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Flying across Europe: the case of the spread of *Chaunocephalus ferox* on a black stork (*Ciconia nigra*)

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Abstract

The annual migration of birds involves a very large number of inter-continental and intra-continental movements in which thousands of bird species participate. These migrations have been associated with the spread of pathogens worldwide, including bacteria, viruses and parasites. This study describes the case of a black stork (*Ciconia nigra*) that was ringed at the nest in Latvia and died five months later in the south-east of the Iberian Peninsula. Post-mortem examination revealed that the cause of death was electrocution. In addition, a massive infection by the trematode *Chaunocephalus ferox* (Digenea: Echinostomatidae) causing severe granulomatous lesions throughout the small intestine was detected. This is the first report of *C. ferox* infection in a black stork in the Iberian Peninsula, a trematode that, due to the severe lesions it causes, can affect the health of *C. ferox*-infected wild birds, particularly in severely infected long-distance migrants. The dispersal of platyhelminths associated with migratory birds is discussed. After the ringing at the nest, the black stork was sighted in Central Europe one month before its capture, and the trematodes found by necropsy were mostly mature adults. Consequently, we estimate that this juvenile animal acquired the infection during its migration in a European area other than the Iberian Peninsula, evidencing a long-distance parasite spread through its migratory host. Our study highlights that bird ringing can be used to understand the epidemiological implications that bird migratory behaviour may have on the dispersal of parasites.

Introduction

Avian migration is a worldwide phenomenon that allows birds to forage in the breeding period and to overcome unfavourable climatic seasons at the expense of high energetic cost (Newton, 2007; Somveille *et al.*, 2013; Flack *et al.*, 2016). In fact, birds in poor condition are unlikely to be able to migrate, since a good body condition is essential to face the migration challenge (Anderson *et al.*, 2019). Many European-breeding species migrate to south in winter through the Palaearctic–African route, thus increasing the connectivity between sedentary and migrant avian populations (Hahn *et al.*, 2009). The black stork (*Ciconia nigra*) is a long-distance migrant that is scarce in western Europe due to the deterioration of habitats and human impacts (Chevallier *et al.*, 2013). Part of the European black stork population, which mostly breeds in central and eastern areas of the continent, but also in the Iberian Peninsula (del Hoyo *et al.*, 1992; Elliott *et al.*, 2020), migrates via the western route that crosses Europe and northern Africa to reach the Sahel. However, in common with other Ciconiidae species in Europe, an increasing number of black storks winter in the Iberian Peninsula (Cano *et al.*, 2014; Rotics *et al.*, 2017).

Migrant birds are exposed to many vectors and pathogens, both in their breeding and wintering areas, where habitat types, diet and, consequently, epidemiological characteristics are often different (Newton, 2007; Altizer *et al.*, 2011; Fecchio *et al.*, 2021). According to the scientific literature, they have been associated with zoonotic and non-zoonotic pathogen dispersion around the world, including not only bacteria and viruses, but also parasites (Literák & Sitko, 2006; Abulreesh *et al.*, 2007; Jourdain *et al.*, 2007; Fuller *et al.*, 2012; Ferraguti *et al.*, 2019). Considering the strong linkages between climate and migration, it is urgent to obtain comprehensive and reliable information about how climate change may modulate the spread of pathogens by migratory birds (Fuller *et al.*, 2012). In this sense, alteration of migratory routes in breeding storks has been detected in the Iberian Peninsula, showing sedentary behaviour due to favourable climatic conditions and food availability (Tortosa

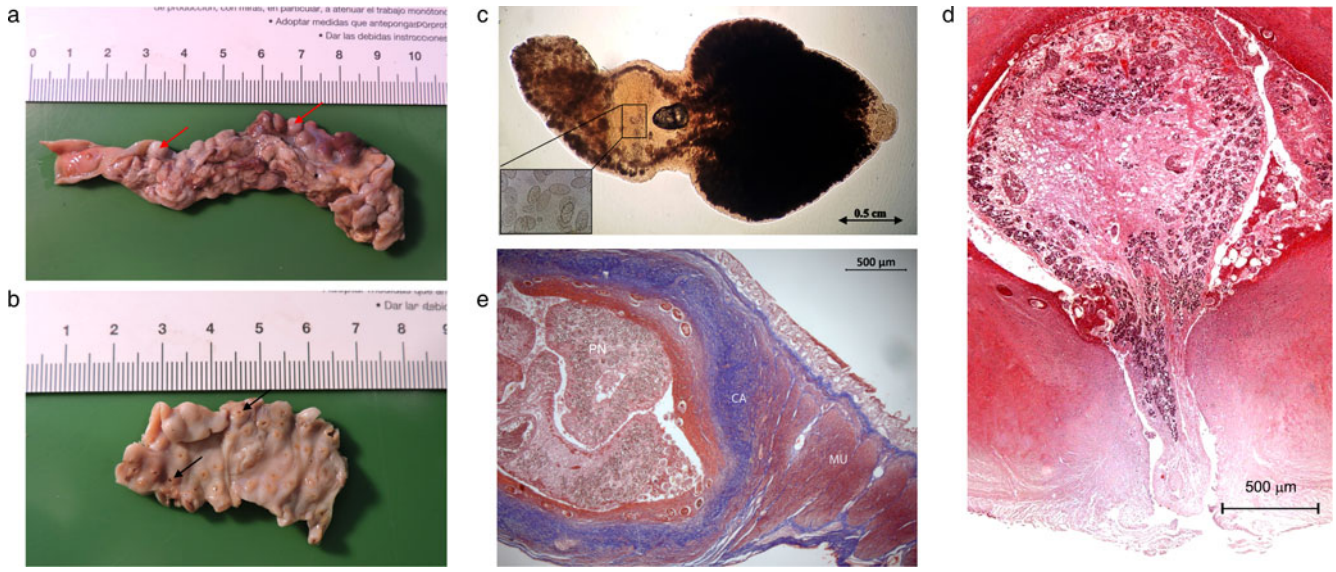

Fig. 1. (a) Granulomatous enteritis caused by *Chaunocephalus ferox* and (b) duodenal mucosa with orifices (black arrow) corresponding to the connection between each parasite nodule (red arrow) with the intestinal lumen; (c) *C. ferox* specimen under stereomicroscope after clearing with Amman's lactophenol, showing eggs within a mature fluke in more detail; (d) microscopic image stained with haematoxylin–eosin of a parasitic nodule in the small intestine with the fluke inside. The nodule is located in the tunica muscularis but is connected to the lumen of the organ by a channel through the submucosal and mucosal tunics; and (e) microscopic image stained by Masson's trichrome staining technique. The disruption of the tunica muscularis (MU) of the intestine due to the presence of a thick connective capsule (CA) of the parasitic nodule (PN) can be observed.

et al., 1995). Large-scale studies using tracking techniques, such as bird ringing, are an effective way to improve our knowledge about disease ecology in wild birds. This case describes a *Chaunocephalus ferox* (Digenea: Echinostomatidae) infection in a black stork ringed in north-eastern Europe and found injured in the south-east of the Iberian Peninsula.

Case report

A five-month-old male black stork (identification ET6251-LATVIA RIGA) was submitted to the Wildlife Rescue and Rehabilitation Centre 'El Valle' (Murcia, south-eastern Spain) on 3 November 2017, being the first documented report of this animal in the Iberian Peninsula. The bird was ringed at the nest in May 2017 in Gavieze Parish (south-western Latvia) and, after leaving the nest, was sighted by ornithologists twice in Poland in mid-September and once in Switzerland in early October before reaching south-east of the Iberian Peninsula. The black stork, which was unable to fly despite having a good body condition (2.2 kg), was found in Jumilla municipality, Murcia province, south-east Spain (38°28.604′N, 1°19.318′W). Clinical examination detected burn wounds in the keel and both tarsometatarsus, and an open fracture in the carpometacarpus. The stork also showed reduced sensitivity and mobility of limbs. All these clinical data were indicative of recent electrocution. Despite treatment, the animal died on the day of admission to the rescue centre.

A detailed post-mortem examination was performed, and all thoracic and abdominal organs and corporal cavities were carefully examined. The most remarkable macroscopic finding was granulomatous enteritis with 143 nodulations of 0.5–0.7 cm diameter in the small intestine, protruding into the intestinal serosa and mucosa (fig. 1a). Inside these nodulations a total of 275 trematodes were isolated, mainly mature stages. Most nodulations (92.3%; 132/143) contained two flukes, although nodules with only one trematode were also described (7.7%; 11/143). Nodules were linked to the intestinal lumen through an orifice (fig. 1b). Trematodes were cleared using Amman's lactophenol and mounted in Hoyer's medium to evaluate their microscopic structures (fig. 1c). The morphological and morphometrical characteristics of parasites corresponded to the trematode *C. ferox* (Patnaik *et al.*, 1970; Choe *et al.*, 2016; Greben *et al.*, 2016). As diagnosis was achieved with mature trematode identification, no coprological examination was performed. Several nodules were fixed in 10% buffered formaldehyde, embedded in paraffin, sectioned, and stained with the haematoxylin–eosin and Masson's trichrome stains, which were performed to describe the morphological characteristics of the parasitic nodule and to evidence the collagen fibres that delimit the nodulation, respectively. Microscopic evaluation revealed the formation of fluke-occupied nodules, mainly located in the intestinal muscular layer causing a focal destruction of the tunica muscularis and forming a thick connective capsule around them. As macroscopically evidenced, nodulations were connected to the lumen of the organ by a pore, where the oral sucker of the parasite was observed (fig. 1d,e).

Discussion

Chaunocephalus ferox is a Digenean trematode with a life cycle in which snails of the family Planorbidae act as first intermediate hosts (Kostadinova, 2005; Saad, 2009), and amphibians and fishes as second intermediate hosts (Patnaik *et al.*, 1970; Höfle *et al.*, 2003). This trematode species has been described in black storks and other Ciconiidae species from Spain and other European and Asian countries (table 1), mainly due to the diet of storks that includes fish and amphibians (Elliott *et al.*, 2020). Most of the studies that have described *C. ferox* in individuals of the family Ciconiidae used morphological criteria to identify this trematode species (table 1).

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Table 1. Epidemiological studies and case reports about *Chaunocephalus ferox* infection in the Ciconiidae family published to date, indicating the number of animals sampled, the prevalence found, the number of intestinal nodules detected, the trematode identification method employed and the use of tools to monitor the bird movements (e.g. bird ringing).

	Ciconiidae species	Country	Number of hosts	Prevalence *C. ferox*	Number of nodules	Identification	Spatial monitoring
present study	*Ciconia nigra*	Spain	1	–	143	morphology	yes
Greben *et al.* (2016)	*C. nigra*	Ukraine	1	–	–	morphology	no
Sitko & Heneberg (2015)	*C. nigra*	Czech Republic	14	71.4%	–	morphology	no
Höfle *et al.* (2003)	*Ciconia ciconia*	Spain	42	23.8%	–	morphology	no
Santoro *et al.* (2013)	*C. ciconia*	Italy	1	–	–	morphology	no
Sitko & Heneberg (2015)	*C. ciconia*	Czech Republic	91	34.1%	–	morphology	no
Girisgin *et al.* (2017)	*C. ciconia*	Turkey	18	37.5%	–	morphology	no
Michalczyk *et al.* (2020)	*C. ciconia*	Poland	38	28.9%	7–15	morphology	no
Choe *et al.* (2016)	*Ciconia boyciana*	Korea	1	–	7	morphology	no
Patnaik *et al.* (1970)	*Anastomus oscitans*	India	10	70.0%	–	morphology	no
Poonswad *et al.* (1992)	*A. oscitans*	Thailand	71	80.0%	1–86	morphology	no

Chaunocephalosis can negatively affect the health status of infected hosts and may hinder successful migration, particularly in individuals with high parasite intensity or concomitant health problems. Cachexia associated with granulomatous enteritis has been previously described in dead storks with *C. ferox* infection (table 1) (Santoro *et al.*, 2013; Choe *et al.*, 2016). Because of these alterations, it is thought that chaunocephalosis produces a weakened state that can reduce flight performance and, consequently, be a predisposing factor for crashes leading to trauma and electrocution (Santoro *et al.*, 2013). However, the two most striking features in this case were: (1) the good body condition of the bird after the migration from northern Europe to south-east Spain; and (2) its ability to complete a long-distance migratory route, in spite of having a high number of parasitic nodules caused by the *C. ferox* infection, compared to other studies (table 1). This report may indicate a non-chronic course of chaunocephalosis in the black stork due to the absence of a cachectic state, which has been only described in sub-adult and adult storks (Santoro *et al.*, 2013; Choe *et al.*, 2016). However, as we have no information on the behaviour of the black stork before it was found in south-east Spain, it is difficult to assess in detail the potential impact that this trematode infection has had on the health of the bird.

As migratory birds may use a variety of habitats, they have a higher risk of being exposed to a parasite infection compared to sedentary species (Koprivnikar & Leung, 2015; Leung & Koprivnikar, 2016). The infection by *C. ferox* found in this black stork is an example of trematode dispersal at an intracontinental scale. The presence of mature *C. ferox* specimens in the small intestine of the stork, along with its sighting in Central Europe in October 2017 before its capture in the Iberian Peninsula in early November 2017, suggest that the infection may have occurred before it arrived in south-eastern Spain, as it has also been stated in the case of the Oriental white stork (*Ciconia boyciana*) (Choe *et al.*, 2016). However, the variation of individual migration patterns described in black storks (https://migrationatlas.org/node/1654), as well as the wide range of prepatent periods reported for parasites belonging to the Echinostomatidae family in intermediate (27–40 days) and definitive hosts (two–four weeks), indicate the need for further research about the biology of chaunocephalosis in wild birds (Huffman & Fried, 1990; Maldonado *et al.*, 2001; Chevallier *et al.*, 2013). Moreover, the knowledge about intermediate host species involved in the life cycle of *C. ferox* is scarce, particularly with regard to amphibians and fish acting as secondary intermediate hosts.

This is the first report of *C. ferox* infection in black storks in the Iberian Peninsula, having been recorded so far only in white storks in this area (Höfle *et al.*, 2003; table 1). Moreover, the fact that it has been possible to obtain precise information on the dates of sighting of the bird during its migration through Europe, as well as the certainty about the age of the host and its origin area thanks to the bird ringing, are the basis that allow us to affirm that the area where the bird acquired the infection is most probably outside the Iberian Peninsula, since otherwise the trematodes found in the intestine of the black stork would not have had enough time to reach the adult stage. Therefore, it is plausible to state that this is a well-documented case of the spread of a parasite through a migratory bird.

Chaunocephalus ferox can reach high prevalence in storks, and although health disorders are usually described in individuals suffering from high parasite intensity, severe problems have been reported in low parasitized storks (Choe *et al.*, 2016; Michalczyk *et al.*, 2020). For these reasons, and taking into account the findings described in our study, it seems relevant to monitor the presence of *C. ferox* in the distribution area of black storks in the Iberian Peninsula, a bird species classified as 'Vulnerable' by the 'Red Book of the birds of Spain – 2021' (López-Jiménez, 2021). This issue is particularly relevant after reporting the highest intensity of infection by *C. ferox* in black storks described to date, highlighting the possibility of massive infections in this host species.

Another highlight of this study is the information provided by bird ringing, which has made it possible to obtain key data to interpret, from an epidemiological viewpoint, the dispersal of *C. ferox* within Europe. In addition, ringing has also revealed that a bird highly parasitized by *C. ferox* may be capable of completing long migratory routes. This demonstrates the utility of bird ringing in the study of parasite spread, as described

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previously for other pathogens (Jourdain *et al.*, 2007). We suggest that information on host migration should be included in epidemiological studies (table 1) to help understand epidemiological aspects of parasitofauna in migratory birds. If other host species are able of migration when infected, it may be possible to create epidemiological risk maps at their breeding, passage and wintering sites.

In summary, the present study highlights the potential relevance of migratory birds as long-distance spreaders of parasites to new areas, as well as the usefulness of bird ringing not only for ecological purposes, as traditionally used (Baillie *et al.*, 2009; Sharp, 2009), but also for epidemiological studies. Our study shows the value of spatial data to understand the epidemiology of parasites hosting wild birds worldwide.

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Conflicts of interest. None.

Ethical statement. No approval of research ethics committees was required to accomplish the goals of this study because the animal manipulation and samples collection were carried out on the dead bird.

Data availability. Data available on request from the authors.

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