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Internal parasites and associated histopathological changes in deceased white storks from Poland

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Abstract. The aim of this study was to analyze the parasitic fauna of dead white storks in nesting sites in different parts of Poland and the associated histopathological changes. Samples from thirty-eight white storks aged 3 weeks to 5 years were examined after their arrival at a stork sanctuary. The presence of *Cathaemasia hians*, *Chaunocephalus ferox*, *Choanotaenia infundibulum*, *Railletina tetragona* and *Syngamus trachea* was confirmed in 17 out of 38 (47.73 %) individuals. *Cathaemasia hians* and *Chaunocephalus ferox* flukes are not endemic to the studied area. The frequency of *C. ferox* was significantly higher in the youngest storks from group I (3–4 weeks old) compared to groups II (11–15 weeks old) and III (older than 2 years). Fluke eggs were only detected with sedimentation method in three samples (group III), while no adult flukes at all were found in the intestines. *Chaunocephalus ferox* was shown to be the most common pathogen in all storks studied when compared to other parasites. A histopathological examination of the jejunum and ileum revealed atherosclerotic changes in the muscular layer, lymphoid infiltration in the mucosa, the presence of adult flukes of *C. hians* and *C. ferox* in the intestinal lumen, and lymphoid infiltration in the muscular layer. Intense lymphoid infiltration in the mucosa was also observed in storks whose intestines were heavily infested with *R. tetragona* and *C. infundibulum.* Parasitic infections compromise the birds' health status and affect the duration of flights, and they can increase the risk of other diseases.

Keywords. *Ciconia ciconia*, stork, parasites, *C. ferox*, *C. hians*, histopathology.

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Introduction

The white stork (*Ciconia ciconia*) is one of the 19 species of the family *Ciconiidae*. The global stork population is estimated at 200,000 pairs (Choe *et al*. 2016). The white stork inhabits humid lowlands with tree clusters and medium-tall vegetation in the Iberian Peninsula, southern Africa, Asia Minor,

Central Asia and Central-Eastern Europe. Storks usually return to Poland from their wintering grounds in March. They breed in Poland and migrate to southern Africa in the second half of August (Tryjanowski *et al*. 2018). Migrating storks cover a distance of 10,000 to 12,000 km and travel as much as 500 km in a single day. The date of their arrival in nesting sites is determined by ambient temperature (Kruszewicz & Czujkowska 2006). Recent years have witnessed a decline in the stork population in nesting sites. This decrease can be attributed to habitat change, excessive pesticide use, electrocution and traffic accidents (BirdLife International 2012). According to Höfle *et al*. (2003) and Santoro *et al.* (2013), the health status and well-being of storks are mainly determined by environmental conditions. Storks feed on small rodents, amphibians and fish which are often sources of pathogens, including parasites. In storks, the most frequently identified internal parasites include protozoa, flukes and tapeworms, whereas lice (*Mallophaga*), mites and ticks are the most prevalent external parasites (Syrota & Kharchenko 2015; Girisgin *et al*. 2017). When present in large numbers, parasites lead to bowel obstruction (Okulewicz & Sitko 2012) and flukes of *Chaunocephalus ferox* (Echinostomatidae) cause nodular lesions in the intestines which compromise bird health and often have lethal consequences (Poonswad *et al*. 1992; Santoro *et al*. 2013). It is believed that parasites directly influence the flight ability of birds, in particular young individuals, premature hatchlings and older birds with chronic parasitic infections (Romaniuk 2016).

The aim of this study was to analyze the parasitic fauna of white storks in nesting sites (places where birds arrive for breeding) and to determine histopathological changes in storks infested by parasites. We hypothesized that young storks (3–4 weeks old) are more likely to carry parasites, which in the future may affect the health of birds and the distance of their migration. It is believed that it is mainly the environment and the immunological status of the storks that affect the frequency of infestation with parasites. Young storks do not have sufficiently developed body defense mechanisms (Romaniuk 2016).

Material and methods

Samples for parasitological analyses were obtained from 38 white storks aged 3 weeks to 5 years in the Azyl Animal Sanctuary in south-eastern Poland (49°47′05″ N, 22°46′02″ E). This study was carried out on fecal samples and organs collected post-mortem from birds, there was no manipulation of any living animals. Feces and organs were collected from the Azyl Animal Sanctuary for research purposes. Birds came to the Center dead or were euthanised, due to severe body injuries. The sanctuary admits storks with health problems, including wing fractures, multiple femoral fractures, limb necrosis, shot wounds and electrocution caused by collision with power lines. Some birds are dead upon arrival, including young individuals killed by hail (Table 1). The collected samples were divided into three groups: group I (13 birds aged 3–4 weeks, fallen out of the nest or killed by hail), group II (12 birds aged 11–15 weeks) and group III (13 birds older than 2 years). Post-mortem examinations were performed to determine the cause of death. The following organs were examined: trachea, lungs, air sacs, kidneys, spleen, liver, gallbladder and the gastrointestinal tract. Parasites were extracted from the intestines, rinsed in saline solution, fixed with 70% ethanol and subjected to further analyses. Fresh feces and intestinal contents were sampled for coproscopic analysis by the Fülleborn floatation method with Darling's solution (saturated NaCl solution and glycerol, 1:1). Fluke eggs were detected by sedimentation (Stefański 1963). Parasites in different developmental stages were observed under a light microscope at a magnification of 200 ×, and their morphometric characteristics were described with the method of Kostadinov (2005).

Samples of intestinal tissue and intestinal contents were subjected to histopathological analyses. Intestines were collected for all studied birds. During necropsy, the intestines were fixed in 10% neutralized formalin

TABLE 1

Cause of death and parasitic species in white stork samples.

group/age		cause of death	parasite
group I (young birds)	3–4 weeks	fell out of the nest	–
	3–4 weeks	fell out of the nest	–
	3–4 weeks	fell out of the nest	*Syngamus trachea* (adults)
	3–4 weeks	fell out of the nest	*Syngamus trachea* (adults)
	3–4 weeks	killed by hail	*Chaunocephalus ferox*
	3–4 weeks	killed by hail	*Chaunocephalus ferox*
	3–4 weeks	killed by hail	*Chaunocephalus ferox*
	3–4 weeks	killed by hail	*Chaunocephalus ferox*
	3–4 weeks	killed by hail	*Chaunocephalus ferox*
	3–4 weeks	killed by hail	*Chaunocephalus ferox*
	3–4 weeks	killed by hail	*Chaunocephalus ferox*
	3–4 weeks	killed by hail	*Chaunocephalus ferox*
	3–4 weeks	killed by hail	*Chaunocephalus ferox*
group II (older storks)	10 weeks	euthanised, due to severe body injuries (limb necrosis)	–
	11 weeks	euthanised, due to severe body injuries (limb necrosis)	–
	12 weeks	dead upon arrival to Animal Sanctuary (health problem)	–
	12 weeks	euthanised, due to severe body injuries (wing fractures)	–
	12 weeks	euthanised, due to severe body injuries (multiple femoral fractures)	–
	12 weeks	dead upon arrival to Animal Sanctuary (health problem)	–
	13 weeks	euthanised, due to severe body injuries (multiple femoral fractures)	–
	13 weeks	euthanised, due to severe body injuries (wing fractures)	–
	13 weeks	dead upon arrival to Animal Sanctuary (health problem)	*Chaunocephalus ferox*
	14 weeks	euthanised, due to severe body injuries (multiple wing fractures)	–
	15 weeks	euthanised, due to severe body injuries (multiple wing fractures)	–
	15 weeks	euthanised, due to severe body injuries (limb necrosis)	–
group III (older storks)	2 years old	electrocution caused by collision with power lines	*Choanotaenia infundibulum* *Railletina tetragona*
	2 years old	electrocution caused by collision with power lines	
	2 years old	electrocution caused by collision with power lines	
	2 years old	euthanised, due to severe body injuries (limb necrosis)	
	2 years old	euthanised, due to severe body injuries (limb necrosis)	
	2 years old	shot wounds	*Choanotaenia infundibulum* *Railletina tetragona* *Cathaemasia hians*
	3 years old	euthanised, due to severe body injuries (multiple femoral fractures)	
	3 years old	euthanised, due to severe body injuries (multiple wing fractures)	*Cathaemasia hians*
	3 years old	shot wounds	
	3 years old	shot wounds	*Chaunocephalus ferox*
	4 years old	euthanised, due to severe body injuries (multiple wing fractures)	
	4 years old	euthansied, due to severe body injuries (multiple wing fractures)	
	4 years old	electrocution caused by collision with power lines	*Choanotaenia infundibulum* *Railletina tetragona*

and embedded in paraffin blocks. Tissue sections (5 μm) were stained with hematoxylin and eosin (HE). Images of tissue sections were acquired with the MIDI 3DHISTECH Panoramic Scanner (3DHISTECH, Budapest, Hungary) and Panoramic Viewer software (3DHISTECH, Budapest, Hungary).

Differences in the frequency of parasitism specific parasites between age groups were statistically analysed with Fisher test. Significance was set at $p < 0.05$.

Results

Parasites in different developmental stages were detected in 17 out of the 38 (44.73%) analyzed individuals. In group I (young birds, sampled from the nest), parasites were identified in 11 out of 13 (84.6%) samples, including 9 (69.23%) samples with adults of *C. ferox* and two (5.26%) with adults of *Syngamus trachea*. In group II (older storks), a fluke of *C. ferox* was detected in one sample (8.33%). In group III (adult birds), eggs or adult parasites were identified in five samples (38.46%), including three samples (23.07%), with flukes of *C. hians* in two samples and *C. ferox* in one sample. Mixed infections with *Choanotaenia infundibulum* and *Raillietina tetragona* tapeworms were noted in three (23.07%) individuals (Table 1). Fluke eggs were only detected with sedimentation method in three samples (group III), while no adult flukes at all were found in the intestines. The prevalence of each parasitic species is presented graphically in Figure 1.

The frequency of appearance of *C. ferox* was significantly higher in storks from group I compared to storks from groups II and III ($p = 0.0009$). *Chaunocephalus ferox* was shown to be the most common pathogen in all storks studied when compared to other parasites ($p = 0.018$) (Table2).

The post-mortem examination did not reveal irregularities or pathological changes in the examined organs. A histopathological examination of the jejunum and ileum revealed atherosclerotic changes in the muscular layer, lymphoid infiltration in the mucosa, the presence of adult *C. hians* and flukes of *C. ferox* in the intestinal lumen, lymphoid infiltration in the muscular layer, and the presence of 'fine' flukes migrating in blood vessels (Figs 2–4). Seven to 15 nodules measuring 5–8 mm were observed on the external surface of the jejunal and ileal segments. The nodules were dissected to reveal 1–2 flukes in the muscular layer, surrounded by a wide band of necrotized muscle and accompanied by hemorrhaging with heterophil and lymphoid infiltration. The muscular layer was locally penetrated by flukes which were observed in the intestinal lumen. Lymphoid infiltration was also noted in the mucosa. Granulomas with central necrosis surrounded by epithelioid cells, multinucleated giant cells and heterophiles were detected in jejunal samples. Lymphoid infiltrates were observed around pathologically changed blood vessels in the muscular layer (Fig. 2). In storks heavily infested with *R. tetragona* and *C. infundibulum*, a histopathological analysis of intestinal samples revealed massive lymphoid infiltration in the mucosa. Adult forms of *S. trachea* were identified in the trachea of two birds.

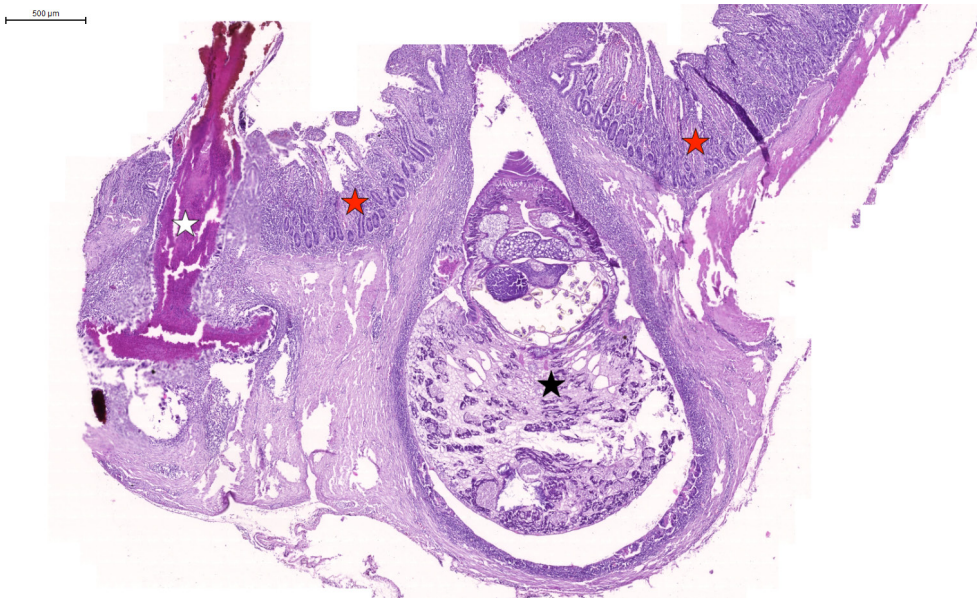

Fig. 1 – Prevalence of parasitic species in the analyzed 38 samples (%).

TABLE 2

Parasitic divisions and species in white stork samples. Fisher test, $p < 0.05$.

Phylum	Species	Group		
		I (n = 13)	II (n = 12)	III (n = 13)
Flukes (*Trematoda*)	*Cathaemasia hians*	–	–	2
	Chaunocephalus ferox	9*	1	1
Tapeworm (*Cestoda*)	*Choanotaenia infundibulum*	–	–	3
	Railletina tetragona	–	–	3
Nematodes (*Nematoda*)	*Syngamus trachea*	2	–	–

* statistical significant between age groups.

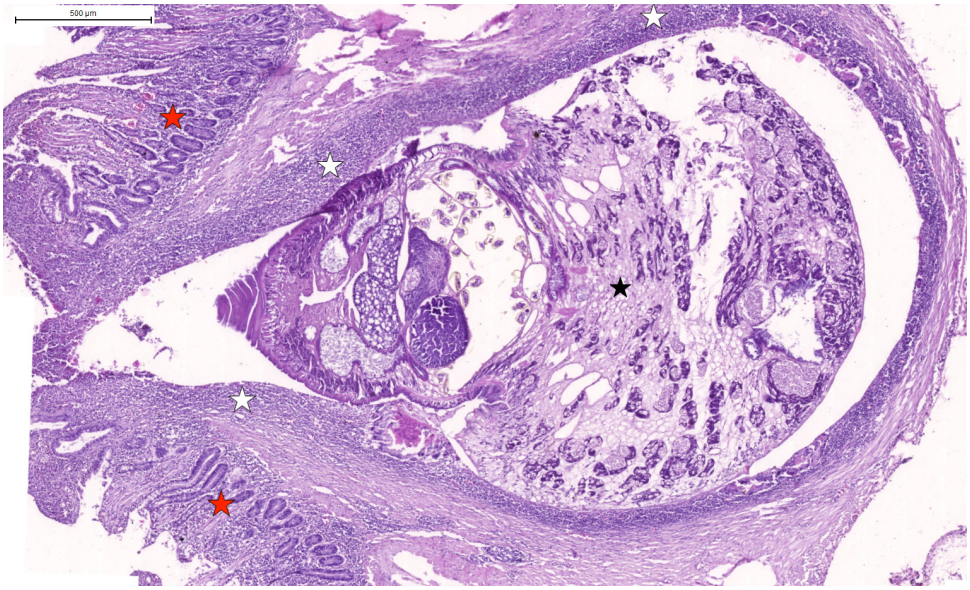

Fig. 2 – Jejunum. Adult flukes in the intestinal wall (black asterisk); infiltration of lymphoid cells around fluke (short black arrows); parasitic spikes (long black arrows). Hematoksylin and eosin staining. Magnification 3 ×.

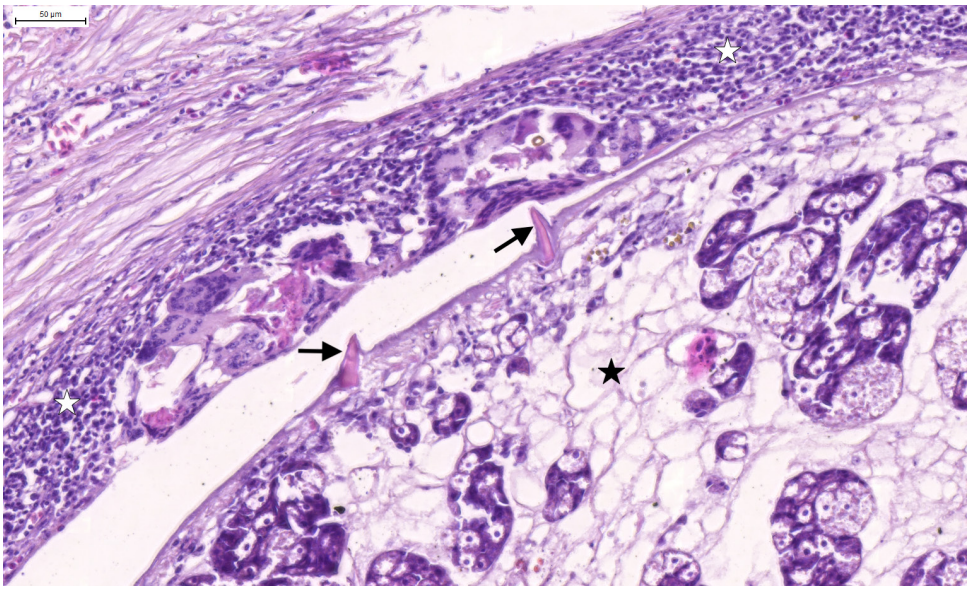

Fig. 3 – Jejunum. Adult flukes in the intestinal wall (black asterisk); necrosis of intestinal wall (dark violet colour); intestinal mucosa (black arrows). Hematoksylin and eosin staining. Magnification 6 ×.

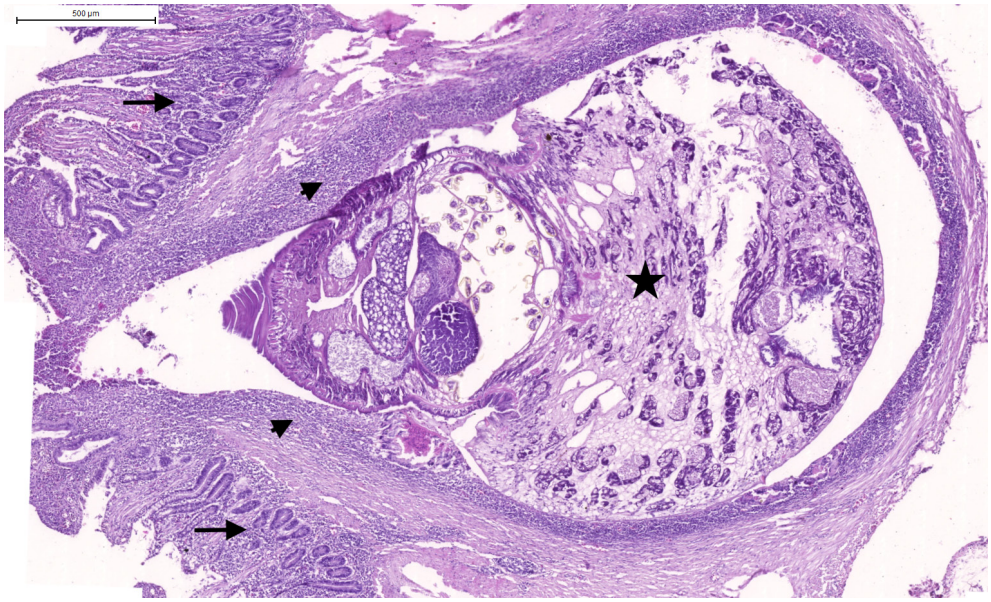

Fig. 4 – Jejunum. Adult flukes in the intestinal wall (black asterisk); infiltration of lymphoid cells around fluke (short black arrows); intestinal mucosa (long black arrows). Hematoksylin and eosin staining. Magnification 23 ×.

Discussion

According to many researchers, stork parasites migrate with the birds and are thus introduced to non-endemic locations. This was first observed in two different studies in 1964 (Grünberg & Kutzer 1964; Van den Broek & Jansen 1964). In the present study, two fluke species that are not endemic to Poland, *C. hians* and *C. ferox*, were detected in the analyzed samples, which confirms the above observations. Interestingly, the sedimentation test revealed the presence of fluke eggs in four samples, but adult flukes were not determined in the intestines. According to Merino *et al*. (2001), *C. hians* requires a warm climate to complete its life cycle, which is why these parasites infest storks migrating to wintering grounds. *Chaunocephalus ferox* is an intestinal parasite of many stork species, including white stork (*C. ciconia*), black stork (*C. nigra*), Eurasian bittern (*Botaurus stellaris*) and black-necked stork (*Xenorhynchus asiaticus*) (Skrjabin & Bashkirova 1956; Poonswad *et al*. 1992; Höfle *et al.* 2003; The Natural History Museum 2013). Its presence has been documented in numerous countries, including Armenia, Spain, India, Japan, Hungary, Italy, Thailand and the Ukraine (Patnaik *et al*. 1970; Höfle *et al*. 2003; The Natural History Museum 2013). Snails of the family *Planorbidae* act as intermediate hosts for cercariae of *C. ferox*, whereas metacercariae are transmitted by amphibians and fish (Kostadinov 2005).

Saad (2009) identified both fluke species in black storks in Egypt. Hölfe *et al*. (2003) also reported these fluke species in white stork and concluded that these parasites can have lethal consequences for birds by penetrating intestinal walls and causing mechanical damage to the digestive tract. The studied storks were emaciated, and their intestinal absorption was compromised. Merino *et al*. (2001) found nodules in the intestinal walls of storks from Spain examined post-mortem, which pointed to the presence of *C. hians*. The size (5–8 mm) and shape of these nodules were similar to those observed in the current study. Moreover, these authors concluded that parasites of *C. hians* are more effectively identified with the sedimentation test, which can be confirmed with the current study.

Patnaik *et al*. (1970) detected *C. ferox* in 545 samples. Nodular enteritis was noted in birds being heavily infested by *C. ferox*, but other pathological changes or internal parasites were not reported. Greben *et al*.

(2016) also identified *C. ferox* flukes in storks from the Ukraine. Hölfe *et al*. (2003) studied 42 storks in a bird rehabilitation center and detected the presence of *C. ferox* in two adults and eight young birds. According to the literature, *C. ferox* is more frequently encountered in storks as compared to others flukes in wild birds. This parasite needs an intermediate host to complete its life cycle, and this role is fulfilled by snails that are abundantly consumed by nesting storks. Santoro *et al*. (2013) reported that a massive infestationby *C. ferox* of a white stork in southern Italy was responsible for pathological changes in the intestines and malabsorption. The parasites compromised the bird's health and prevented it from reaching the nesting site. Poonswad *et al*. (1992) found that the pathogenicity of *C. ferox* was determined by the number of flukes in the host. In the studied population, 57 (80%) out of 71 birds were infested with 2 to 180 parasites. Similar observations were here, because nodular enteritis was noted in birds infested by *C. ferox*, but no other pathological changes were observed.

Storks have a diverse diet, which could explain the wide range of parasitic species infesting these birds (Carrascal *et al*. 1993). Storks feed on earthworms which act as intermediate hosts for tapeworms (Melendro *et al*. 1977). Two species of tapeworms (*C. infundibulum* and *R. tetragona*) and one nematode species (*S. trachea*) were identified in the current study. Girisgin *et al*. (2017) detected as many as eight intestinal helminth species in storks (*Dictymetra discoidea*, *C. ferox*, *Schistocephalus solidus*, *Stephanoprora spinulosa*, *Echinopharyphium*, *Tylodelphys excavata*, *T. clavata*, *Syncunaria ciconiae*). Their results validate the observation that migration and a diverse diet both contribute to mixed parasitic infections in storks.

According to Okulewicz & Sitko (2012), the size of individual parasites in birds poses a smaller threat than the actual number of parasites. In the current study, tapeworms (*C. infundibulum*, *R. tetragona*) present in the jejunum and ileum caused bowel obstruction. The identified tapeworms were small in size, but they occurred in sufficiently high numbers to block the intestinal lumen. In the examined birds, bowel obstruction most probably had lethal consequences. Symptoms of cachexia were also observed in all storks infested with tapeworms.

In our study, the presence of *S. trachae* (single nematodes) was confirmed in two out of 38 birds. Similarly, Krone *et al*. (2007) detected *S. trachae* in the respiratory tract of only one of the 549 examined storks.

In a study by Santoro *et al*. (2013), a histopathological examination of intestinal nodules confirmed the presence of flukes responsible for granulomatous lesions throughout the intestinal tract. The morphological features of the parasites were similar to those of *C. ferox* extracted from granulomas or found in the intestinal lumen. A histological analysis of the site of parasite penetration revealed tissue damage, hemorrhaging, thickened mucosa and submucosa. The presence of flukes was accompanied by inflammatory infiltrate composed of heterophils, lymphocytes and histiocytes with necrosis of the muscular layer around *C. ferox*. Apparently, older lesions were characterized by the accumulation of necrotic material, degenerate heterophils and cellular debris. Similar results were noted in the current study where adults of *C. ferox* were also detected in the muscular layer, surrounded by a wide band of necrotized tissue with hemorrhaging. Lymphoid infiltration was also observed in the mucosa.

In the literature, descriptions of parasitic fauna are mainly based on the results of studies conducted in bird rehabilitation centers. Birds are usually admitted to sanctuaries because of health problems sustained during migratory flights, including wing and limb fractures or malnutrition. The presence of intestinal parasites in storks undoubtedly reduces their body weight gain and negatively affects the flight duration (Hölfe *et al*. 2003). Birds admitted to rehabilitation centers are often exposed to chronic stress caused by multiple factors, which compromises their immune status and exacerbates coexisting infections, including parasitic infestation. Hölfe *et al*. (2003) therefore recommended that fecal samples should be analyzed with the use of the flotation technique first before birds are admitted to rehabilitation centers.

Our study revealed the presence of numerous parasitic species, including *C. hians*, *C. ferox*, *C. infundibulum*, *R. tetragona* and *S. trachea*, and associated histopathological changes in white storks nesting in Poland. Parasitic infections compromise the birds' health status and affect the duration of flights, and they can increase the risk of other diseases (Illescas-Gomez *et al.* 1993; Coulson *et al.* 2010; Santoro *et al.* 2010). Therefore, parasitic fauna should be monitored in stork populations for all life stages, and infested storks should receive the appropriate treatment in bird sanctuaries and rehabilitation centers.

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