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Short Communication

Could introducing confiscated parrots to zoological collections jeopardise conservation breeding programmes?

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Summary

Confiscated parrots are frequently introduced to captive populations in zoological institutions, regularly with insufficient health screening. This short communication describes a case where 25 confiscated parrots, from four different locations, were brought to the same zoological institution within two years, where they were kept under quarantine conditions. A year after the last birds arrived, several birds died due to either proventricular dilatation disease or herpesvirus infection. As all individuals belonged to rare species, the surviving birds were transferred to the Justus-Liebig-University in Giessen, Germany, for thorough diagnostics including parrot bornavirus, psittacine herpesvirus 1, adenovirus, polyomavirus, circovirus, *Chlamydia psittaci*, and mycobacteria. Birds that tested negative for all pathogens were transferred to captive breeding programmes, whereas pathogen carriers were paired up in collections of a similar pathogen status. This case report highlights the dangers of latent infections with different pathogens and the importance of managed screening programmes if such populations are to be considered for conservation.

Introduction

Parrots are one of the most threatened bird orders with 28% of species listed as globally threatened and 56% of species in decline (Olah *et al.* 2016). For some highly endangered species, captive breeding programmes might be the only solution for recovery (Earnhardt *et al.* 2014). Parrots are frequently brought into zoological institutions through confiscations or as rescue birds in the form of unwanted pets from private aviculture. In Europe, a number of such birds are subsequently introduced into breeding programmes of the European Association of Zoos and Aquaria (EAZA), regularly without any or only insufficient previous health screening. Some of these breeding programmes are working towards reintroductions as part of species recovery programmes (Sanz and Grajal 1998, Woolaver *et al.* 2000, Collazo *et al.* 2003). In the past there may have been insufficient focus on infectious diseases when considering reintroductions (White *et al.* 2012, Collar *et al.* 2015).

Case study: disease in a collection of confiscated parrots

Between April 2009 and February 2011, a total of 24 living and one dead parrot, representing 11 species, arrived at one institution from four different locations in Denmark (Table 1). All species involved, apart from Long-billed Corella *Cacatua tenuirostris*, are considered to have declining *in situ* populations, one is listed as 'Endangered' (IUCN 2016) and all are considered rare in

Table 1. Parrots confiscated between April 2009 and February 2011, transferred to the same institution and housed in one room.

Species	Common Name	Date in	Date out	Reason: †/JLU*	IUCN Status
Primolius couloni	Blue-headed Macaw	Apr 25, 2009	Jul 11, 2012	JLU	Vulnerable
Primolius couloni	Blue-headed Macaw	Apr 25, 2009	Jul 11, 2012	JLU	Vulnerable
Primolius couloni	Blue-headed Macaw	Apr 25, 2009	Jul 11, 2012	JLU	Vulnerable
Amazona leucocephala	Cuban Amazon	Feb 11, 2010	Jul 11, 2012	JLU	Near Threatened
Amazona leucocephala	Cuban Amazon	Feb 11, 2010	Jul 11, 2012	JLU	Near Threatened
Cacatua g. eleonora	Eleonora Cockatoo	Feb 11, 2010	May 20, 2011	† - PDD	Least Concern (*C. galerita*)
Cacatua g. eleonora	Eleonora Cockatoo	Feb 11, 2010	Jul 11, 2012	JLU	Least Concern (*C. galerita*)
Cacatua g. eleonora	Eleonora Cockatoo	Feb 11, 2010	Jul 11, 2012	JLU	Least Concern (*C. galerita*)
Amazona tucumana	Tucuman Amazon	Feb 11, 2010	Jul 11, 2012	JLU	Vulnerable
Amazona dufresniana	Blue-cheeked Amazon	Dec 22, 2010	May 24, 2011	† - Herpes-virus	Near Threatened
Amazona dufresniana	Blue-cheeked Amazon	Dec 22, 2010	Jul 11, 2012	JLU	Near Threatened
Amazona dufresniana	Blue-cheeked Amazon	Dec 22, 2010	Jul 11, 2012	JLU	Near Threatened
Amazona dufresniana	Blue-cheeked Amazon	Dec 22, 2010	Jul 11, 2012	JLU	Near Threatened
Amazona collaria	Jamaican Amazon	Feb 1, 2011	Feb 1, 2011	† - avian TB	Vulnerable
Amazona collaria	Jamaican Amazon	Feb 1, 2011	Apr 13, 2011	† - killed by marten	Vulnerable
Amazona f. festiva	Festive Amazon	Feb 1, 2011	May 23, 2011	†- Herpes-virus	Near Threatened
Amazona f. festiva	Festive Amazon	Feb 1, 2011	May 28, 2011	† - Herpes-virus	Near Threatened
Amazona f. festiva	Festive Amazon	Feb 1, 2011	Jul 11, 2012	JLU	Near Threatened
Amazona o. ochrocephala	Yellow-crowned Amazon	Feb 1, 2011	Apr 13, 2011	† - killed by marten	Least Concern
Amazona o. ochrocephala	Yellow-crowned Amazon	Feb 1, 2011	Apr 13, 2011	† - killed by marten	Least Concern
Amazona o. ochrocephala	Yellow-crowned Amazon	Feb 1, 2011	Apr 13, 2011	† - killed by marten	Least Concern
Amazona diadema	Diademed Amazon	Feb 1, 2011	May 28, 2011	† - Herpes-virus	Endangered
Amazona diadema	Diademed Amazon	Feb 1, 2011	Jul 11, 2012	JLU	Endangered
Cacatua tenuirostris	Long-billed Corella	Feb 1, 2011	Jul 11, 2012	JLU	Least Concern
Psittrichas fulgidus	Pesquet's Parrot	Feb 1, 2011	Jul 11, 2012	JLU	Vulnerable

*the bird has died († cause) or was transferred to Justus-Liebig University in Giessen, Germany (JLU).

captivity (Zootierliste 2016). The birds were either confiscated on the same day as arrival or had been confiscated earlier and were relocated from other institutions due to space limitations.

Upon arrival, all animals were identified, checked for body condition and endoparasites. The dead Jamaican Amazon *Amazona collaria* was kept frozen for further investigation. Due to restricted space, all birds were housed in one 100-m² room in c.4-m² cages. All parrots were housed in the same pairs or social groups as previously kept. The caretakers only entered the room with exclusive protective clothing. All removed food dishes were disinfected (Vircon S®)

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and waste was disposed of for incineration. In April 2011 a marten *Martes* sp. entered one of the cages and killed four amazons (see Table 1 for species). Four weeks later, in May 2011, having shown no prior sign of disease, five birds died within one week: one cockatoo confirmed due to proventricular dilatation disease (PDD) and four Amazons due to herpesvirus infection (Pacheco's disease). All remaining birds were treated immediately for 14 days orally with acyclovir (100 mg/kg BID) for herpesvirus infection and no further deaths occurred.

The authorities agreed to transfer the remaining 15 parrots to the Justus-Liebig-University (JLU) in Giessen, Germany, in July 2012, because of their importance for managed populations. At JLU they received thorough diagnostics and further transfer to adequate holdings according to the test results. An overview on tests and results is provided in Table 2.

The major finding was parrot bornavirus (PaBV), with 10 of the 15 birds either demonstrating specific anti-PaBV antibodies and/or shedding PaBV-RNA. Additionally, three birds from three different origins tested positive for adenovirus in the PCR, and four demonstrated specific antibodies against *Chlamydia psittaci*. Avian tuberculosis was diagnosed microscopically in the Amazon which was dead upon arrival. In contrast, although Pacheco's disease was diagnosed in the four dead Amazons one year earlier, none of the remaining birds showed antibodies against psittacine herpesvirus 1 or was shedding psittacine herpesvirus DNA. Four of the 15 parrots did not test positive for any of the tests applied.

As a result of these efforts, and with defined health status, all birds were moved to new holders; the four negative birds were included in conservation breeding programmes at zoological institutions, and all others were moved to private holders with birds of same pathogen status in their collection.

Discussion

The considerable risks of introducing captive animals, particularly when having been kept in close proximity to other parrots, have been identified as a concern (Snyder *et al.* 1996), especially since some diseases can remain latent in asymptomatic carriers for long periods (Partington *et al.* 1989). Twenty years ago, it was suggested that captive populations should be screened intensively for diseases and only animals that had a long history without exposure to potential disease carriers should be chosen for reintroduction (Snyder *et al.* 1996). A programme to certify disease-free collections was proposed to simplify exchanges between holders (Greenwood 1992).

Since then, serological and PCR testing methods for most diseases concerning psittacines (Avian polyomavirus, circo-, herpes-, adeno-virus, *Chlamydia psittaci*) have been established, and are performed by commercial laboratories. Even screening for PaBV, the causative agent of proventricular dilatation disease, is now possible for viral RNA by PCR (Honkavuori *et al.* 2008) and specific serum antibodies by indirect immunofluorescence assay (Herzog *et al.* 2010).

Four of 15 birds in this study tested negative for pathogens despite their close proximity to positive individuals, highlighting the importance of individual susceptibility and indicating that virus-free populations may be achievable. In species where every individual is important to the future of a population, population screening is essential in establishing clinically healthy breeding pairs (Lierz *et al.* 2011). This study illustrates that without appropriate testing, 11 birds carrying infectious pathogens could have transmitted diseases to other collections. Moreover, such unscreened birds could potentially impact wild populations if considered for reintroduction programmes. In-depth screenings were performed in the present case due to the perceived importance of the species for captive breeding; less conservation-relevant species would likely have been euthanized after the exposure to PDD and Pachecos' disease.

The influence of the marten attack four weeks prior to the disease outbreak is unclear, but it could have potentially stressed one herpesvirus carrier bird enough to shed virus in vast amounts, resulting in disease and eventual death of sensitive animals. No matter what caused the outbreak, the time delay since the birds' arrival demonstrates clearly the insufficiency of physical quarantine without any specific testing.

Ex situ breeding has played an important role in the recovery of a number of vertebrate species in recent years, contributing to a reduction in their IUCN threat level (Conde *et al.* 2011). Yet, of

Table 2. Scientific and common name of 15 parrots from the same institution transferred on July 11, 2012 to Giessen University, Germany. Results from serological and PCR examinations for different pathogens.

Scientific name	Common name	Herpesvirus		Adeno-virus	Parrot bornavirus		Polyoma-virus		Circo-virus	Chlamydia sp.		Mycobact-eriosis
		Serol	PCR	PCR	Serol	PCR	Serol	PCR	PCR	Serol	PCR	Serol
Primolius couloni	Blue-headed Macaw	neg	neg	neg	**pos**	neg	neg	neg	neg	neg	neg	neg
Primolius couloni	Blue-headed Macaw	neg	neg	neg	**pos**	neg	neg	neg	neg	**pos**	neg	neg
Primolius couloni	Blue-headed Macaw	neg	neg	**pos**	**pos**	neg	neg	neg	neg	**pos**	neg	neg
Amazona leucocephala	Cuban Amazon	neg	neg	**pos**	**pos**	**pos**	neg	neg	neg	neg	neg	neg
Amazona leucocephala	Cuban Amazon	neg	neg	neg	**pos**	**pos**	neg	neg	neg	**pos**	neg	neg
Cacatua g. eleonora	Eleonora Cockatoo	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
Cacatua g. eleonora	Eleonora Cockatoo	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
Amazona tucumana	Tucuman Amazon	neg	neg	neg	**pos**	**pos**	neg	neg	neg	neg	neg	neg
Amazona dufresniana	Blue-cheeked Amazon	neg	neg	neg	**pos**	**pos**	neg	neg	neg	neg	neg	neg
Amazona dufresniana	Blue-cheeked Amazon	neg	neg	neg	neg	**pos**	neg	neg	neg	**pos**	neg	neg
Amazona dufresniana	Blue-cheeked Amazon	neg	neg	neg	neg	**pos**	neg	neg	neg	neg	neg	neg
Amazona f. festiva	Festive Amazon	neg	neg	**pos**	neg	neg	neg	neg	neg	neg	neg	neg
Amazona diadema	Diademed Amazon	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
Cacatua tenuirostris	Long-billed Corella	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
Psittrichas fulgidus	Pesquet's Parrot	neg	neg	neg	**pos**	neg	neg	neg	neg	neg	neg	neg

neg = Negative, **pos** = Positive; Negative serology = no specific antibodies against the pathogen were found; Negative PCR = no DNA/RNA of the pathogen was present in the sample. Positive serology = specific antibodies against the pathogen present; Positive PCR = DNA/RNA of the pathogen was present in the sample.

24 EAZA parrot studbooks, only one, Lilacine Amazon *Amazona lilacina*, has up-to-date Best Practice Guidelines with preventive veterinary recommendations (Pilgrim and Biddle 2016) which includes testing requirements for all the pathogens mentioned in this case report. Considering that EAZA zoos keep on average 12 different species of Psittacines, routinely catching and screening all parrots in an institution may be beyond available budgets and willingness. Existing concerns regarding individuals that escape detection of disease, despite repeated testing (Collar *et al.* 2015), can be minimised through multiple testing of entire collections which helps identify individuals that only sporadically shed pathogens. Additionally, test sensitivities are constantly improving.

Looking at publications regarding releases of captive reared or confiscated parrots, animals have often been released with only selective screening (Brightsmith *et al.* 2005, Saidenberg *et al.* 2015). In consequence, the possible threat of introducing pathogens to naïve species has already been demonstrated. PBFD is considered to have emerged in the wild population of the endangered South African Cape Parrot *Poicephalus robustus*, probably from exposure to infected captive-bred animals (Regnard *et al.* 2015).

In conclusion, in order to ascertain the conservation value of individuals and their reintroduction potential, we recommend the following at all zoological facilities: (1) establish pathogen-testing guidelines for target conservation species; (2) implement regular, mandatory health screening for the pathogens discussed above (see Table 2); and (3) for large collections, perform periodic collection-wide disease-risk assessments and implement strict quarantine and screening protocols. Ideally, untested Psittacines should not be housed at facilities with high-conservation-priority parrots. Specimen transfers should depend upon individual health status and be coordinated by the studbooks accordingly.

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