Preventing the extinction of the Dinaric-SE Alpine lynx population through reinforcement and long-term conservation

LIFE16 NAT/SI/000634

Health surveillance during the LIFE Lynx project (2017 – 2023)

Action C.5

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Contents

Introduction

Animal health can have important implications not only for the establishment and survival of the reintroduced populations but also for individual welfare (Cunningham, 1996, Viggers et al., 1993). Translocated individuals may expose target populations to diseases for which they have no resistance, which is especially problematic for inbred populations with low levels of immunity. Conversely the reintroduced individuals may be affected by diseases already present in the remnant population (Thorne and Williams, 1988). Translocated animals may be particularly vulnerable because of a lack of acquired immunity from previous exposure, and stress-induced immunosuppression. Translocations are therefore regarded as the movement of a package of living organisms, rather than the mere movement of the individuals of the translocated species (Dalziel et al., 2017).

There is a general acceptance that pre-release health-screening is good practice, however, there is no clear consensus on the protocols (Mathews et al. 2006). For instance, the choice of analyzed pathogens varies, even between different reintroduction schemes for the same species. Most of the available data focuses on diseases of zoonotic concern, or those that directly threaten populations with extinction (Mathews et al. 2006). Additional problem is that for the most species of conservation concern, the baseline data needed to determine which agents to monitor are lacking.

Leighton (2002) suggests that a screening program related to wildlife translocations and reintroductions should have three functions: first to protect the recipient population from new pathogens; secondly to create awareness of the stress-induced risks to the animals being moved; and thirdly to establish the risks to the founder group from pathogens endemic in the recipient population. "Translocation Disease Risk Analysis" is a method to evaluate the health risks associated with any animal movement and identify mitigation measures to reduce them (Jakob-Hoff et al., 2014; Sainsbury and Vaughan Higgins, 2012; OIE, 2017).

LIFE Lynx project was implemented in the period July 2017 – March 2024, with 11 partner institutions from five countries. Eighteen Eurasian lynxes (*Lynx lynx*) were captured in Carpathian Mountains (Slovakia and Romania) and translocated to Dinaric range (Slovenia and Croatia) and south-east Alps (Slovenia). This report presents methods and results of health monitoring program conducted during the LIFE Lynx project. Additionally, data about two animals translocated to Italy from Romania, and one Croatian orphan lynx translocated to Italy within the Ulyca 2 project were included in this report.

LIFE Lynx project health protocol

Health protocol and disease risk analysis for the translocation of Eurasian lynxes from Carpathian Mountains (Slovakia and Romania) to the Dinaric and southeastern Alps (Croatia and Slovenia) were prepared based on the review of published literature and consultations with relevant experts. There was almost no data published about infectious agents present among the either of the lynx populations involved in the project, so efforts were made to gain insight into the health status of the donor and receiver population before the inclusion of the translocated animals. This was challenging due to the low number of available samples, so additional samples were collected during the entire project implementation.

Project protocols for capturing, quarantine and transport of lynxes were prepared at the beginning of project implementation for each source country (Kubala et al. 2018). These documents contain detailed description of capture, tranquilization, transport and quarantine (and those procedures are not the focus of this report). First part of the health screening of the captured individuals starts at the capturing location, focusing to prevention of translocation of animals that are clinically diseased or asymptomatic carriers of pathogen that might be potential risk to other lynxes, wild or domestic animals and humans. Main veterinary procedures at capture location include clinical examination, animals were weighted, and body measurements taken, vaccination against rabies and antiparazitic drugs were administered, blood samples, feces (if possible) and mucosa swabs were collected. Lynx in a normal body condition, aged more than one year but not more than approximatively 12 years, without significant clinical abnormalities were considered as adequate for translocation and transported to the quarantine. Health surveillance was continued during the quarantine, animals were monitored continuously, and analysis were performed on samples collected at capturing location. Additionally, if necessary additional samples could be collected non-invasively (for example feces from the enclosure) or if necessary, animals were tranquilized. Thirty days after the capturing lynx were tranquilized again and blood samples were analyzed for rabies antibody titter by EU accredited laboratory (antibody titration must be at least equal to 0.5 IU/ml). According to translocation disease risk analysis it was planned that animals that tested positive for Feline Leukemia Virus (FeLV), panleukopenia virus (FPV) and Feline Immunodeficiency Virus (FIV) will not be translocated, and further actions will be discussed with the responsible authorities. So, depending on the results of viral agents and rabies antibody titer, animals were declared suitable for translocation or required additional health testing and/or received treatment.

In the recipient countries, Slovenia and Croatia, health protocols were focused to monitoring of mortality, clinical examination of live captured animals and monitoring of infectious diseases based on samples collected from all possible sources. Carcasses were found by chance or recovered based on radio-tracking. Morphological data, pictures of coat pattern of both sides of the body, samples (blood, mucosa swabs, hair, feces) were collected from both live and dead animals, stored and analyzed. Protocols for necropsy and clinical examination of live animals prepared at the beginning of the project were adjusted according to Ryser-Degorgis et al. (2021) and were constantly improved.

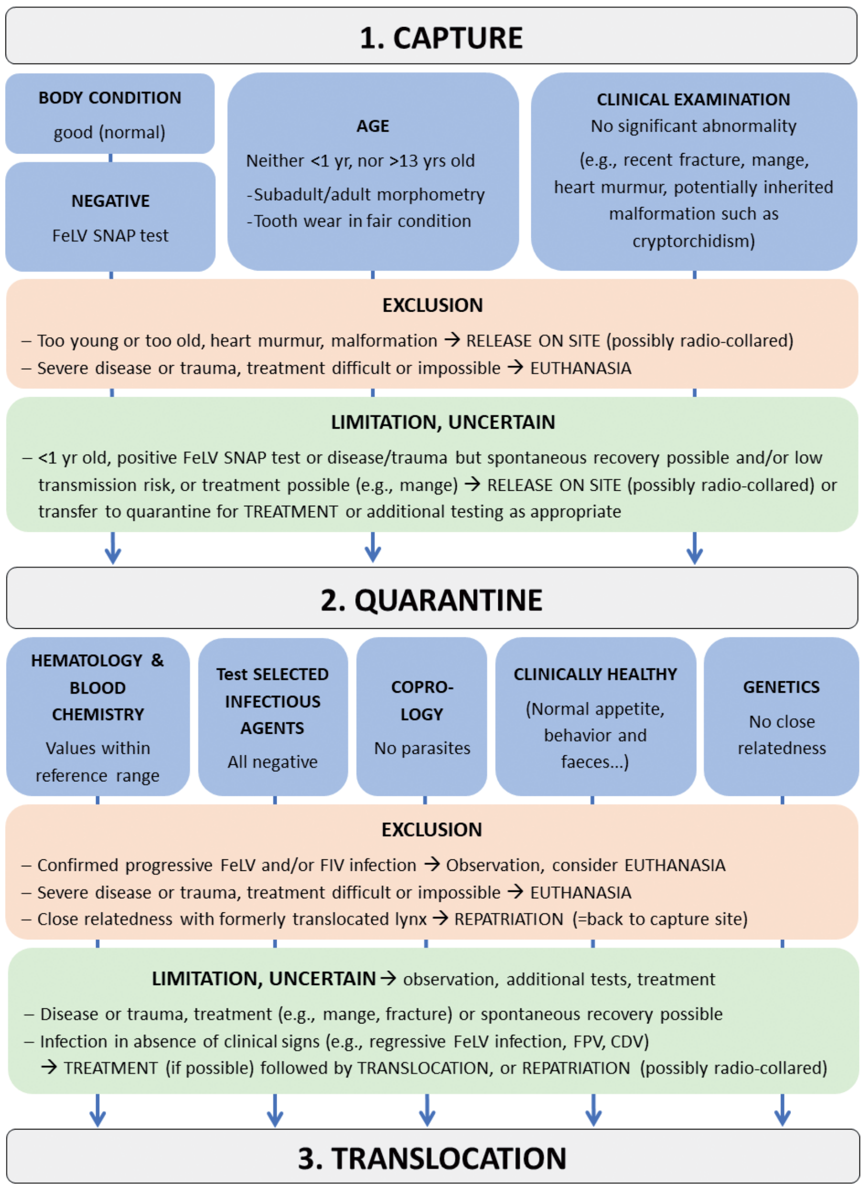

Figure 1. Selection criteria applied for lynx translocation (Ryser-Degiorgis et al. 2021)

Health status of the resident Dinaric and SE Alpine population

Mortality in the Dinaric lynx population

During the six years of LIFE Lynx project implementation (July 2017 – June 2023) all available lynx carcasses were collected and pathology examination were performed at the Veterinary faculty University of Ljubljana and Zagreb. In that period a total of 16 dead lynxes were recorded in Croatia (9) and Slovenia (7), and none in Italy (Table 1). Additionally, we received anonymous reports about 3 cases of lynx poaching in Croatia, but none of them could be confirmed. The highest yearly mortality was recorded in 2021 with 4 confirmed and 1 unconfirmed case, while the annual average lynx mortality for confirmed cases was 2,7 for Croatia and Slovenia together (1.5 for Croatia and 1.2 for Slovenia). Among the 16 animals, there were 7 females, 7 males, while for 2 animals sex could not be determined. Eight animals were adults, 6 juvenile and in 2 cases age could not be determined.

Causes of death were various. In five cases (31,3%) cause of death was caused by humans - 4 collision with cars (2 in Slovenia (2019 and 2021) and 2 in Croatia (2017 and 2018)) and one poaching case in Slovenia. Female lynx Neža was captured on 13.2.2022 on Mala gora. She was one of the three kittens from the litter of translocated lynx Goru and Teja born in the season 2021-2022. At the time of the capture Neža weighed 12 kg and she was eight months old. In the end of March 2022, she started to show first signs of dispersion from her mother. She moved a couple of times different ways mostly towards the east, but each time she came back. On May 11th 2022 a mortality signal was received and the collar was found ripped off the lynx. With the help of a trained detection dog, blood traces were found nearby and genetic analysis showed that the blood was Neža's. With these proofs, the police unit trained for investigation of illegal killing, started with the investigation. The case has been reported to the court, however, the process has not been finalized yet.

One case of drowning was recorded – juvenile male lynx, aged approximately 6 months, was found in an abandoned and uncovered well in Croatia, near Donji Lapac, Lika - Senj county, on November 5th 2020. Necropsy confirmed that drowning was the cause of death. Similar case happened in January 2022 when a live lynx was found in an empty underground water tank near an abandoned house, also in Lika – Senj county, Croatia. LIFE Lynx team immediately went to the location, tranquilized the animal and evacuated it from the tank. Lynx was a juvenile male, underweight and dehydrated but without any visible injuries. First aid was provided, lynx was rehydrated, fed and kept in a transport box indoors and monitored for 24 hours. As he started to consume food and show signs of aggression towards people it was concluded he should be released back to nature. Additional food was provided on the release site and the animal was equipped with a telemetry collar. Unfortunately, he was found dead three days later. Pathology exam confirmed the animal died due to malnutrition. So, although the final causes of the death were not the same (drowning and malnutrition), the downfall into a man-made object (water tank) is what has actually caused the death of those two juveniles.

Another case was connected to human activity. In January 2020 we got info that in Lika – Senj county in Croatia, in the surrounding of town Gospić (exact location unknown) guarding dogs (breed Anatolian shepherd) killed an adult lynx. We got a photo of the lynx but could not retrieve the carcass.

Indications of death due to health issues were detected in 5 cases (31,25%).

Male juvenile lynx was found alive on December 7th 2017 near Novi Vinodolski in Croatia. He was near the settlement, showed no fear of humans, was malnourished and had injured front leg. So the lynx was presumed the be orphan, was captured and transported to Zagreb ZOO. Despite therapy lynx died the next day. Necropsy showed generalized anemia, cachexia, purulent necrotic hepatitis, inflammation of the lymph nodes, purulent bronchopneumonia and sepsis.

Juvenile female (5,5 kg) was found dead on 19.2.2019 near Ložine, Slovenia by a dog walker. The lynx died at least few days before, since the body already started to decay. The lynx kitten was in poor body condition. Skin was crusted and thickened on several locations and parasitological examination of skin samples was positive for the causative agent of scabies, *Sarcoptes scabiei*. Bacteriological analysis of the lungs confirmed the presence of *Staphylococcus felis* bacteria. Molecular tests of various tissues and matrices for the presence of viral genetic material of more common viruses were negative. Based on the results of the examination it was concluded that the cachexia and death of the lynx was due to infection/sepsis with the *Staphylococcus felis* that affected the lungs. Purulent inflammation of the skin, a consequence of a scabies infection, has persisted for an extended period of time, causing the animal to stop eating, as evidenced by marked bloating and dehydration and severe constipation.

Adult female lynx Viva (17 kg) died in Slovenia on March 23rd 2021, a day after she was captured in a box-trap, sedated, collared and released. This female was known from systematic camera trapping and other photo records since 2016 while genetic analysis later showed that she was at least 10 years old (her genetic sample was found in Snežnik area in 2011). She was a territorial female in the area of Glažuta and Kočevska Reka and had been recorded with two cubs in lynx year 2020-2021. The animal was in good body condition and the teeth were badly worn. The heart was of normal size and irregular round shape with two apices. There was an oval opening,17 mm in diameter in the wall between the left and right atria. Histopathologic examination revealed severe adiposis and fibrosis of the myocardial tissue, mild thickening and necrosis of the myocardial cells, and severe fibrosis of the inner pericardium. Molecular tests of various tissues and matrices for the presence of viral genetic material of common viruses were negative. The female lynx died due to the heart failure caused by ostium secundum atrial septal defect. Ostium secundum atrial septal defect (ASDs) is a congenital heart defect that allows animals reach sexual maturity due to its location, but often causes shorter lifespan. Death is often result of decompensation and heart failure due to the abnormal or strenuous physical or psychical exercise. The very same congenital heart defects have been found in Florida panthers in high prevalence (Cunningham 2016). Moreover, histopatological examination revealed severe degeneration and fibrosis of the myocardium and liver, severe anemia in the lungs, and small groups of glial cells in the brain. Chronic inflammation and a single small renal stone were found in the kidneys, while numerous large hemorrhages were found in the small intestine and small hemorrhages in the uterine wall.

Adult female lynx was found dead on October 3rd 2021 in Gorski Kotar, Croatia. We have monitored this lynx with camera traps since November 2019 and she was named Jela. The last time she was photographed was in March 2021 and no signs of problems were observed. Necropsy showed lynx was cachectic, dehydrated and anemic, and was infected with scabies. Also mild pneumonia was diagnosed and chronic degenerative changes on the heart muscle. Pathologist concluded that heart condition was not the primary cause of the death but contributed to the overall bad health.

A remnant male lynx named Igi was captured on 17th of February 2022 in Mokrc area, Slovenia. He was monitored with camera traps during the previous year. He was estimated to be around 5 years old and weighed 20 kg. During the immobilization a heart murmur was detected, also he had two tufts on one ear. Ultrasound on the anaesthetized animal has confirmed the same congenital defect – atrial septal defect (ASDs) as in female Viva. Modified anesthesiologic protocol has been used and recovery has been prolonged, however uneventful otherwise. This type of the heart defect causes the mixing of oxygenized blood and its recirculation through lungs which might alter the pharmacokinetics of anesthesiologic agents. Partial opacity on the lens of one eye has also been found during the clinical examination, where neither traumatic nor degenerative origin could be confirmed. His home range was quite small, compared with other males and measured around 60 km^2. During his telemetry monitoring, we surveyed some of his kill sites, where he mostly preyed on roe deer, however we also found one consumed feral cat close to the village, probably killed by him. In the beginning of May 2022, a mortality signal from the collar was received. From the telemetry data we could see that lynx Igi was confined to a small area of about 1 km^2 for two weeks before his death. The activity data from the telemetry collar further confirmed his stagnation as the activity almost halved after he made his last roe deer kill. Necropsy confirmed the same type of congenital heart defect – atrial septal defect, type secundum, as found in female Viva. Molecular testing of various tissues and matrices for the presence of viral genetic material of more common viruses was negative. We assume his death is a consequence of heart failure, which appeared to have been triggered during the hunting attempt.

Figure 1. Lynx Igi at the time of his capture, with his special feature: two ear tufts on the same ear.

In three cases (18.8%) the cause of death could not be determined.

In August 2018 a policeman found a dead lynx on mountain Plešivica in Croatia and photographed it. Unfortunately, he did not report about this finding trough obligatory channels and by the time we were informed the carcass was gone, so we could not retrieve it to the cause of death is unknown.

Female lynx named Martina was found orphaned in Croatia in August 2019. Rehabilitation was attempted in enclosure built in Risnjak National Park and the animal was released in February 2020. Martina dispersed to Slovenia, but her collar soon failed. On August 6th 2020 only bone remains and collar were found in Pivka valley, in an open agriculture landscape and the cause of death could not be determined.

On April 20th 2021 young male was found by a hunter in northern Dalmatia, south of Sinj and this is the most southeastern verified (C1 category) evidence of lynx presence in Croatia. The carcass was already decayed so a certain cause of death could not be determined. Bullet wounds or casings were not detected, but the ears were cut with knife or scissors pointing that a human was in contact with the carcass, so poaching is not excluded.

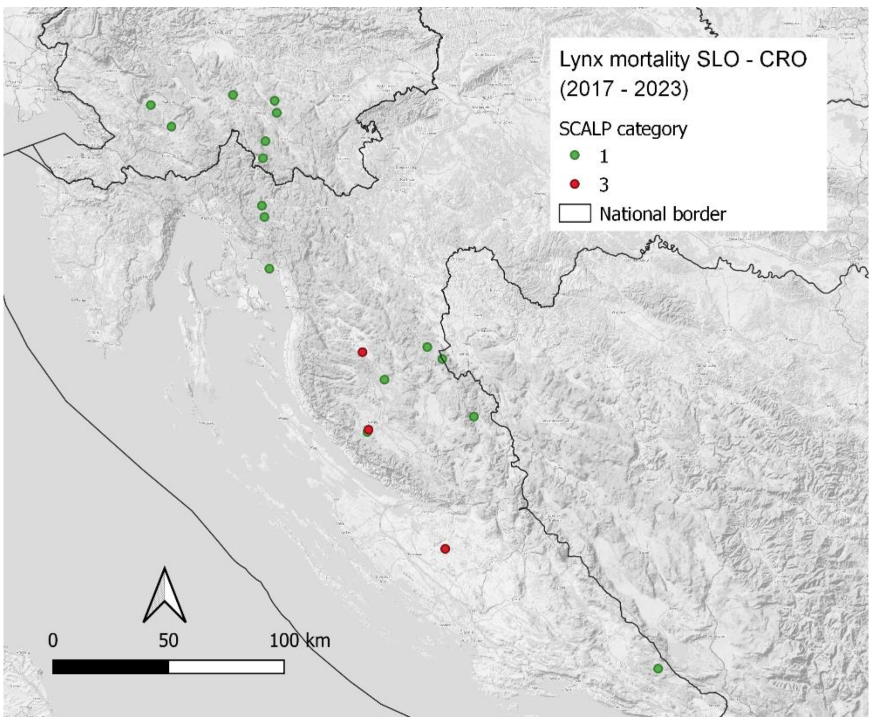

Figure 2. Map with locations of lynx mortality recorded in Croatia and Slovenia in the 2017 – 2023 period. Green dots represent cases confirmed with hard evidence, categorized as SCALP 1, while red dots represent unconfirmed cases (SCALP 3).

Infectious diseases

Retroviruses

Feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) were identified as one of the most hazardous viruses for the lynx translocation programme (Meli et al., 2009), so before the translocation we analyzed the prevalence of retroviruses in the Dinaric lynx population. Samples from 17 lynxes from Croatia collected in the period 2001 – 2019 were available before the translocations started and were tested using two different approaches – ELISA and molecular analysis. Two commercial immunochromatographic assays were used - FASTest® FeLV- FIV, MEGACOR Diagnostik GmbH and IDEXX SNAP Combo Plus, IDEXX Laboratories. Also, molecular diagnostics for FIV infection were performed with two different PCR protocols - for detection of proviral gag and env genes. For FeLV infection nested PCR was performed for gag gene amplification. All samples tested negative (Gomerčić et al. 2021).

Additionally, 4 resident animals captured in Croatia during the project were tested for FIV and FeLV using the same protocol and all were negative.

Canine protoparvovirus 1

Canine protoparvovirus 1 encompasses canine parvovirus type 2 (CPV-2) and feline panleukopenia virus (FPLV), causing agents of severe, often deadly disease of terrestrial carnivores. Dogs are susceptible only to the CPV-2 and all its variants, but cats are susceptible to both FPLV and CPV-2 variants. Presence of CPV-2 and FPLV was analyzed in 6 lynxes captured in Croatia using PCR amplification of the NS gen. All samples tested negative

Feline herpesvirus 1

Herpesviruses can cause latent infections and disease in a wide variety of mammals. They are generally showing low pathogenicity but can cause severe illness and even mortality under immunosuppressive conditions. Feline herpesvirus causes infection of upper respiratory tract and eyes. Clinically recovered cats become latently infected carriers which undergo periodic episodes of virus reactivation, particularly after a stress. A total of 5 swabs of nasal cavity and eyes collected from lynxes captured in Croatia were tested using PCR and all samples were negative.

Leptospirosis

Leptospirosis is a re-emerging veterinary and public health problem caused by different pathogenic serovars of the genus *Leptospira*. Disease is usually endemic and sustained in a certain environment by variety of animal species with rodents as primary reservoirs. Serological tests were conducted by the microscopic agglutination test (MAT) with 12 *L. interrogans* serovars on 14 animals captured in Croatia before the reintroductions started. All tested negative.

Francisella tularensis

Francisella tularensis is the causative agent of the zoonotic illness tularemia. Although natural infections by *F. tularensis* are sporadic and generally localized, the low infectious dose, with the ability to be transmitted to humans via multiple routes and the potential to cause life-threatening infections, is the reason why a national monitoring program was implemented for tularemia in Croatia in 2018. As rodents, hares and rabbits are the main reservoir, lynx as their predator was also included in the monitoring program. Serum samples of 14 lynxes from Croatia were analyzed using agglutination test and 1:20 dilatation all were negative for *Francisella tularensis*.

Sarcoptic mange

Sarcoptic mange (or scabies) is a highly contagious skin disease caused by parasitic mite *Sarcoptes scabiei*. Sarcoptic mange has been identified as the most common infectious cause of death in lynx in Sweden (Ryser Degiorgis et al., 2005), and has already been classified as a population hazard for Eurasian lynx translocation by Mayhew et al (2017).

Traditionally sarcoptic mange is monitored by examination of dead animals and confirmation of the parasitic infestation, but recently non-invasive camera trap monitoring is being implemented for wolves and foxes. As the number of carcasses examined during the project was low (13, out of which two animals were diagnosed with sarcoptic mange) we also used lynx images from camera traps to monitor the presence of skin lesions, possibly associated with sarcoptic mange in the Dinaric lynx population. Out of almost 5000 lynx pictures collected in Croatia and Slovenia during the March 2018 – March 2022 period skin lesions that could be caused by scabies were detected in three animals, one in Croatia (Figure 3) and two in Slovenia (Figure 4). Besides scabies, skin lesions noticed on camera trap photos might be caused by heavy flea infestation or congenital hypotrichosis, commonly known as rat-tail syndrome.

Figure 3. Camera trap images of lynx with skin lesions, photographed in Croatia in January 2023 and March 2023

Figure 4. Camera trap image of lynx with thin tail, photographed in Slovenia in June 2022

Parasitology

Detection of parasites can be done on feces collected from rectum of captured animals or found at the capturing location, or on the carcasses of dead animals. Collection of feces in the nature is challenging, as lynxes live on large territories and often hide their feces. Also scats found in the nature are primarily used as a source of DNA for the monitoring of genetic variability. When animals are captured and tranquilized their rectum is often empty, so both invasive and non-invasive sampling of lynx feces is challenging. Due to those reasons our analysis was based on a small sample size. Collected feces was analyzed using the flotation method with a saturated $ZnSO_4$ solution (S.G.=1.3). Also, to detect *Giardia* sp. and *Cryptosporidium* sp. cysts, fecal samples prepared using the flotation technique with sucrose are analyzed with the direct immunofluorescence method (Meridian Bioscience, Inc., Merifluor® *Cryptosporidium/Giardia*).

Details insight in internal and external parasites can be done as a part of necropsy of dead animals. The gastrointestinal tract (stomach and intestine) are removed from the animal, stored in separated plastic container, opened along its length and flushed with water, while its mucosa was scraped and separated in another plastic container. Content of the stomach and intestines are examined in Petri dishes on a dark background under the stereomicroscope, while scraped mucosa is checked for parasites under the microscope. Parasites were then washed out with saline to remove the gastrointestinal contents to facilitate the determination to the genus or species level, depending on conservation condition.

In Croatia out of 9 dead animals that were examined during the project period, parasitology analysis was performed on 5 as others were in advanced state of decomposition. Three animals had no intestinal parasites, one had nematodes in the stomach and intestine (which could not be determined to the species level due to the decomposition) and one animal had a single *Toxascaris leonine* in the intestine. Out of 4 dead animals examined in Slovenia, only one had single parasites of genus Ascaris. As already mentioned previously, two dead animals also had sarcoptic mange.

Only one sample of captured animals was collected in Croatia, the one of orphan lynx Karlo found in 2022. Flotation method showed he had individual eggs of *Toxocara cati* and *Isospora* sp. oocysts. *Giardia dudodenalis* was also identified.

Health status of the lynxes translocated from Carpathians

Infectious diseases

All 18 lynxes included in the translocation program were tested for feline immunodeficiency virus (FIV), feline leukemia virus (FeLV), Canine protoparvovirus 1 and feline herpesvirus 1 (methodology described in the previous chapter). Also, two animals captured in Romania and translocated to Italy were included in the analysis. All testes were negative, except lynx Doru from Romania that tested positive for the Canine protoparvovirus 1 and lynx Zois also from Romania that tested positive for the feline herpesvirus 1. Both lynxes did not have any clinical symptoms and their blood values were within the normal range. So, after consultations with literature and experts it was concluded that animals will be kept in the quarantine and retested each week, until negative.

Parasitology

At capturing, scat sample was successfully collected from only one lynx from Slovakia. He was diagnosed with Trichuris sp. eggs and was positive for *Giardia duodenalis*.

Conclusion

Health status of the source and target population are essential info for the health management plan of the reintroduction/reinforcement projects. Unfortunately, we were faced with the same issues as many previous reintroduction/reinforcement projects – lack of data about the prevalence of infectious and non-infectious diseases both at the source and target population. The only available source of info was analysis of mortality causes during the 40-year period in Croatia (Sindičić et al. 2016) and research of the prevalence of FIV and FeLV published just before the first translocations (Gomerčić et al. 2021). So during the project implementation our health surveillance was focused to both the source Carpathian, and the target Dinaric population. For the source population examination of carcasses and clinical examination and laboratory analysis of samples of live captured animals was the main source of info, while for the Carpathian population we were focused to captured animals.

Monitoring of mortality in the Dinaric lynx population showed that the most frequent (31,3%) cause of mortality among the 16 recorded cases relates to human activity (4 car accidents and one poaching case). With 2 additional cases of death due to the fall into man-made water tanks and 1 death due to shepherd dog attack, the total of human induced mortalities is as high as 50%. Even though the number of carcasses (9 in Croatia, 4 in Slovenia) is very small and not representative, it is very worrying that indications of death due to health issues were detected in 5 cases (31,25%). Also, especially alarming are proofs of congenital heart defects and heart failure as a cause of death.

Animals that were live captured in both the source and target countries were tested for a panel of pathogens and showed predominantly negative results. So, results of our health monitoring are indicating that infectious diseases are not a significant threat, but that further attention should be paid to congenital diseases and possible impaired immunity in the Dinaric lynx population. Our findings suggest that in animals with congenital heart defects stress caused by hunting the prey or by being captured for telemetry purpose might be deadly.

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