is further increased by the possibility of indirect transmission through mites surviving for several days outside the host in conditions of high humidity and moderate temperatures. Mild winter conditions are a factor favouring mite survival outside the host. Also, the lack of snow cover as another environmental condition of a mild winter made it difficult for wolves to find food, and changed them from predators to scavengers, and thus may indirectly have had a significant impact on the persistence of infestations in the host population. Furthermore, as described previously (37), the predation of red deer by wolves in the BF depends on the snow cover depth. In warmer months wolves would choose wild boar over deer (37). However, the wild boar population in the area has declined dramatically, which may have further depleted the wolves' food base and exacerbated the poor health of the sarcoptic individuals.

An assessment of the epidemiological situation should also take into account the presence of other predators that may be both competitors and sources of infection. As shown recently for foxes in Spain (11), camera health monitoring should be considered an effective tool for the conservation of protected species such as the wolf and has the advantage of being non-invasive. The wolf's conservation is a matter with a rising profile because the increasing population sizes of the species in many parts of Europe and environmental changes may promote the spread of pathogens and increase the risks of an epidemic, which is shown here at the micro-scale of the BFo. Furthermore, as predators, wolves are a good model species for exposure to infectious and non-infectious threats, as their food is mostly wildlife. An additional motivation for their surveillance is that wolves can potentially contribute to the spread of diseases, even those that are not a threat to them (5, 12, 13, 39, 40, 62, 65, 69).

Conclusion

An outbreak of severe cases of mange related to mixed infestation of *Sarcoptes* and *Demodex* mites, which caused higher wolf morbidity and mortality in the Białowieża Forest in the winter of 2021/2022, was the result of several cofactors. Climate alterations such as rises in air and ground temperatures, a decrease in humidity, a thinning of the snow cover and shortening of its period in recent years may have contributed to the emergence of mange by influencing the ecology and behaviour of wolves and their prey. Other potential drivers included the growth of the wolf population and the disturbances in their habitat by anthropogenic factors such as the migration crisis on the border between Poland and Belarus. The crisis, which started in 2021 with an influx of thousands of refugees from Asia and Africa through the BF, has also entailed increased activity by the military and border services. This could have affected the general health status of wolves by causing them stress-induced immunosuppression and increasing their susceptibility to sarcoptic mange in the favourable weather conditions.

Video S1: Camera trap C1 (Table 1) footage of the wolf showing severe alopecia the day before it died. Above, on the right, another wolf with minor skin lesions indicating scabies may be observed. **Video S2:** Camera trap C2 trap (Table 1) footage of a wolf pack. The second and third wolf passing show some coat changes indicative of mange (alopecia on the hind legs, tail and head).

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Financial Disclosure Statement: This research received no external funding. The publication costs were covered by the funds from the ENETWILD project (EFSA framework contract “Wildlife: collecting and sharing data on wildlife populations, transmitting animal disease agents”, OC/EFSA/ALPHA/2016/01).

Animal Rights Statement: The study was performed in accordance with a Resolution of the Polish Ministry of Climate and Environment to grant a dispensation from the prohibitions referred to in Article 52 (1), (4) and (6) of the Nature Conservation Act (No. DZP-WG.6401.107.2024.KS).

Acknowledgements: We would like to thank Wiesław Klimiuk from the Białowieża National Park for the technical assistance in climate data accusation and Andrzej Milko, a forester of the Cupryki Protective District, who reported the dead wolf which was used in the diagnostic investigation.

References

1. Alasaad S., Ndeereh D., Rossi L., Bornstein S., Permunian R., Soriguer R.C., Gakuya F.: The opportunistic *Sarcoptes scabiei*: a new episode from giraffe in the drought-suffering Kenya. Vet Parasitol 2012, 185, 359–363, doi: 10.1016/j.vetpar.2011.10.039.
2. Alasaad S., Rossi L., Heukelbach J., Pérez J.M., Hamarsheh O., Otiende M., Zhu X.Q.: The neglected navigating web of the incomprehensibly emerging and re-emerging *Sarcoptes* mite. Infect Genet Evol 2013, 17, 253–259, doi: 10.1016/j.meegid.2013.04.018.
3. Alcayna T., Fletcher I., Gibb R., Tremblay L.L., Funk S., Rao B., Lowe R.: Climate-sensitive disease outbreaks in the aftermath of extreme climatic events: A scoping review. One Earth 2022, 5, 336–350, doi: 10.1016/j.oneear.2022.03.011.
4. Almberg E.S., Cross P.C., Dobson A.P., Smith D.W., Hudson P.J.: Parasite invasion following host reintroduction: a case study of Yellowstone’s wolves. Philos Trans R Soc Lond B Biol Sci 2012, 367, 2840–2851, doi: 10.1098/rstb.2011.0369.
5. Alsarraf M., Dwużnik-Szarek D., Hildebrand J., Mierzejewska E.J., Kloch A., Kot K., Kurek K., Nowak S., Mysłajek R.W., Myśliwy I., Popiołek M., Rodo A., Alsarraf M., Tołkacz K., Topolnytska M., Wężyk D., Bajer A.: Occurrence of *Dirofilaria repens* in wild carnivores in Poland. Parasitol Res 2023, 122, 1229–1237, doi: 10.1007/s00436-023-07823-5.
6. Andriantsoanirina V., Fang, F., Ariey F., Izri A., Foulet F., Botterel F., Bernigaud C., Chosidow O., Huang W., Guillot J., Durand R.: Are humans the initial source of canine mange? Parasit Vectors 2016, 9, 177, doi: 10.1186/s13071-016-1456-y.
7. Arahal D.R., Bull C.T., Busse H., Christensen H., Chuvochina M., Dedysh S.N., Fournier P., Konstantinidis K.T., Parker C.T., Rossello-Mora R., Ventosa A., Göker M.: Judicial Opinions 123–127. Int J Syst Evol Microbiol 2022, 72, 005708, doi: 10.1099/ijsem.0.005708.
8. Arlian L.G., Morgan M.S.: A review of *Sarcoptes scabiei*: Past, present and future. Parasit Vectors 2017, 10, 297, doi: 10.1186/s13071-017-2234-1.
9. Arlian L.G., Morgan M.S., Neal J.S.: Extracts of scabies mites (Sarcoptidae: *Sarcoptes scabiei*) modulate cytokine expression by human peripheral blood mononuclear cells and dendritic cells. J Med Entomol 2004, 41, 69–73, doi: 10.1603/0022-2585-41.1.69.
10. Arlian L.G., Runyan R.A., Estes S.A.: Cross infestivity of *Sarcoptes scabiei*. J Am Acad Dermatol 1984, 10, 979–986, doi: 10.1016/s0190-9622(84)80318-7.
11. Barroso P., Palencia P.: Camera traps reveal a high prevalence of sarcoptic mange in red foxes from northern Spain. Res Vet Sci 2024, 166, 105098, doi: 10.1016/j.rvsc.2023.105098.
12. Bień J., Moskwa B., Goździk K., Cybulska A., Kornacka A., Welc M., Popiołek M., Cabaj W.: The occurrence of nematodes of the genus *Trichinella* in wolves (*Canis lupus*) from the Bieszczady Mountains and Augustowska Forest in Poland. Vet Parasitol 2016, 231, 115–117, doi: 10.1016/j.vetpar.2016.04.010.
13. Borecka A., Gawor J., Zieba F.: A survey of intestinal helminths in wild carnivores from the Tatra National Park, southern Poland. Ann Parasitol 2013, 59, 169–172.
14. Brandell E.E., Dobson A.P., Hudson P.J., Cross P.C., Smith D.W.: A metapopulation model of social group dynamics and disease applied to Yellowstone wolves. PNAS 2021, 118, e2020023118, doi: 10.1073/pnas.2020023118.
15. Chief Inspectorate for Environmental Protection (GIOŚ): Pilotażowy monitoring wilka i rysia w Polsce realizowany w ramach Państwowego Monitoringu Środowiska (Pilot study of wolf and lynx monitoring in Poland realized as a part of the National Monitoring of the Environment Programme (POIiŚ) – in Polish) https://www.gov.pl/web/gios/poiis---monitoring-wilka-i-rysia.
16. Cohen J.M., Sauer E.L., Santiago O., Spencer S., Rohr J.R.: Divergent impacts of warming weather on wildlife disease risk across climates. Science 2020, 370, eabb1702, doi: 10.1126/science.abb1702.
17. Cunningham A.A., Daszak P., Wood J.L.N.: One Health, emerging infectious diseases and wildlife: two decades of progress? Philos Trans R Soc Lond B Biol Sci 2017, 372, 20160167, doi: 10.1098/rstb.2016.0167.
18. DeCandia A.L., Schrom E.C., Brandell E.E., Stahler D.R., vonHoldt B.M.: Sarcoptic mange severity is associated with reduced genomic variation and evidence of selection in Yellowstone National Park wolves (*Canis lupus*). Evol Appl 2020, 14, 429–445, doi: 10.1111/eva.13127.
19. Directorate-General for Environment of the European Commission, Directorate D – Biodiversity, Unit ENV.D.3 – Nature Conservation, N2K Group EEIG, Blanco J.C., Sundseth K.: The situation of the wolf (*Canis lupus*) in the European Union – An In-depth Analysis. A report of the N2K Group for DG Environment, European Commission. Publications Office of the European Union, Luxembourg, 2023, https://op.europa.eu/en/publication-detail/-/publication/5d017e4e-9efc-11ee-b164-01aa75ed71a1.
20. El-Sayed A., Kamel M.: Climatic changes and their role in emergence and re-emergence of diseases. Environ Sci Pollut Res 2020, 27, 22336–22352, doi: 10.1007/s11356-020-08896-w.
21. Esenkaya Taşbent F., Dik B.: A dog related *Demodex spp*. infestation in a student: a rare *Demodex* case (in Turkish). Mikrobiyoloji Bul 2018, 52, 214–220, doi: 10.5578/mb.66410.
22. Etiz P., Altunsu A.T.: The Scabies Epidemic During the Covid-19 Pandemic. Turkiye Parazitol Derg 2023, 47, 235–239, doi: 10.4274/tpd.galenos.2023.44154.

23. Fuchs B., Zimmermann B., Wabakken P., Bornstein S., Månsson J., Evans A.L., Liberg O., Sand H., Kindberg J., Ågren E.O., Arnemo J.M.: Sarcoptic mange in the Scandinavian wolf *Canis lupus* population. BMC Vet Res 2016, 12, 156, doi: 10.1186/s12917-016-0780-y.
24. Gakuya F., Ombui J., Maingi N., Muchemi G., Ogara W., Soriguer R.C., Angelone S.: Sarcoptic mange and cheetah conservation in Masai Mara (Kenya): epidemiological study in a wildlife/livestock system. Parasitol 2012, 139, 1587–1595, doi: 10.1017/S0031182012000935.
25. Gakuya F., Rossi L., Ombui J., Maingi N., Muchemi G., Ogara W., Soriguer R.C., Alasaad S.: The curse of the prey: *Sarcoptes* mite molecular analysis reveals potential prey-to-predator parasitic infestation in wild animals from Masai Mara, Kenya. Parasit Vectors 2011, 4, 193, doi: 10.1186/1756-3305-4-193.
26. Government of the Republic of Poland: Rozporządzenie Ministra Środowiska z dnia 7 listopada 2014 w sprawie ustanowienia planu ochrony dla Białowieskiego Parku Narodowego (Decree of the Minister of the Environment of 7 November 2014 on the establishment of a protection plan for the Białowieża National Park – in Polish), Dz U 2014 poz. 1735 (Journal of Laws 2014, item 1735).
27. Griffin L.R., Pender E.K., Laing M.E., Markham T.: Unexpected consequences of SARS-CoV-2 pandemic: scabies infestation. Clin Exp Dermatol 2022, 47, 1196–1197, doi: 10.1111/ced.15151.
28. Gundłach J.L., Sadzikowski A.B., Tomczuk K.: Świerzb świń (Pig Scabies – in Polish). Med Weter 1996, 52, 416–419.
29. Gundłach J.L., Sadzikowski A.B., Tomczuk K.: Odczyny skórne z antygenem świerzbowcowym u świń (Skin tests with mite antigen in pigs – in Polish). Ann Univ Mariae Curie-Skłodowska DD Med Vet 1997, 5, 139–142.
30. Hindrikson M., Remm J., Pilot M., Godinho R., Stronen A.V., Baltrūnaité L., Czarnomska S.D., Leonard J.A., Randi E., Nowak C., Åkesson M., López-Bao J.V., Álvares F., Llaneza L., Echegaray J., Vilà C., Ozolins J., Rungis D., Aspi J., Paule L., Skrbinšek T., Saarma U.: Wolf population genetics in Europe: a systematic review, meta-analysis and suggestions for conservation and management. Biol Rev 2017, 92, 1601–1629, doi: 10.1111/BRV.12298.
31. Iacopelli F., Fanelli A., Tizzani P., Berriatua E., Prieto P., Martínez-Carrasco C., León L., Rossi L., Candela M.G.: Spatio-temporal patterns of sarcoptic mange in red deer and Iberian ibex in a multi-host natural park. Res Vet Sci 2020, 128, 224–229, doi: 10.1016/j.rvsc.2019.11.014.
32. Izdebska J.N., Fryderyk S.: Diversity of three species of the genus *Demodex* (Acari, Demodecidae) parasitizing dogs in Poland. Polish J Environ Stud 2011, 20, 565–569.
33. Jędrzejewska B., Jędrzejewski W., Bunevich A.N., Minkowski L., Okarma H.: Population dynamics of wolves *Canis lupus* in Bialowieża Primeval Forest (Poland and Belarus) in relation to hunting by humans, 1847–1993. Mammal Rev 1996, 26, 103–126, doi: 10.1111/j.1365-2907.1996.tb00149.x.
34. Jędrzejewski W., Branicki W., Veit C., MeĐugorac I., Pilot M., Bunevich A.N., Jędrzejewska B., Schmidt K., Theuerkauf J., Okarma H., Gula R., Szymura L., Förster M.: Genetic diversity and relatedness within packs in an intensely hunted population of wolves *Canis lupus*. Acta Theriol 2005, 50, 3–22, doi: 10.1007/BF03192614.
35. Jędrzejewski W., Jędrzejewska B., Okarma H., Ruprecht A.L.: Wolf predation and snow cover as mortality factors in the ungulate community of the Bialowieża National Park, Poland. Oecologia 1992, 90, 27–36, doi: 10.1007/BF00317805.
36. Jędrzejewski W., Niedziałkowska M., Hayward M.W., Goszczyński J., Jędrzejewska B., Borowik T., Bartoń K.A., Nowak S., Harmuszkiewicz J., Juszczyk A., Kałamarz T., Kloch A., Koniuch J., Kotiuk K., Mysłajek R.W., Nędzyńska M., Olczyk A., Teleon M., Wojtulewicz M.: Prey choice and diet of wolves related to ungulate communities and wolf subpopulations in Poland. J Mammal 2012, 93, 1480–1492, doi: 10.1644/10-MAMM-A-132.1.
37. Jędrzejewski W., Schmidt K., Theuerkauf J., Jędrzejewska B., Selva N., Zub K., Szymura L.: Kill rates and predation by wolves on ungulate populations in Białowieża Primeval Forest (Poland). Ecology 2002, 83, 1341–1356, doi: 10.2307/3071948.
38. Jimenez M.D., Bangs E.E., Sime C., Asher V.J.: Sarcoptic mange found in wolves in the Rocky Mountains in western United States. J Wildl Dis 2010, 46, 1120–1125, doi: 10.7589/0090-3558-46.4.1120.
39. Kaczmarek A., Sobociński W., Wesołowska M., Gołąb E., Kołodziej-Sobocińska M., Sałamatin R.: *Blastocystis* occurrence and subtype diversity in wild European terrestrial mammals – The case of Białowieża Primeval Forest (NE Poland). Int J Parasitol Parasites Wildl 2021, 16, 120–125, doi: 10.1016/j.ijppaw.2021.08.010.
40. Karamon J., Samorek-Pieróg M., Sroka J., Bilska-Zając E., Dąbrowska J., Kochanowski M., Różycki M., Zdybel J., Cencek T.: The first record of *Echinococcus ortleppi* (G5) tapeworms in grey wolf (*Canis lupus*). Pathogens 2021, 10, 853, doi: 10.3390/pathogens10070853.
41. Kołodziej-Sobocińska M., Zalewski A., Kowalczyk R.: Sarcoptic mange vulnerability in carnivores of the Białowieża Primeval Forest, Poland: underlying determinant factor. Ecol Res 2014, 29, 237–244, doi: 10.1007/s11284-013-1118-x.
42. Kwak R., Kamal K., Charrow A., Khalifian S.: Mass migration and climate change: Dermatologic manifestations. Int J Women Dermatol 2021, 7, 98–106, doi: 10.1016/j.ijwd.2020.07.014.
43. Loredo A.I., Rudd J.L., Foley J.E., Clifford D.L., Cypher B.L.: Climatic suitability of San Joaquin kit fox (*Vulpes macrotis mutica*) dens for sarcoptic mange (*Sarcoptes scabiei*) transmission. J Wild Dis 2020, 56, 126–133, doi: 10.7589/2019-02-035.
44. Martínez I.Z., Oleaga Á., Sojo I., García-Iglesias M.J., Pérez-Martínez C., García Marín J.F., Balseiro A.: Immunohistochemical Assessment of Immune Response in the Dermis of *Sarcoptes scabiei*—Infested Wild Carnivores (Wolf and Fox) and Ruminants (Chamois and Red Deer). Animals 2020, 10, 1146, doi: 10.3390/ani10071146.
45. Metz M.C., Smith D.W., Vucetich J.A., Stahler D.R., Peterson R.O.: Seasonal patterns of predation for gray wolves in the multi-prey system of Yellowstone National Park. J Animal Ecol 2012, 81, 553–563, doi: 10.1111/j.1365-2656.2011.01945.x.
46. Morgan M.S., Arlian L.G., Markey M.P.: *Sarcoptes scabiei* mites modulate gene expression in human skin equivalents. PLoS One 2013, 8, e71143, doi: 10.1371/journal.pone.0071143.
47. Moroni B., Rossi L., Bernigaud C., Guillot J.: Zoonotic Episodes of Scabies: A Global Overview. Pathogens 2022, 11, 213, doi: 10.3390/pathogens11020213.
48. Mysłajek R.W., Romański M., Kwiatkowska I., Stępniak K.M., Figura M., Nowak-Brzezińska A., Diserens T.A., Nowak S.: Temporal changes in the wolf *Canis lupus* diet in Wigry National Park (northeast Poland). Ethol Ecol Evol 2021, 33, 628–635, doi: 10.1080/03949370.2021.1907787.
49. Niedringhaus K.D., Brown J.D., Sweeley K.M., Yabsley M.J.: A review of sarcoptic mange in North American wildlife. Int J Parasitol Parasite Wildl 2019, 9, 285–297, doi: 10.1016/j.ijppaw.2019.06.003.
50. Niedringhaus K.D., Brown J.D., Ternent M.A., Peltier S.K., Yabsley M.J.: Effects of temperature on the survival of *Sarcoptes scabiei* of black bear (*Ursus americanus*) origin. Parasitol Res 2019, 118, 2767–2772, doi: 10.1007/s00436-019-06387-7.
51. Nowak S., Szewczyk M., Stępniak K.M., Kwiatkowska I., Kurek K., Mysłajek R.W.: Data for: Wolves in the borderland – changes in population and wolf diet in Romincka Forest, along the Polish-Russian-Lithuanian state borders (Dataset). Dryad 2024, doi: 10.5061/dryad.qfttdz0qn.
52. Okarma H.: *Wilk*. Wydawnictwo H2O, Kraków, Poland, 2015.
53. Okołów Cz.: *Białowieski Park Narodowy w Pigułce* (Key Facts About the Białowieża National Park – in Polish). Białowieski Park Narodowy, Białowieża, Poland, 2021.
54. Oleaga A., Alasaad S., Rossi L., Casais R., Vicente J., Maione S., Soriguer R.C., Gortázar C.: Genetic epidemiology *of Sarcoptes*

scabiei in the Iberian wolf in Asturias, Spain. Vet Parasitol 2013, 196, 453–459, doi: 10.1016/j.vetpar.2013.04.016.
55. Oleaga A., Casais R., Balseiro A., Espí A., Llaneza L., Hartasánchez A., Gortázar C.: New techniques for an old disease: sarcoptic mange in the Iberian wolf. Vet Parasitol 2011, 181, 255–266, doi: 10.1016/j.vetpar.2011.04.036.
56. Oleaga A., Vicente, J., Ferroglio E., Pegoraro de Macedo M. R., Casais R., del Cerro A., Espí A., García E.J., Gortázar C.: Concomitance and interactions of pathogens in the Iberian wolf (*Canis lupus*). Res Vet Sci 2015, 101, 22–27, doi: 10.1016/j.rvsc.2015.05.010.
57. Parizi O., El-Adawy H., Melzer F., Roesler U., Neubauer H., Mertens-Scholz K.: Seroprevalence and Molecular Detection of Bovine Anaplasmosis in Egypt. Pathogens 2020, 9, 64, doi: 10.3390/pathogens9010064.
58. Pisano S.R.R., Zimmermann F., Rossi L., Capt S., Akdesir E., Bürki R., Kunz F., Origgi F.C., Ryser-Degiorgis M.-P.: Spatiotemporal spread of sarcoptic mange in the red fox (*Vulpes vulpes*) in Switzerland over more than 60 years: lessons learnt from comparative analysis of multiple surveillance tools. Parasit Vectors 2019, 12, 521, doi: 10.1186/s13071-019-3762-7.
59. Robardet E., Andrieu S., Bruun Rasmussen T., Dobrostana M., Horton D.L., Hostnik P., Jaceviciene I., Juhasz T., Müller T., Mutinelli F., Servat A., Smreczak M., Vanek E., Vázquez-Morón S., Cliquet F.: Comparative assay of fluorescent antibody test results among twelve European National Reference Laboratories using various anti-rabies conjugates. J Virol Meth 2013, 191, 88–94, doi: 10.1016/j.jviromet.2013.03.027.
60. Sastre N., Francino O., Curti J.N., Armenta T.C., Fraser D.L., Kelly R.M., Hunt E., Silbermayr K., Zewe C., Sánchez A., Ferrer L.: Detection, prevalence and phylogenetic relationships of *Demodex* spp. and further skin prostigmata mites (Acari, Arachnida) in wild and domestic mammals. PLoS One 2016, 11, e0165765, doi: 10.1371/journal.pone.0165765.
61. Smreczak M., Orłowska A., Trębas P., Rola J.: Rabies in Poland in 2021 (in Polish). Med Weter 2022, 78, 279–283, doi: 10.21521/mw.6646.
62. Stojecki K., Sroka J., Cacció S.M., Cencek T., Dutkiewicz J., Kusyk P.: Prevalence and molecular typing of *Giardia duodenalis* in wildlife from eastern Poland. Folia Parasitol 2015, 62, 042, doi: 10.14411/fp.2015.042.
63. Sunde P., Collet S., Nowak C., Thomsen P.F., Møller Hansen M., Schulz B., Matzen J., Michler F.-U., Vedel-Smith C., Olsen K.: Where have all the young wolves gone? Traffic and cryptic mortality create a wolf population sink in Denmark and northernmost Germany. Conserv Lett 2021, 14, e12812, doi: 10.1111/conl.12812.
64. Szewczyk M., Nowak S., Niedźwiecka N., Hulva P., Špinkytė-Bačkaitienė R., Demjanovičová K., Bolfíková B.Č., Antal V., Fenchuk V., Figura M., Tomczak P., Stachyra P., Stępniak K.M., Zwijacz-Kozica T., Mysłajek R.W.: Dynamic range expansion leads to establishment of a new, genetically distinct wolf population in Central Europe. Sci Rep 2019, 9, 19003, doi: 10.1038/s41598-019-55273-w.
65. Tołkacz K., Kretschmer M., Nowak S., Mysłajek R.W., Alsarraf M., Wężyk D., Bajer A.: The first report on *Hepatozoon canis* in dogs and wolves in Poland: clinical and epidemiological features. Parasit Vectors 2023, 16, 313, doi: 10.1186/s13071-023-05928-5.
66. Unterköfler M.S., Schausberger M., Deutz A., Gressmann G., Kübber-Heiss A., Ferroglio E., Joachim A.: Sarcoptic mange in wild ungulates in the European Alps – A systematic review. Int J Parasitol Parasite Wildl 2023, 22, 121–125, doi: 10.1016/j.ijppaw.2023.10.003.
67. Valldeperes M., Granados J.E., Pérez J.M., Castro I., Ráez-Bravo A., Fandos P., López-Olvera J.R., Serrano E., Mentaberre G.: How sensitive and specific is the visual diagnosis of sarcoptic mange in free-ranging Iberian ibexes? Parasit Vectors 2019, 12, 405, doi: 10.1186/s13071-019-3665-7.
68. World Organisation for Animal Health (OIE): Part 3, Section 3.1, Chapter 3.1.18. Rabies (Infection with rabies virus and other Lyssaviruses). In: *Manual of diagnostic tests and vaccines for terrestrial animals*, Sixth Edition, WOAH, Paris, 2008.
69. Wymazał A., Nowak S., Mysłajek R.W., Bajer A., Welc-Falęciak R., Szewczyk M., Kwiatkowska I., Stępniak K.M., Figura M., Kloch A.: Tick-borne infections in wolves from an expanding population in Eastern Europe. Ticks Tick-borne Dis 2024, 15, 102272, doi: 10.1016/j.ttbdis.2023.102272.
70. Zub K., Theuerkauf J., Jędrzejewski W., Jędrzejewska B., Schmidt K., Kowalczyk R.: Wolf pack territory marking in the Białowieża Primeval Forest (Poland). Behaviour 2003, 140, 635–648, doi: 10.1163/156853903322149478.