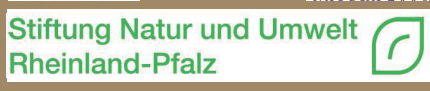


# Special Issue

N° 14 | Autumn 2021

# CAT FLOWS

## The Eurasian lynx in Continental Europe





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Original contributions and short notes about wild cats are welcome

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**Cover Photo:** Camera trap picture of two Eurasian lynx kittens in north-eastern Switzerland. 11 December 2014 (Photo KORA).

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# Health surveillance in wild felid conservation: experiences with the Eurasian lynx in Switzerland

**Switzerland has become an important source of Eurasian lynx *Lynx lynx* for reintroduction projects in Europe. It is now widely accepted that translocations of animals are associated with a serious health risk. Therefore, the development of multidisciplinary expertise and the elaboration of veterinary protocols are needed, which require knowledge on the health status of the source population and information on potential health risks at the release site. Here, both disease cases and carriers of potentially threatening pathogens have to be taken into consideration. In Switzerland, a range of infectious agents circulate within lynx populations apparently without associated morbidity. However, genetic analyses combined with health investigations have pointed at a possible inbreeding depression. Furthermore, unexpected health issues arose in the framework of translocations. Overall, the Swiss experiences underline the necessity of long-term health surveillance of reintroduced and small isolated wildlife populations, the usefulness of well-established veterinary protocols in the framework of translocation projects, the value of multidisciplinary collaborations and of sample archives for further analyses, and the need for adaptive management based on scientific data. For a conservation programme of the Eurasian lynx on a pan-European level, procedure harmonisation should be sought.**

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Defaunation is a rather new term that aims at raising awareness for the ongoing unprecedented species loss worldwide (Dirzo et al. 2014). Attempts to counteract this dramatic phenomenon include conservation efforts through species reintroductions and population reinforcement (Seddon et al. 2014). However, it is now widely accepted that translocations of animals are associated with a serious health risk (Daszak et al. 2000, Kock et al. 2010) because relocation of an animal always entails relocation of a “biological package” (the animal together with its “passenger organisms”). Further health aspects to consider in this context are stress-induced increased susceptibility to disease and injuries associated with capture, transport, and confinement in a quarantine enclosure. Here, not only health but also animal welfare requirements must be fulfilled (Kock et al. 1999, Kock et al. 2010, Ryser-Degiorgis 2009a). Additionally, genetic considerations are required, particularly when dealing with populations arising from only a few individuals (Trinkel et al. 2011, Brambilla et al. 2015, Pelletier et al. 2017, Grossen et al. 2018, Bozzuto et al. 2019). The development of veterinary protocols requires knowledge on species susceptibility to infection and di-

sease, causes of mortality and health risks, both in the source population and at the release site, including non-infectious health issues such as inbreeding depression. The prerequisite to access this information is the existence of a health surveillance programme for the species of interest, consisting at least in necropsies of dead animals as well as pathogen and serological surveys, especially at the source (Ryser-Degiorgis 2009a).

The Eurasian lynx was reintroduced to Switzerland in the 1970s. Meanwhile Switzerland is considered having a great responsibility regarding the conservation of the Alpine lynx population (Zimmermann et al. 2011), and the two Swiss lynx populations (one in the Alps, the other in the Jura Mountains; Breitenmoser et al. 1998, Chapron et al. 2014) have become an important source for reintroduction and reinforcement projects in neighbouring countries. The aim of this article is to share the implemented procedures and acquired experience in the framework of the veterinary supervision of lynx translocation in Switzerland (2000–2020).

## Lynx health surveillance programme in Switzerland

Surveillance of lynx health in Switzerland has been carried out for several decades, im-

plying the close collaboration of veterinarians, biologists, wildlife managers and museums. The programme currently in place includes (1) the pathological examination of all lynx found dead (whether diseased, poached or traffic-killed). Carcasses may be found by chance or recovered thanks to radio-tracking; and (2) the clinical examination of live lynx (orphans and older animals captured for management, conservation or research purposes).

The costs of post-mortem investigations have been covered by the long-term mandate of the Swiss Federal Office of the Environment (FOEN) to the Centre for Fish and Wildlife Health (FIWI) at the University of Bern for general surveillance of wildlife health in Switzerland (Ryser-Degiorgis and Segner 2015). The FOEN has also supported the lynx population monitoring carried out by the Foundation KORA (Carnivore Ecology and Wildlife Management) and attributed mandates to both the FIWI and KORA for the translocation programmes. Research grants have additionally contributed to capture costs and genetical analyses, and the laboratory analyses have been supported by the Clinical Laboratory of the University of Zurich. An additional contribution has consisted of non-remunerated personal investment by multiple collaborators.

Morphological data, pictures of the coat patterns and samples such as blood are collected from both dead and live animals. Faeces from live animals are collected from the ground, either in the field (on tracks or around a prey of radio-marked animals) or during quarantine. Samples are subsequently analysed and/or archived. Tentative rehabilitation of lynx orphans has been carried out for decades, with disappointing results. The main limitations have been the political situation hindering releases into the wild, the lack of appropriate enclosures, captivity stress resulting in severe teeth damages, and post-release issues such as traffic accidents or predation on domestic animals. Independent diseased lynx were either treated in the field and released on site (two cases affected by sarcoptic mange) or euthanised in quarantine because of severe debilitation (two cases with suspected Feline Immunodeficiency Virus (FIV) infection; Ryser-Degiorgis et al. 2017).

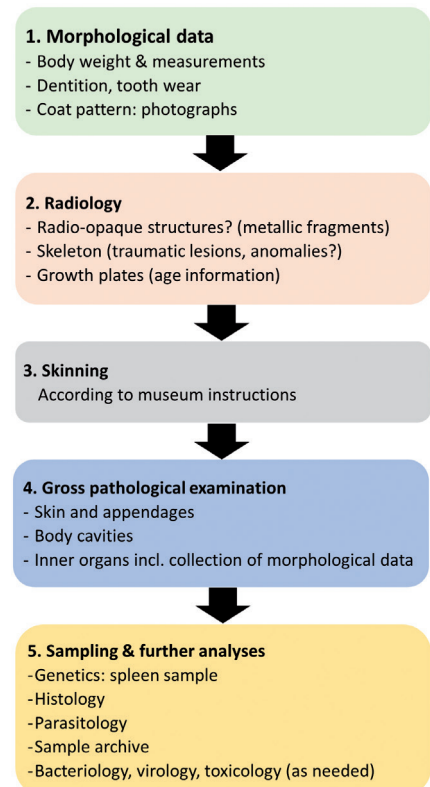
## Dead lynx

Post-mortem examinations on Eurasian lynx found dead, culled or euthanised in Switzerland have been carried out since the 1970s (earliest reports in the FIWI archives) and the necropsy findings compiled from 1987

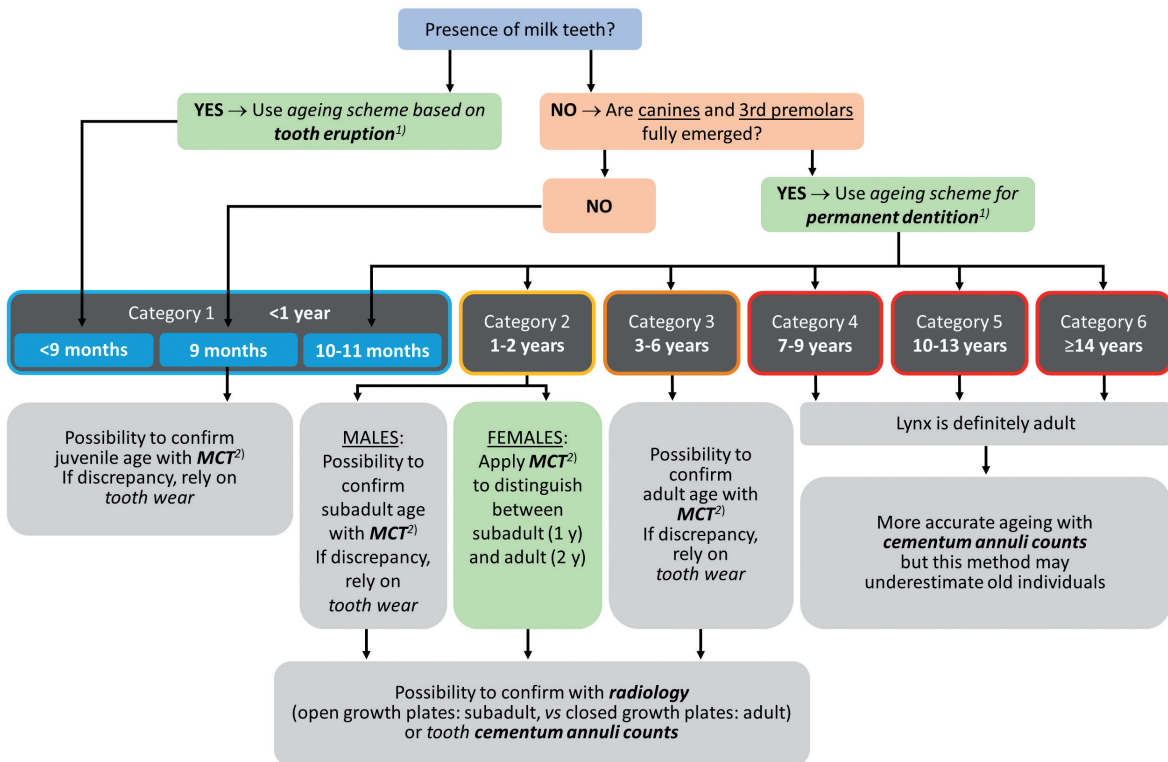
(Schmidt-Posthaus et al. 2002). An extended necropsy protocol including sample collection for systematic histological analyses (collection of baseline data) and for archive purposes was introduced in 2002. The 2004 update of the official management plan (Swiss lynx concept, originally implemented in 2000), required the submission of all dead lynx to a single institution (FIWI). Since then, the FIWI has been officially responsible for lynx veterinary examinations and for hosting a sample archive.

Necropsy and sampling protocols have been improved over years. The current protocol includes the following steps (Fig. 1): lynx are photographed on both sides to record the individual coat pattern to identify individual animals for comparison with photo-trapping data (Thüler 2002, Pesenti & Zimmermann 2013), sex and body condition are determined, the body weight is recorded, and standard morphological measurements are taken (Marti & Ryser-Degiorgis 2018b). Age is estimated mainly based on dentition, tooth wear and body size (Fig. 2; Marti & Ryser-Degiorgis 2018a, 2018b) but also considering maturity of genital organs and season.

All animals are systematically radiographed to search for foreign bodies such as ammunition fragments and for skeletal anomalies. After complete skinning according to museum instructions for subsequent taxidermic preparation, a thorough gross necropsy is performed without damaging the skeleton. A careful macroscopic inspection of the thoracic and abdominal cavities as well as of all internal organs is carried out; pictures of any abnormality are taken. Weight and other morphological data of selected organs are collected, and multiple organ samples fixed in 4% buffered formalin for histological examination. Additional native samples (blood, selected organs) are stored frozen at -20°C for genetic analysis and at both -20°C and -80°C for archive purposes. The brain is only collected if required to achieve a diagnosis, after consultation and agreement of the local hunting authorities who submitted the case (otherwise, skeletons are left intact for taxidermy). Samples of the diaphragm, tongue and/or masseter muscle, as well as faecal samples from the rectum, are immediately submitted to parasitological examination, namely for the search for *Trichinella* sp.



**Fig. 1.** Necropsy protocol for Eurasian lynx established at the Centre for Fish and Wildlife Health, University of Bern, Switzerland.



**Fig. 2.** Decision tree to determine the age of Eurasian lynx (developed for *Lynx lynx carpathicus* in Switzerland). <sup>1)</sup> Marti and Ryser-Degiorgis 2018a (age estimation based on tooth eruption and tooth wear); <sup>2)</sup> MCT = morphology classification tree described in Marti and Ryser-Degiorgis 2018b (age estimation based on morphological measurements). These two methods have the advantages that they are noninvasive, costless, deliver immediate results, and can be applied both intra-vitam and postmortem, in any working place. Green boxes are necessary steps, light grey boxes correspond either to possible confirmatory steps or to a more accurate but invasive ageing procedure.

(Frey et al. 2009) and for gastrointestinal helminths and protozoa, respectively. If necessary, bacteriological, virological or toxicological analyses are initiated to determine the cause of death.

*Live lynx*

A procedure similar as that applied to dead lynx is used for live lynx, starting with the collection of morphological data including body weight and photographs of the coat pattern (Fig. 3). Blood has been collected for genetic analyses since 1993. Since 1997, additional blood samples have been taken for health investigations and archiving. Since 2000, a thorough clinical analysis and close anaesthesia monitoring has been performed on every lynx manipulated alive (Supporting Online Material Figure SOM F1). The corresponding data have been recorded on paper and the main information transferred into a digital database. Since 2013, heart sounds have been recorded by means of an electronic stethoscope with record function (3M™Littmann® 3200; [https://www.littmann.com/3M/en\\_US/littmann-stethoscopes/](https://www.littmann.com/3M/en_US/littmann-stethoscopes/)), and since 2018 echocardiographies have additionally been performed, using a portable ultrasound device (Logiq\_e BT12 with transducer 3S-RS (1,5–4,0 MHz), scil animal care company GmbH, Germany; SOM F2). Blood samples

and by necessity (according to clinical signs or translocation protocols) other clinical samples (e.g., oropharyngeal, conjunctival or rectal/faecal swabs) have been sent to the Clinical Laboratory of the University of Zurich for immediate analysis (haematology, blood chemistry, serology, and molecular methods for pathogen detection).

**Lynx protocols for translocation**

The first translocation project of Eurasian lynx from Switzerland (2000–2008) aimed at reintroducing animals in an area within country borders (Ryser-Degiorgis et al. 2002a, Zimmermann et al. 2011). Subsequently, a few lynx were translocated to Austria for population reinforcement (2011, 2013 and 2017: [www.kalkalpen.at/de/Luchse\\_in\\_den\\_OOe\\_Kalkalpen](http://www.kalkalpen.at/de/Luchse_in_den_OOe_Kalkalpen)) and Italy (2014: Molinari et al. 2021), followed by a larger reintroduction project to southern Germany (2016–2020: Idelberger et al. 2021). Crossing national borders implied the fulfilling of additional requirements by international regulations (Convention on International Trade in Endangered Species of Wild Fauna and Flora, CITES) and the veterinary authorities of the destination countries. Over the years, health protocols have evolved based on the acquired experience and on the health and genetic data collected.

in the health screening of wild felids to be translocated is also recommended by international organisations (International Union for the Conservation of Nature, IUCN; World Organization for Animal Health, OIE; and European Association of Zoo and Wildlife Veterinarians, EAZWV; Woodford 2000).

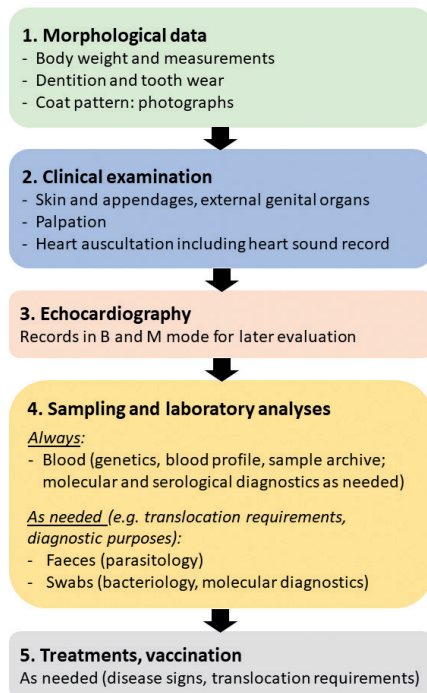
*Disease risk in the destination environment*

Secondly, information was gathered on potential health risks associated with the destination environment. This was most difficult to access, as documentation (scientific literature, unpublished project reports) was poor or non-existent. Consequently, the risk evaluation in foreign countries largely relied on the official epizootic disease status of the concerned country and personal communications from project partners. Nevertheless, for the first project within Switzerland (2000–2008), data on prey species from the general wildlife health surveillance programme were taken into account, and for the last project (Germany, 2016–2020), the release area being in geographical continuity with the source population, it was assumed that the risk of pathogen exposure would be comparable to the situation in the capture area.

*Criteria for translocation*

The selection of individuals aims at: (1) preventing the introduction of lynx either clinically diseased or carrying pathogens representing a potential threat to other lynx, other animals (wild or domestic) and humans at the release site [Figs 4 and 5 (1)]; (2) increasing the chance of survival of the individuals being translocated [Fig. 5 (2a, 2b)]; (3) increasing the chances of reproduction of the released lynx after translocation; and (4) optimizing the genetic pool of lynx moved for reintroduction or reinforcement. These four aspects refer to health both on an individual (1, 2, 3) and on a population level (1, 3, 4). Further important health considerations on an individual level include acting with respect of animal welfare, i.e., selecting appropriate methods for safe and effective capture/anaesthesia, stress management and maximal possible reduction of the risk of injuries.

Criteria for selection revised in 2015 include the absence/presence of disease signs or other abnormal observations at clinical examination, the estimated age (based on the methods described for dead lynx, see above), the genetic profile of the animal, and the results of diagnostic analyses (haematology, blood chemistry, coprology, and pathogen



**Fig. 3.** Capture protocol for Eurasian lynx in Switzerland with focus on veterinary procedures. Details on samples, blood profile and molecular diagnostics are given in Table 1.

*Disease susceptibility of Eurasian lynx*

Firstly, the available published and grey literature was reviewed, completed by personal communications from ongoing studies or unpublished data, to provide an overview of the knowledge on pathogens potentially affecting or carried by lynx (Ryser-Degiorgis 2001, 2009b, Ryser-Degiorgis et al. 2002a).

The main disease of concern was sarcoptic mange, which emerged in lynx in the Swiss Alps in the late 1990s (Ryser-Degiorgis et al. 2002b, Schmidt-Posthaus et al. 2002, Munson et al. 2010). This disease is typically observed in lynx in geographical areas where mange affects the local fox population (Ryser-Degiorgis 2009b, Munson et al. 2010) but at the time there was no mange epidemic in red foxes *Vulpes vulpes* in the release area in north-eastern Switzerland (Pisano et al. 2019). No specific bacteria was of particular concern (Ryser-Degiorgis 2001, 2009b) but feline viruses (Table 1) were considered a potential threat, considering their significance in both domestic and wild felids (Lutz 2005; Leutenegger et al. 1999, Meli et al. 2009). The emphasis on mange and viruses

**Table 1.** Testing scheme for Eurasian lynx to be translocated from Switzerland. Since the purpose is, on the one hand, to assess the health status of each individual, and on the other hand, to prevent the “export” of infectious agents potentially relevant to the (new) population at the release site, investigations target mainly direct pathogen detection rather than antibodies, because antibodies indicate past or present exposure of the individual to the microorganism(s) but do not deliver information on its current infection status.

Parameter	Target	Sample	Details/methods
Blood profile	Haematology	EDTA whole blood + fresh thin blood smears	Complete blood cell count and white blood cell differential
	Blood chemistry	Serum	Chemistry profile parameters*
FeLV <sup>1</sup>	Free FeLV p27 antigen	Serum	ELISA <sup>2</sup>
	FeLV whole virus (FL74) and/or FeLV p15E antibodies	Serum	ELISA
	Proviral DNA	EDTA whole blood	Real-time TaqMan qPCR <sup>3</sup>
FIV <sup>4</sup>	FIV antibodies	Serum	FIV-Westernblot
Feline Herpesvirus	Viral DNA	Conjunctival swab	Real-time TaqMan qPCR
Feline Calicivirus	Viral RNA	Oropharyngeal swab	Real-time TaqMan RT <sup>5</sup> -qPCR
Canine Distemper Virus	Viral RNA	Oropharyngeal swab	Real-time TaqMan RT-qPCR
Feline Parvovirus	Viral DNA	Rectal or faecal swab	Real-time TaqMan qPCR
Feline Coronavirus	Viral RNA	Rectal or faecal swab	Real-time TaqMan RT-qPCR

<sup>1</sup> FeLV: Feline Leukaemia Virus. <sup>2</sup> ELISA Enzyme-linked immunosorbent assay. <sup>3</sup> qPCR: quantitative polymerase chain reaction. <sup>4</sup> FIV: Feline Immunodeficiency Virus. <sup>5</sup> RT: reverse transcriptase. \* Parameters: total bilirubin, glucose, urea, creatinine, total protein, albumin, globulin, cholesterol, triglycerides, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, lipase, creatine kinase, calcium, phosphorus, sodium, potassium, chloride

screening as presented in Table 1). The selection of animals is a process that takes place in two steps (Fig. 6). A first selection occurs in the field after examination at the capture site. Animals considered suitable for the translocation programme are moved to quarantine facilities.

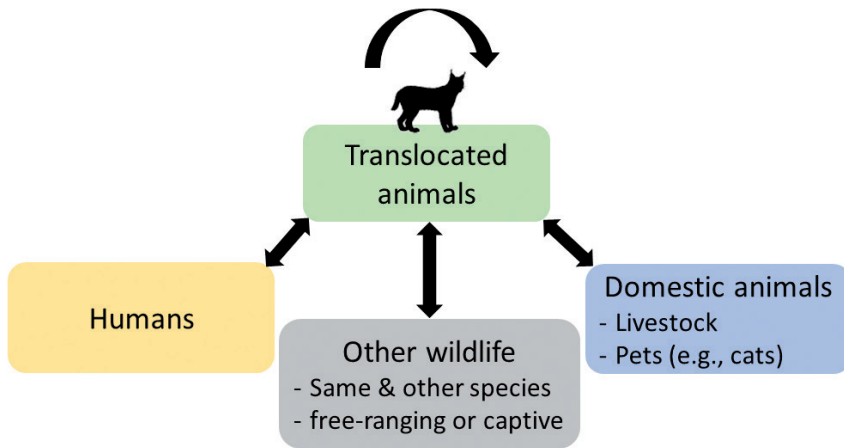
During quarantine, lynx are observed by video-surveillance while their samples are tested in the laboratory (blood and genetic profiles, selected infectious agents and endoparasites). Depending on the results, the lynx will either qualify for immediate translocation, or may undergo additional testing and a prolonged observation period, and/or receive a specific treatment. In some cases, repatriation to the original capture site or even euthanasia may have to be considered (Fig. 6). During the first translocation project (2000–2008), quarantine duration used to be 2–3 weeks. Others recommend a minimum of 30 days for wild felids (Woodford 2000). However, experience showed that quarantine duration shall be reduced to a minimum because of the considerable stress experienced by lynx caught in the wild and the resulting self-inflicted injuries when they are kept in captivity. Besides improvement of the enclosures (size, structure, materials, sliding

doors between adjacent enclosures, video-surveillance), nowadays the quarantine lasts only until all laboratory results are available and the release logistics (border paperwork, transport to release site) is set up; all this takes about a week.

#### Field procedures

Lynx are captured with foot snares, box traps or a remote-controlled injection system as previously described (Breitenmoser et al. 2014, Ryser et al. 2005, Vogt et al. 2016). Until 2019, subadult and adult lynx were anaesthetised with an intramuscular injection of medetomidine hydrochloride (Domitor<sup>®</sup>, Orion Corporation, Espoo, Finland) in the rear muscles of a hindleg, followed by ketamine hydrochloride (Ketazol<sup>®</sup>-100, Dr. E. Gräub AG, Bern, Switzerland) 15–20 minutes later. Since the weight of the animal is not known before anaesthesia, lynx were administered a standard dose of 2.8 mg medetomidine and 80 mg ketamine (i.e., approx. 0.13–0.17 mg/kg medetomidine and 3.6–5.0 mg/kg ketamine depending on the weight; Marti & Ryser-Degiorgis 2018a). This is normally sufficient for a safe anaesthesia until the end of the manipulations. If necessary 0.1–0.2 mg medetomidine and/or 10–20 mg ketamine

was subsequently injected in the shoulder musculature. Atipamezole hydrochloride (Antisedan<sup>®</sup>, Orion Corporation, Espoo, Finland) at a dose of five times the medetomidine dosage (in mg) was used as an antagonist for medetomidine and was injected at least 1 hour after the last ketamine injection. The effect of ketamine can last up to approximately 1 hour and cannot be antagonised. If medetomidine is antagonised too early, there is a risk of rough recovery due to the residual effects of ketamine (Kreeger & Arnemo 2007). Drug injection in the shoulder results in faster absorption (Kreeger & Arnemo 2007), which can be useful in emergency situations. Besides emergencies, our experience has shown that shoulder injections are efficient for drug supplementation during manipulations (see above). However, for recovery under normal conditions, antagonist injection in the hind leg musculature is preferable because it results in a smoother recovery than if the drug is administered in the shoulder muscles. This anaesthesia protocol is well established and no adverse effects have been recorded, neither in previous studies (Vogt et al. 2016) nor in the past few years. However, since 2020, a single intramuscular injection of 2.2 mg medetomidine mixed with 80 mg ketamine



**Fig. 4.** Health risks associated with lynx translocations at population level. Moved wild animals can bring microorganisms or deleterious genes to the destination environment, which may cause disease in individuals of the same species, in other wildlife, in domestic animals or in humans.

has been used per lynx to reduce the induction time, followed by the same procedure for reversal as before. This new protocol has been used for only a few lynx so far but appears to be promising.

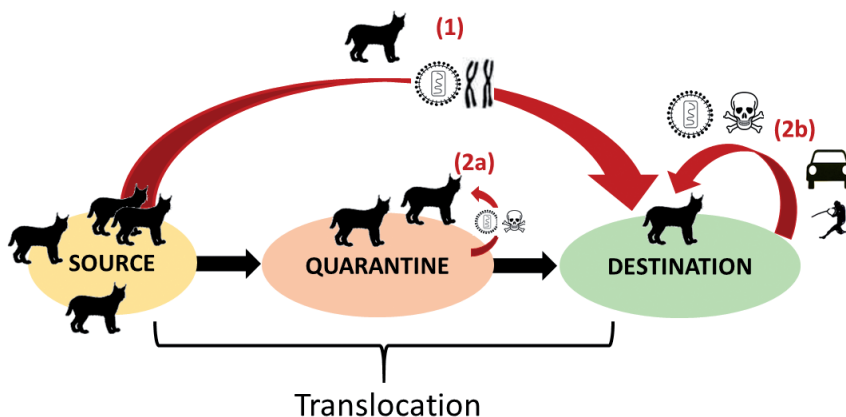
During anaesthesia (SOM F2), respiratory rate, heart rate, pulse rate, mucous membranes (capillary refill time and colour), rectal temperature and reflexes are continuously monitored by a person previously designated for this task. Blood oxygen saturation is measured with a portable pulse oximeter (Pulse Oximeter, CONTEC Medical Systems Co., LTD, Qinhuangdao, China). All values and observations are recorded in an anaesthesia datasheet. The most critical point encountered during field anaesthesia of lynx has been related to the body temperature, name-

ly hypothermia during cold days, especially in rainy or snowy weather and hyperthermia at mild environmental temperatures. In these situations, prevention is crucial as it is very challenging to reverse the development in one direction or the other. Details on manipulation and emergency procedures can be found elsewhere (Breitenmoser et al. 2014, Kreeger & Arnemo 2007).

Captured animals are examined clinically, with particular attention to their general appearance, body condition, size and weight, tooth wear and genitals. Animals in a normal body condition (considering that adult males may be thinner during the mating season, aged more than one year but no more than 13 years old, and without significant clinical abnor-

malities (such as a recent fracture, infected wounds, mange lesions, a heart murmur or a potentially inherited malformation such as cryptorchidism), are considered as adequate for a transfer to quarantine facilities (Fig. 6). By contrast, obviously old lynx (based on tooth wear, e.g., severely worn or discoloured teeth; Marti & Ryser-Degiorgis 2018b), lynx with a heart murmur or a nonlethal malformation of potential genetic origin, are directly released on site; those with a heart murmur may be radio-collared to follow the evolution of their condition and to eventually recover their carcasses for pathological examination. Lynx younger than one year or presenting a disease or trauma with good chances of healing (e.g., mange after appropriate treatment) may be released on site with a GPS collar to be re-captured at a convenient time. In some cases, a transfer to the quarantine station for more intensive care may be considered. However, animal welfare aspects must be taken into account (e.g., stress induced by transport and captivity may have a negative impact on health), as well as the risk that an animal suffering from an infection may represent to other lynx already present in the quarantine facility (although this risk largely depends on the building structure and quarantine management).

All lynx selected for translocation receive an antiparasitic treatment (single subcutaneous injection of praziquantel: Caniquantel pro Inj., Dr. E. Gräub AG, Berne, Switzerland, at a dosage of 5.68 mg/kg; and of doramectin: Dectomax®, Elanco Tiergesundheit AG, Basel, Switzerland, at a dosage of 1 mg/kg; this doramectin dosage has led to a full recovery of lynx heavily affected by mange; Ryser-Degiorgis 2013) to reduce the risk of translocating apparently healthy lynx infested with mange mites (early disease stage or healthy carriage; Munson et al. 2010) and in the hope to decrease their helminth burden (Woodford 2000), which may have a greater health impact under stressful conditions. Any necessary wound treatment is made at this time point. Other medication (including antibiotics) is not administrated unless it appears appropriate based on the clinical findings. Vaccination is only foreseen if authorities of the recipient country require it. The rationale behind this decision is that (1) vaccination provides protection only for a limited amount of time; since both repeated vaccination boost(s) and systematic vaccination of offspring are unpracticable in a free-living population, animals unable to cope with the infection risks in

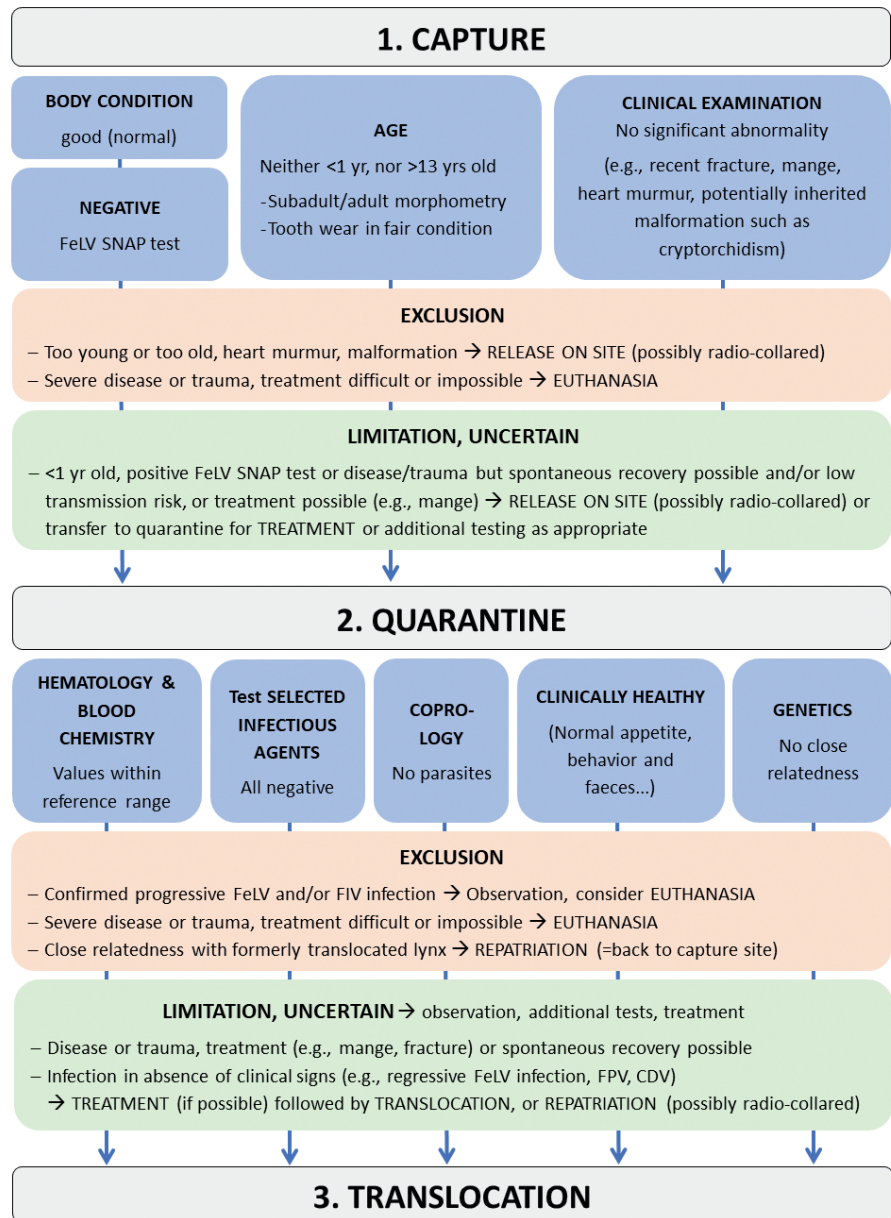


**Fig. 5.** Health risks associated with lynx translocations include the potential introduction of a pathogen (infectious agent with the potential to cause disease) or deleterious genes into the destination population (to be reinforced or reintroduced) by the animals being translocated (1); and the potential transmission (by other animals or the environment) of a pathogen new to the lynx being translocated as well as the exposure to toxic compounds from the environment or other non-infectious causes of disease or mortality during (2a) or after (2b) the translocation process.

their new environment would not survive on the long term; and (2) vaccination with inactivated vaccines offer only limited protection, while the use of live vaccines in wildlife species may cause disease or even death (Connolly et al. 2015).

All captured lynx are marked with a subcutaneous transponder (microchip; DATAMARS, <https://datamars.com/>) implantation in the midway region on the left side of the neck (according to the standards of the Global Veterinary Community for domestic cats and other companion animals in continental Europe; <https://www.wsava.org/Global-Guidelines/Microchip-Identification-Guidelines>) and blood-sampled. Pharyngeal, conjunctival and rectal dry swabs are collected. In recent years a point-of-care test (i.e., a fast field test) for Feline Immunodeficiency Virus (FIV) antibody and Feline Leukaemia Virus (FeLV) antigen detection validated for domestic cats (SNAP FIV/FeLV Combo Test, IDEXX, Switzerland) has been used in the field, as the selection criteria foresee to exclude individuals with a positive result. However, experiences with testing for FIV in 2016 and 2017 have shown that this fast field test may deliver false negative results when applied on lynx samples and that testing in the laboratory is required to obtain reliable data on FIV infection. As experiences in the Iberian lynx suggest that the test can detect progressive FeLV infections that might end fatally, lynx fulfilling criteria for translocation are brought to quarantine without SNAP testing and tested in the laboratory. Lynx not fulfilling translocation criteria and planned to be released on site should be tested with the SNAP test and taken to captivity in case of a positive result. If a progressive infection is confirmed by laboratory testing, they should be extracted from the population (Meli et al. 2010a).

Lynx transport has proven to be a more challenging step than originally thought. If anaesthetised and slowly recovering during transport, constant monitoring of vital parameters is required until recovery, and lynx tend to develop hypothermia even in a heated vehicle. If transported after anaesthesia reversal, lynx might be stressed and thus at risk of injuries (splitted claws, broken teeth, skin abrasion on the forehead) and cardio-respiratory distress (hyperthermia, hyperventilation). However, anaesthesia reversal is preferred, as risks of stress and injuries also concern lynx recovering from anaesthesia during transport and in case of transboundary translocations transport may be very long. There are marked and unpredictable inter-



**Fig. 6.** Selection criteria currently applied for translocation of lynx from Switzerland. FIV: Feline Immunodeficiency Virus; FeLV: Feline Leukaemia Virus; FPV: Feline Parvovirus; CDV: Canine Distemper Virus.

dividual differences in behaviour and stress-susceptibility but since interventions cannot be performed on a conscious lynx, once more, prevention is key. It is important for humans accompanying the animal during transport to stay quiet (no loud voices or sudden noises) and to cool down the interior of the vehicle. Furthermore, over the years, the transport boxes have been improved to be able to interchange doors without opening the boxes, and thus have the possibility to either keep the animal in the dark (full door) or to improve ventilation (metal bar door) by interchanging door types (SOM F3). Additionally, active ventilation into the box with an external device has had a calming effect on stressed lynx. Extensive general guidelines on the transport of

live animals can be found elsewhere (e.g., the "IATA Live Animals Regulations" (LAR), which is the global standard and the essential guide to transporting animals by air in a safe, humane and in a cost-effective manner (<https://www.labeline.com/product/iata-live-animal-regulations-lar-46th-edition-2020>); and the CITES guidelines for the non-air transport of live wild animals and plants ([https://cites.org/sites/default/files/eng/resources/transport/transport\\_guidelines\\_2013-english.pdf](https://cites.org/sites/default/files/eng/resources/transport/transport_guidelines_2013-english.pdf)).

#### *Pre-release procedures*

Blood samples taken at capture are analysed in the laboratory. Haematology and blood chemistry values are compared with reference values obtained from clinically healthy



free-ranging Eurasian lynx from Switzerland. The first faeces found in the enclosure is collected and analysed for lung and gastrointestinal parasites by coprology. Blood and swab samples are tested by molecular methods and/or serology for selected infectious agents (Table 1).

Based on the data collected in Switzerland since the first lynx reintroductions and on studies published on wild felids elsewhere in Europe, we classified microorganisms potentially occurring in lynx into three risk levels: (1) High risk: Only infections with FeLV and FIV were considered as a criterion for exclusion, as these viruses had not previously been detected in free-ranging or captive populations of Eurasian lynx and were considered having the potential to seriously harm infected animals (Meli et al. 2009, Geret et al. 2011, Troyer et al. 2011).

(2) Mild to moderate risk: In the case of other agents such as Canine Distemper Virus (CDV) or Feline Parvovirus (FPV), which may cause disease in lynx (Stahl and Vandell 2009, Wasieri et al. 2009, Meli et al. 2010, Origgi et al. 2012) but are also known to occur (FPV PCR-positive, CDV-seropositive) without associated morbidity and mortality in apparently healthy lynx populations, or such as Feline Calicivirus, Feline Herpesvirus and Feline Coronavirus, for which there is serologic evidence of exposure but no known morbidity (Meli et al. 2009; Ryser-Degiorgis and Meli, unpubl.), the clinical status and blood parameters of the animals are more relevant criteria to evaluate their individual health status than the detection of the pathogen. On a population/ecosystem level, the relevance of pathogen detection depends on the harm it may cause at the release site.

(3) Minimal risk: Infectious agents such as *Cytauxzoon* spp., feline haemotropic mycoplasmas and intestinal endoparasites are widespread in clinically healthy lynx (Valdmann et al. 2004, Willi et al. 2007, Millán et al. 2007, Meli et al. 2009, Ryser-Degiorgis et al. 2010a, Deksne et al. 2013) and not expected to be a threat to lynx or to represent a serious risk for other felid species in the framework of Eurasian lynx translocations. Their detection should serve as a documentation for the long-term health monitoring of the source and of the re-introduced populations but is currently not considered a criterion for selection of lynx in the framework of translocations. Therefore, samples for additional tests of scientific value but not relevant to immediate translocation are stored for potential later analysis.

Animals with blood values significantly diverging from reference data or with infections of unclear clinical significance are observed more closely, possibly longer, and submitted to additional testing as appropriate. The genetic profile of all animals is determined during the quarantine period. In case of close relatedness (brother and sister; mother/father and offspring) with other lynx already translocated or simultaneously kept in quarantine for translocation, the animal is excluded from the programme and repatriated to the capture site.

Before transfer to the release site, lynx are anaesthetised to be fitted with a GPS collar and undergo another clinical check before transportation. They are blood-sampled for archive purposes, i.e., no test is performed at this point without a specific indication. If a reason for exclusion (see above) not noticed earlier is detected, experts in charge consider three options: (1) repatriation to the original capture site, (2) prolongation of the quarantine (with treatment as appropriate and subsequent re-assessment), or (3) euthanasia. Radiographs or additional laboratory tests are performed only in case of specific indication. No treatment is administered at the time of release unless indicated by clinical findings. If the suitability of an individual is questionable, the decision whether to translocate it or not is taken together with the project partners in the recipient country.

A summary of the identified health risks and the corresponding management measures is presented in Table 2.

### Translocation challenges encountered in Switzerland

Sarcoptic mange was first detected in lynx in Switzerland in 1999 (Ryser-Degiorgis et al. 2002b, Schmidt-Posthaus et al. 2002). At the time, there was no indication of another health issue relevant to translocations in the Swiss lynx population (Ryser-Degiorgis et al. 2002a). More cases of mange were diagnosed since then, including captured lynx that were successfully treated (Ryser-Degiorgis 2013). This occurred simultaneously with the Swiss-wide spread of sarcoptic mange in the red fox (Pisano et al. 2019). Similarly, in the framework of the large canine distemper outbreak that has affected Swiss wildlife since 2009, an infected Eurasian lynx was observed with clinical signs (Origgi et al. 2012). *Cytauxzoon* spp. was found for the first time in a severely debilitated lynx in 2006, but the hypothesis of its causal role was discarded

by the pathological examination that followed euthanasia. As the significance of this pathogen was still unclear, raising questions regarding the suitability of positive animals for translocation programmes, a retrospective study on archived samples and systematic testing of lynx to be translocated were initiated, which showed that this haemoparasite is widespread in lynx from Switzerland (Ryser-Degiorgis et al. 2010b). Feline Parvovirus infection (viraemia and faecal excretion) without associated disease signs was first found in an orphaned lynx in 2012, and again in an adult lynx to be translocated to Austria (viraemia only) in 2013 (Ryser-Degiorgis & Meli, unpubl.). In 2017 a lynx was diagnosed with ocular chlamydiosis (Marti et al. 2019), and two animals with unspecific disease signs were suspected to be infected with FIV (Ryser-Degiorgis et al. 2017). In 2019, an apparently healthy male was confirmed to be latently infected with FeLV (detection of proviral DNA and anti-whole virus and p15E antibodies, i.e., regressive FeLV infection) and was repatriated to the capture site fitted with a radio-collar; no disease sign development has subsequently been observed (Marti et al. unpubl.). Concerning non-infectious diseases, sporadic congenital malformations have been observed over the past decades (Morend 2016, Ryser-Degiorgis et al. 2004). Genetic analyses have revealed a loss of variability and increasing inbreeding mainly in the Alps (Breitenmoser-Würsten & Obexer-Ruff 2003). Heart murmurs have increasingly been detected in Alpine lynx since 2001, after a lynx with such a murmur was translocated and died of cardiac failure due to a cardiomyopathy two years after release (Ryser-Degiorgis et al. 2020). A few more fatal cardiomyopathies have been diagnosed since then, and meanwhile there are indications that the observed heart anomalies (murmurs, histological cardiac lesions), whether associated with disease signs or not, may be related to inbreeding (Ryser-Degiorgis et al. 2018). As new knowledge has been gathered on this issue, it has progressively led to the exclusion of individual lynx with heart murmurs from translocation programmes, and since 2015 even of the whole Alpine lynx population. Other aspects of the health screening protocol (selected agents, collected samples and applied laboratory tests) have been improved and selection criteria for individual lynx have been refined (Table 2, Fig. 6). The management of health-relevant findings detected in individual lynx

**Table 2.** Health risk management in the framework of translocation of free-ranging Eurasian lynx from Switzerland.

Health issue	Risk management
<b>POTENTIALLY INHERITED</b>	
Heart murmurs of potential genetic origin incl. a few fatal cases (Alpine population)	Exclusion of all Alpine lynx as well as individuals from other populations presenting with a heart murmur
Sporadic malformations of various body parts	Documentation of visible anomalies at clinical examination; exclusion if suspected inheritance (e.g., cryptorchidism)
<b>INFECTIOUS</b>	
<b>High risk:</b> FeLV <sup>1</sup> , FIV <sup>2</sup> (no infection known in the source population, potential threat to both the source and destination)	Systematic testing; if confirmed positive for FIV or progressive FeLV infection: observation, with additional testing if deemed appropriate, followed by repatriation or euthanasia
<b>Moderate risk:</b> Canine Distemper Virus (moderate disease risk), Feline Parvovirus (low disease risk but cannot be excluded: see Stahl & Vandell 2009), Feline Herpesvirus, Feline Calicivirus and Feline Coronavirus	Systematic testing, decision based on clinical status and re-testing (aim: to minimize the risk of excretion)
<b>Minimal risk:</b> <i>Cytauxzoon</i> spp., feline haemotropic mycoplasmas, among other microorganisms (no known cases of clinical disease)	No systematic testing (scientific documentation only and/or surveillance data collection)
Sarcoptic mange (also notoedric, otodectic)	Systematic treatment against mites (aim: to eliminate mites)
Endoparasites (gastrointestinal helminths)	Systematic treatment and testing (aim: to minimize the risk of increase of endoparasite burden and associated apparition of clinical signs potentially resulting from increased stress)
<b>OTHERS</b>	
Any other viral or bacterial infection; ecto- or endoparasitic infestation; trauma; or detection of unspecific disease signs	Treatment if available and appropriate (on medical, financial, logistical and animal welfare points of view), observation of clinical status, additional testing if deemed useful; depending on disease course: translocation, repatriation or euthanasia

<sup>1</sup>FeLV: Feline Leukaemia Virus. <sup>2</sup>FIV: Feline Immunodeficiency Virus

during translocation projects since 2013 is summarized in Table 3.

Detailed records of clinical findings and anaesthesia procedures have contributed to the improvement of capture methods and prevention measures aimed at decreasing capture-related risks. In particular, awareness was raised regarding the elevated risk of injuries when using box traps made of metallic grid, of hypothermia in winter and of hyperthermia during transport as well as when using foot snares in the spring. Already after the first year of the first project (i.e., at the end of 2001), improvements were made to box traps to reduce the risk of injuries at capture and to housing conditions to reduce the risk of injuries and stress during quarantine, followed later on by modifications of transport boxes to reduce the risk of injuries, stress and associated hyperthermia and to provide possibilities to better ventilate and observe the animals during transport. Importantly, as stated above, the duration of the quarantine has been drastically shortened, being now limited to the time

required for relevant laboratory results to be available, unless there are indications for a prolongation.

Of the few females diagnosed as pregnant at the end of the quarantine, some gave birth after translocations, others did not, suggesting that the stress caused by transport and release in a new environment, alone or in addition to that induced by the initial capture, first transport and quarantine period (i.e., additive stressful situations), might cause abortion. However, to our knowledge, to date there is no scientific evidence supporting this hypothesis (Vié et al. 1998, Kreeger 2012, Nagel et al. 2019), hinting at a minimal risk of abortion due to translocating procedures. Since the period of the year associated with the highest lynx capture success is the mating season (males on the move for reproduction purposes, snowy landscapes resulting in a higher likelihood for prey to be found and a frequent use of existing paths where box traps are placed), it is inevitable to capture and move potentially pregnant females.

## Conclusions

Health risk analysis in the framework of lynx translocations has proven to be an important tool to reduce the risk of project failure, considering that a range of pathogens have been detected, which required case-specific management measures. These experiences have also underlined the importance of a health surveillance programme starting prior to a translocation project and of the usefulness of a sample archive. Furthermore, the lack of data on disease risk at the release sites pointed at the necessity to carry out health surveillance in both domestic animals and wildlife to provide data useful to the planning of species conservation projects.

It is important to remember that it will never be possible to work with zero risk, and that one needs to be ready to experience the unexpected and to show adaptation potential after careful planning. Although not mentioned further here, post-release monitoring not only of the lynx behaviour, reproduction and population genetics but also of health issues (particularly the thorough examination

**Table 3.** Health issues encountered during capture and quarantine of Eurasian lynx caught for translocation in Switzerland, 2001–2020.

Health issue	Decision criteria	Case management and decision	Reference
Heart murmur (in absence of associated clinical signs)	<ul style="list-style-type: none"> <li>- DS<sup>1</sup>: None; TH<sup>2</sup>: None</li> <li>- Previous data: suspected inherited cardiomyopathy</li> </ul>	REPATRIATION	(Ryser-Degiorgis et al. 2018)
Parvovirus infection and excretion (faeces) <sup>3</sup>	<ul style="list-style-type: none"> <li>- DS: None; TH: None</li> <li>- Not all Parvoviruses cause disease</li> <li>- Previously same situation in orphaned lynx: remained healthy, excretion stopped, was released and followed up</li> </ul>	Retesting, negative in faeces (no excretion) and TRANSLOCATION	(Ryser-Degiorgis & Meli, unpubl.)
<i>Cytauxzoon</i> spp. infection <sup>3</sup>	<ul style="list-style-type: none"> <li>- DS: None; TH: None</li> <li>- Retrospective analysis: many Swiss lynx infected, no known fatal cases in domestic cat in Europe at the time<sup>4</sup> (questionable parasite pathogenicity)</li> <li>- Widespread in healthy bobcats and Iberian lynx<sup>5</sup></li> </ul>	TRANSLOCATION	(Ryser-Degiorgis et al. 2010a)
Suspected FIV <sup>6</sup> infection	<ul style="list-style-type: none"> <li>- DS: present and potentially associated with FIV infection</li> <li>- TH: None available</li> <li>- Deterioration of health status in quarantine</li> <li>- Retrospective analysis with reliable test (gold standard): Absence of positive lynx in source population confirmed</li> </ul>	EUTHANASIA	(Ryser-Degiorgis et al. 2017)
Chlamydiosis	<ul style="list-style-type: none"> <li>- DS: Yes, typical for infection; TH: Yes, available and feasible</li> <li>- No other known disease case in source population, infection status population unknown (neither previous data nor appropriate samples available)</li> </ul>	TREATMENT, observation until total recovery and TRANSLOCATION (retesting at release and post-release observation by photo-trapping)	(Marti et al., 2019)
FeLV <sup>7</sup> infection	<ul style="list-style-type: none"> <li>- DS: None; TH: None</li> <li>- Previous data: No infection in source population</li> <li>- Regressive infection, high antibody titre, no virus shedding</li> </ul>	REPATRIATION and follow up (GPS collar, photo-trapping)	(Marti et al., unpubl.)

<sup>1</sup>DS: Disease signs; <sup>2</sup>TH Available therapy; <sup>3</sup>These experiences contributed to the classification of the corresponding infectious agents in the currently used risk category (see Table 2). <sup>4</sup>Since then, *Cytauxzoon* spp. has been shown to occur in both wild and domestic felids in Europe, with varying pathogenicity (from asymptomatic to fatal infections) and sometimes uncertain causal relationship between the infection and observed clinical signs (Nentwig et al. 2018, Panait et al. 2021). <sup>5</sup>Since then, *Cytauxzoon* spp. has also been detected in Eurasian lynx in Romania (Gallusová et al. 2016) and reported in European wildcats by multiple authors (Panait et al. 2021). <sup>6</sup>FIV: Feline Immunodeficiency Virus; <sup>7</sup>FeLV: Feline Leukaemia Virus.

of dead lynx) is a crucial point to evaluate the success of the project on a longer term and to determine the need for additional management measures.

Twenty years ago, when veterinary supervision was first implemented for lynx translocation projects in Switzerland, hardly any health issue was a limiting factor and infections seemed to be of minor importance, but

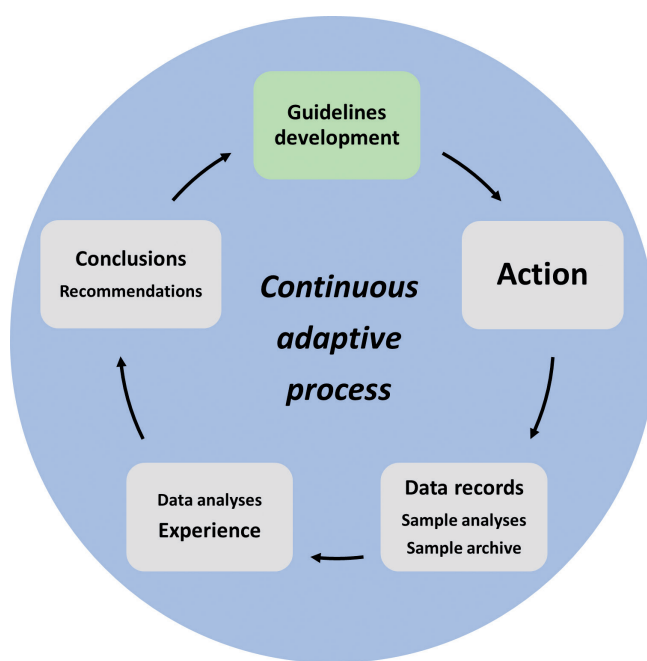
over the past 10 years a range of microorganisms with pathogenic potential have been newly detected. The change from serological investigations to pathogen detection may have favoured this situation, however, pathogen detection partly followed the detection of associated clinical signs (Origgi et al. 2012, Ryser-Degiorgis et al. 2017, Marti et al. 2019). While both antigen and

antibody detection are essential to study pathogen/disease dynamics in a population, only pathogen detection is relevant for decision-making in the framework of translocations (risk of excretion potentially leading to disease in stressed animals or resulting in contamination/transmission at the release site). On the same line, it is crucial to distinguish infection and exposure from

disease and not to be misled by results of serosurveys (Munson et al. 2010).

The infections documented in lynx in Switzerland were most likely sporadic and related to the occurrence of the corresponding infectious agents in sympatric hosts such as foxes [sarcoptic mange (Ryser-Degiorgis et al. 2002b, Pisano et al. 2019); distemper (Origi et al. 2012)] and possibly domestic cats and/or European wildcats *Felis sylvestris* [FIV (Ryser-Degiorgis et al. 2017); FeLV (Leutenegger et al. 1999, Meli et al. 2009, Geret et al. 2011, Hofmann-Lehmann et al. 2018); *Chlamydia felis* (Martí et al. 2019)]. Current information on Swiss stray and feral domestic cats is limited (Berger et al. 2015, Hofmann-Lehmann et al. 2018; Novacco et al., 2019), but personal communications from clinical pathologists at the Clinical Laboratory of the University of Zurich support the occurrence of FeLV and suggest the presence also of other viruses such as FIV in feral and stray cats potentially sharing lynx habitat (B. Riond, pers. comm.). The regular record of heart anomalies possibly associated with a loss of genetic variability (Ryser-Degiorgis et al. 2018), have added concerns regarding the health status of the source populations and underlined the necessity of considering also non-infectious diseases in health risk assessments in the context of translocation projects.

Health surveillance and retrospective studies require access to a sample size sufficient for inference at population level (Ryser-Degiorgis 2013). In protected secretive species, the access to samples is typically difficult, as animals found dead and captured individuals represent the only possible sources of material. From a strategic viewpoint, three components of health surveillance appear to be particularly important: (1) long-term data and sample collection; (2) interdisciplinary collaboration and a combination of multiple diagnostic approaches (e.g., clinical and post-mortem examinations, laboratory tests, observations of disease signs by photo-trapping; examination of both marked animals and those found by chance; examination of diseased and of apparently healthy animals such as traffic kills, which can provide baseline data); (3) harmonization of data collection over time and among study areas to allow for comparisons. Last but not least, data need to be regularly compiled to improve protocols and procedures as appropriate. Overall, the aim is to carry out adaptive management based on scientific data (Fig. 5). For a pan-European conservation programme of Eurasian lynx,



**Fig. 7.** Conservation of lynx populations, including but not limited to translocation procedures, requires adaptive management based on scientific data. This corresponds to a never ending learning process translating into continuous protocol adaptation.

coordinated efforts are advisable. Among others, the harmonization of veterinary protocols and genetic investigations is desirable, as well as the exchange of information on detected health issues.

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Supporting Online Material SOM Figures F1-F3 are available at [www.catsg.org](http://www.catsg.org)

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