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## TRICHOMONIASIS IN A BONELLI'S EAGLE POPULATION IN SPAIN

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**ABSTRACT:** During 1980–97, trichomoniasis was detected in nestlings of Bonelli's eagle *Hieraetus fasciatus* in Catalonia (Spain). In 1993 *Trichomonas gallinae* was isolated in 36% of nestlings ( $n = 39$ ) and affected 41% of broods ( $n = 22$ ). Overall, trichomoniasis was one of the most important single nestling mortality factor, accounting for 22% of total chick mortality, and causing the death of 2% of chicks. Trichomoniasis deaths took place during the second half of the nestling period. The median age at death was 45.5 days. Although the presence of the parasite was not related to the composition of the diet or parental age, pairs that developed the disease ate more pigeons and included more often non-adult birds. At present trichomoniasis apparently has little demographic impact on the Bonelli's eagle population in Catalonia, but the eventual spread of this disease in chicks and its unknown effects on adults might be of concern.

**Key words:** Birds of prey, Bonelli's eagle, *Hieraetus fasciatus*, nestlings, survey, *Trichomonas gallinae*, trichomoniasis.

### INTRODUCTION

Feral pigeons (*Columba livia*) and other columbiform birds are the main primary hosts of *Trichomonas gallinae*, a sarcosporidial parasite that causes trichomoniasis in birds (Stabler, 1951; Locke and James, 1962). Most of the strains of the parasite are found in the upper digestive tract of their hosts and are considered to be non-pathogenic (Stabler, 1969; Honigberg, 1970). However, others are highly virulent and are responsible for proliferative lesions in the oropharynx that cause the death of a non-immune bird by starvation or secondary bacterial infections (Stabler, 1954; Mesa et al., 1961; Cooper and Petty, 1988; Samour et al., 1995).

Trichomoniasis has been reported frequently in birds of prey from several areas (Stabler, 1969; Keymer, 1972; Beecham and Kochert, 1975; Rettig, 1978; Tangredi, 1978; Stone and Nye, 1981; Redig, 1987; Pepler and Oetlé, 1992; Samour et al., 1995; Boal et al., 1998), but there is little information on the prevalence of infection or disease incidence of this parasite in wild populations (Beecham and Kochert, 1975; Cooper and Petty, 1988; Kietzmann, 1988; Boal et al., 1998). The occurrence of trichomoniasis in this group of birds is re-

lated to the ingestion of infected pigeons (Stabler and Shelanski, 1936; Stabler, 1969; Boal et al., 1998) or other infected birds (Halliwell, 1979). In some species, there is increasing concern that the expansion of the disease in the population may impair or reduce population growth rate, or even put the population at risk (Cooper and Petty, 1988; Boal et al., 1998).

The Bonelli's eagle (*Hieraetus fasciatus*) is an endangered bird of prey whose populations have undergone a marked decline in Europe in recent years (Rocamora, 1994; Real and Mañosa, 1997). During the last decades, in some areas the eagles have increased the consumption of feral pigeons as a consequence of the decline on wild prey and the increase of feral pigeon populations (Real, 1991; Del Hoyo et al., 1994). This change increases the risk of exposure to *T. gallinae* and nestling mortality, which has been shown to entail the decline of some populations (Cugnasse, 1989; Real 1991; Fernández et al., 1998). The objectives of this paper are to describe some cases of trichomoniasis in wild Bonelli's eagle nestlings, to quantify the prevalence of infection of *T. gallinae* among nestlings, and to analyze the influence of diet and eagle age on the presence

TABLE 1. Cases of trichomoniasis detected in Bonelli's eagle chicks in Catalonia (Spain) during 1980–97.

Case	Year	Sex	Hatching order	Weight (g)	Age (days)	Status at finding <sup>a</sup>	Fate <sup>b</sup>	Number of siblings and fate
1	1987	F	1	1,150 <sup>d</sup>	58	D	D	1 (dead at postnestling <sup>c</sup> )
2	1992	M	2	1,010 <sup>d</sup>	47	A	D	1 (fledged)
3	1992	M	—	1,350 <sup>d</sup>	48	A	D	0
4	1993	F	—	—	28	D	D	1 (case 5)
5	1993	M	—	1,480	43	A	F	1 (case 4)
6	1995	F	1	—	44	D	D	1 (dead at 32 days <sup>c</sup> )
7	1997	M	—	1,690	43	D	D	0

<sup>a</sup> D = dead, A = alive.

<sup>b</sup> D = dead, F = fledged.

<sup>c</sup> Unknown cause of death.

<sup>d</sup> Underweight.

of the parasite and on the development of the disease. The potential impact of the disease in the Bonelli's eagle breeding success and conservation also is discussed.

#### MATERIALS AND METHODS

Between 1980–97 the breeding success of the Bonelli's eagle population was monitored in Catalonia (northeastern Spain), where about 70 pairs still remain (Real et al., 1997). A preliminary monitoring scheme was conducted during 1980–85 involving only six pairs to determine nestling mortality and breeding success. From 1986–93, an intensive study was undertaken on a large sample. During these years, the nests of 41 breeding sites were regularly checked for hatching success and nestling survival. Nests were visited 20, 35, 55, and 65 days after hatching to determine the levels and causes of nestling mortality during the nestling period. When possible, the age and sex of the nestlings were determined following Mañosa et al. (1995).

Clinical trichomoniasis in nestlings (living or dead) was verified by visual inspection of the buccal cavity, where characteristic *T. gallinae* lesions can be observed (Cooper, 1978; Ward, 1986; Cooper and Petty, 1988).

The prevalence of infection by *T. gallinae* was assessed in nestlings aged 32 to 46 days from 22 nests in 1993. Culture specimens were obtained by swabbing the surface areas of the upper crop, pharynx, palatin region, and mouth with a cotton-tipped swab previously soaked in culture medium. The samples were immediately transferred to a 5 ml of CPLM (Cysteine, Peptone, Liver extract and Maltose) *Trichomonas* broth with CAF (Chloramphenicol) (Bioline, le Monza, Milano, Italy) medium, (supplemented with 0.1 g mycostatin, 0.061 g penicillin, 0.1 g streptomycin and 5 ml of inactivated rabbit serum/100 ml). The cultures

were stored at room temperature and taken to the laboratory the same day, where they were incubated at 36 C. Each sample was examined under an inverted microscope (100×) after 24 to 48 hrs of incubation and checked again after 120 to 144 hrs. If no trichomonads were detected at this time, the sample was considered free of *T. gallinae*.

The diet of nestlings was assessed by collecting prey remains and pellets in the nest, which were analysed and quantified following Real (1996). Breeding birds were classified as adults (those with full adult plumage, >4-yr-old) or non-adults (≤4-yr-old) based on plumage characteristics (Parellada, 1984; J. Real, pers. data). We used chi-square and Fisher's exact tests for comparisons (Zar, 1984). The level of  $\alpha$  was established at  $P \leq 0.05$ .

#### RESULTS

Between 1980–97, seven cases of trichomoniasis were recorded (Table 1, 2). Of those cases, three involved females and four involved males. Of these nestlings, six died before leaving the nest, and the remaining fledged.

The nestling period of Bonelli's eagle lasts for 65 to 70 days. Most deaths (five of six) associated with trichomoniasis occurred in the second half of this period, when nestlings were between 28-days and 58-days-old, usually after 40 days of life. The median age at death was 45.5 days ( $n = 6$ ). Three of five nestlings were underweight (Mañosa et al., 1995) (Table 1, 2). All the nestlings had buccal cavity nodular lesions of different size, usually more than 2 cm long (Table 2). These nodules caused

TABLE 2. Characteristics of Bonelli's eagle chicks affected by trichomoniasis during 1980-97 in Catalonia (Spain).

Case	Nodule size	Nodule position in buccal cavity	Esophagus obstruction	External swelling	Weight loss	Rotted food into the mouth	Deteriorated plumage
1	>3 cm	Lower jaw and laterally	Total	Yes	Yes	Yes	No
2	3 cm	Lower jaw and laterally	Important	Yes	Yes	No	No
3	>3 cm	Superior jaw and posterior	Important	No	Yes	No	Yes
4	>3 cm	Lower jaw	Total	Yes	?	—	No
5	2 cm	Lower jaw and posterior	Little	No	No	—	No
6	2 cm 3 nod.	Lower jaw and posterior	Important	Yes	?	No	No
7	2 cm	Lower jaw near glottis	No	No	No	No	No

TABLE 3. Causes of nestling Bonelli's eagle mortality in relation to age during 1986–93.

Causes	1–20 days	21–40 days	41–60 days	Total chicks
Trichomoniasis	0	1	3	4
Starvation	1	0	0	1
Hatching asynchrony	4	0	0	4
Predation	1	0	0	1
Accident	0	0	2	2
Other diseases	0	1	0	1
Unknown	2	1	2	5
Total	8	3	7	18

moderate to severe occlusion of the esophagus and prevented the passage of food. One nestling may have died by asphyxia caused by a large nodule on the inferior surface of the tongue, which occluded the glottis. This chick was in good physical condition and the crop and stomach were full of recently ingested food. One of the underweight nestling showed deficient feather growth (remiges, tail feathers and coverts), consisting in bands without cornial deposits as a result of malnutrition (Grubb, 1995). The other underweight nestlings did not have deteriorated plumage but one had delayed development of the feathers.

The presence of *T. gallinae* was checked in 22 broods in 1993; one had 3 chicks, 15 had 2, and 6 consisted of one, involving 21 males and 18 females. The parasite was isolated in 13 live nestlings and diagnosed in one dead nestling (36%, 7 males and 7 females), corresponding to 9 broods (41%) (two single broods and seven double broods). Only two (5%) of these 14 infected chicks had lesions clearly related to

the disease and only one (3%) died. For the 105 broods checked in the 1986–93 intensive study period, involving 179 nestlings, trichomoniasis caused the death of 2% of chicks, accounting for 22% of total nestling mortality (Table 3). Mortality affected 0% of chicks and broods in 1986 ( $n = 8$  chicks, and  $n = 5$  broods), 1988 ( $n = 18$ , and  $n = 10$ ), 1989 ( $n = 14$ , and  $n = 8$ ), 1990 ( $n = 14$ ,  $n = 9$ ), and 1991 ( $n = 13$ ,  $n = 8$ ) respectively, 2% of chicks ( $n = 54$ ) and 3% of broods ( $n = 30$ ) in 1993, 5% of chicks ( $n = 41$ ) and 8% of broods ( $n = 25$ ) in 1992, and 6% of chicks ( $n = 17$ ) and 10% of broods ( $n = 10$ ) in 1987.

There were no differences in diet composition between broods that were positive or negative for *T. gallinae* in 1993 ( $\chi^2 = 9.3955$ ,  $df = 6$ ,  $P = 0.1525$ ) (Table 4). However, the development of the disease was related to the consumption of pigeons. Taking into account only the four pairs that developed the disease at least once between 1980 to 1997, and where dietary information could be collected, differences in the consumption of *C. livia* were ob-

TABLE 4. Diet of pairs of Bonelli's eagle monitored in 1993 related to the presence of *Trichomonas gallinae*.

	Infected		Uninfected	
	<i>n</i>	%	<i>n</i>	%
European rabbit ( <i>Oryctolagus cuniculus</i> )	39	28	57	25
Other mammals	26	18	23	10
Domestic pigeon ( <i>Columba livia</i> )	15	11	22	9
Woodpigeon ( <i>Columba palumbus</i> )	17	12	34	15
Phasianidae	17	12	32	14
Other birds	16	11	45	19
Ocellated lizard ( <i>Lacerta lepida</i> )	12	8	19	8

served between three broods developing the disease (31%,  $n = 26$ ) and four broods without the disease (11%,  $n = 73$ ) ( $\chi^2 = 4.187$ ,  $df = 1$ ,  $P = 0.04$ ). No differences were detected in the consumption of *C. palumbus*, 4% and 10% respectively ( $\chi^2 = 0.254$ ,  $df = 1$ ,  $P = 0.61$ ).

In 1993 no difference of infection was found between adult or non-adult pairs (formed at least by one non-adult individual) (40%,  $n = 15$  and 43%,  $n = 7$ , respectively, Fisher Exact Test,  $P > 0.05$ ). However, when we take into account only the five pairs in which trichomoniasis developed at any time from 1980–97, we found that the disease developed more often when the breeding attempt involved a non-adult bird (83%  $n = 6$ ), than when both parents were adult (5%,  $n = 20$ , Fisher Exact Test,  $P = 0.0005$ ).

#### DISCUSSION

Despite the high prevalence of *T. gallinae* in nestling Bonelli's eagles, only a small proportion developed clinical trichomoniasis and died during the nestling period. This may indicate that most strains of *T. gallinae* are non-pathogenic or that nestlings of Bonelli's eagle have certain immunity, as happens in pigeons (Stabler, 1969; Honigberg, 1970) or other raptors (Samour et al. 1995; Boal et al. 1998). In spite of that, trichomoniasis appears as one of the most important causes of chick death and might produce up to 6% nestling mortality in particular years.

Although the presence of the parasite in a brood seems to be independent of the proportion of domestic pigeons or wood-pigeons in the diet or parental age, the development of the disease in infected chicks is more frequent in broods consuming more domestic pigeons or where non-adult breeders are involved. This suggests that although most of the population is exposed to the protozoan (since most pairs consume pigeons to some extent), pairs consuming large amounts of domestic pigeons have a higher probability of being exposed to a virulent strain (Boal et al.,

1998). Also, the disease would mainly develop in broods raised by non-adult birds, which might be related to lower experience (Newton, 1979), resulting in poor chick condition, or to non-adult birds consuming more feral pigeons than adult birds.

Although the monitoring of Bonelli's eagle in Catalonia began in 1980, trichomoniasis was not detected until 1987, which might be related to the changes in feeding habits of Bonelli's eagles which took place in late 1980s (Real, 1991). Whatever the causes of these changes are (decline in wild prey populations, increased feral pigeon availability, increased proportion of non-experienced parents), increased consumption of feral pigeons may have led to an increased prevalence of the disease and chick mortality, since the prevalence of the parasite in rural pigeons *C. livia* from the area (90 to 97%) is far higher than that exhibited by woodpigeons *C. palumbus* (50%) (Muñoz, 1995). Increased trichomoniasis prevalences in raptor populations consuming large proportions of columbiform birds have repeatedly been reported (Cooper and Petty, 1988; Boal et al., 1998).

Although chick mortality associated with *T. gallinae* in Catalonia is low at present and may have low demographic effects (Real and Mañosa, 1997), it may be of future concern if natural prey continue to be replaced by infected prey such as domestic pigeons in eagle's diet. In Portugal, where Bonelli's eagle consume large amounts of domestic pigeons (Palma et al. 1984), nestling mortality as high as 14% associated with the disease has been reported (L. Palma, pers. comm.).

Future monitoring and research is needed to understand the susceptibility of adult birds to trichomoniasis, as well as the ecological factors and kind of prey related to the spread of the disease. In the mean time, the most sensible measure to be undertaken must be the restoration of wild prey populations (rabbits and red-legged partridges), in order to ameliorate the

availability of food to the eagles and to reduce the risk of exposure to the parasite. Further studies should indicate the eventual need of feral pigeon control and prophylaxis in some areas where prevalence of the disease is high.

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