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Carriage of *Rickettsia* spp., *Coxiella burnetii* and *Anaplasma* spp. by endemic and migratory wild birds and their ectoparasites in Cyprus

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INTRODUCTION

Three hundred and sixty-eight (368) species of birds (local, endemic and migratory) have been recorded in Cyprus. According to a rough estimate, nearly a quarter of a billion migratory birds fly through the island during the two periods of migration, thus supporting the role of the island as a stopover of great importance.

Despite the fact that the role of migratory birds in the transmission of pathogens from the endemic source to Europe has been recognized, the connection between birds, dispersal of ticks infected with medically important pathogens and outbreaks of certain zoonoses remains a hypothesis [1].

MATERIALS AND METHODS

We conducted a survey from October 2004 to October 2006, in order to investigate the presence of *Rickettsia* spp., *Coxiella burnetii* and *Anaplasma* spp. in migrating and local birds and their ectoparasites in Cyprus. Bird trapping took place through the use of nylon nets and traps. Trapped birds were checked for the presence of any ectoparasites, which were removed, placed in 1, 5ml Eppendorfs tubes, and stored at −20°C until processing. Data regarding each bird were recorded, and a small amount of blood was drawn into filter papers and stored as above. Occasionally, sampling took place from wounded or dead birds brought to the laboratory of Veterinary Services or from birds killed by hunters.

Filter papers were cut into pieces of 1 × 1 cm and stored in separate Eppendorfs tubes. Filters were treated with 490 μL of phosphate-buffered saline + 10 μL of fetal bovine serum, and incubated at 4°C overnight. Pools were prepared by removal of 200 μL of the solution into new Eppendorfs tubes. All Eppendorfs tubes were stored at −20°C.

Ectoparasites were split into different Eppendorfs tubes according to species and gender, disinfected using 70% ethanol, and triturated along with 200 μL of phosphate-buffered saline. Pools (final volume 200 μL) were created on the basis of ectoparasite species and bird origin, and stored at −20°C. All DNA extractions were carried out using the QIAamp DNA blood mini kit (Qiagen, Hilden, Germany). PCR amplifications were performed using specific primers for *Rickettsia* spp. (RpCS 877p-RpCS 1258n, and Rr19070p-Rr190602n, targeting the *glta* and *ompA* genes, respectively), *C. burnetii* (CB1–CB2, targeting the *SOD* gene), and *Anaplasma* spp. (EHR16SD–EHR16SR, targeting the 16S rRNA gene).

RESULTS

In total, 557 bird samples, representing 51 bird species, were collected (Table 1). Of the 131 pools prepared from bird filters, four (3%) were positive for *Rickettsia* spp., 56 (43%) for *C. burnetii*, and 64 (49%) for *Anaplasma* spp. Fifteen ticks (all *Ixodes ventalloi* and all removed from the species *Alectoris chukar*) and 18 lice were removed from the above birds. *Rickettsia* spp. and *C. burnetii* were detected in three *I. ventalloi* ticks. All ectoparasites were negative for *Anaplasma* spp.

DISCUSSION

The first report of wild bird infection by *C. burnetii* was made by Babudieri *et al.* (1952) [2]. In a survey conducted in Japan [7], as many as 22% of 167 sera of wild birds were found to be positive for *C. burnetii* by PCR, and the presence of the pathogen has also been recorded in a brown pelican (*Pelecanus occidentalis*) in the USA [3]. The detection of *Rickettsia* spp. in wild bird ticks dates back to 1969 [4]. The apparent increase in granulocytic ehrlichiosis in animals and humans during the last few decades may be the result of several factors, which could include wild birds. This report, in agreement with others [1], suggests that birds may be important in the dispersal of *Anaplasma* spp.

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Table 1. Bird species collected and positive pools detected for each of the pathogens tested in the current survey

Bird species	No. of samples	No. of pools	*Coxiella burnetii*		*Rickettsia* spp.		*Anaplasma* spp.	
			Positive pools	Percentage of positive pools	Positive pools	Percentage of positive pools	Positive pools	Percentage of positive pools
Accipiter gentilis	6	3	3	100			1	33
Accipiter nisus	6	2					2	100
Alcedo atthis	2	2						
Alectoris chukar	2	2					2	100
Anas crecca	1	1						
Anas platyrhynchos	7	1	1	100				
Ardea cinerea	10	4	1	25			4	100
Ardea purpurea	1	1						
Asio otus	17	4	1	25			1	25
Athene noctua	5	2						
Botaurus stellaris	4	1						
Burhinus oedicnemus	3	1					1	100
Buteo buteo	12	4	1	25			3	75
Buteo rufinus	11	3	1	33			1	33
Caprimulgus europaeus	3	3	1	33			1	33
Ciconia ciconia	4	1	1	100				
Circus aeruginosus	23	5						
Circus cyaneus	6	3	2	67			2	67
Columba palumbus	67	10	3	30			8	80
Coturnix coturnix	12	3					1	33
Crex crex	5	2					2	100
Egretta garzetta	2	1						
Falco cherrug	1	1						
Falco eleonorae	1	1						
Falco peregrinus	6	4	2	50			2	50
Falco subbuteo	1	1						
Falco tinnunculus	80	11	4	36			5	45
Falco vespertinus	7	1						
Francolinus francolinus	11	2						
Fulica atra	20	5	1	20				
Gallinula chloropus	30	5	1	20	1	20	2	40
Grus grus	2	1	1	100			1	100
Hieraaetus fasciatus	2	2	1	50			1	50
Himantopus himantopus	3	1						
Ixobrychus minutus	6	1	1	100			1	100
Lanius minor	2	1					1	100
Larus cachinnans	17	4	1	25			1	25
Larus fuscus	3	2	1	50				
Larus ridibundus	9	1						
Merops apiaster	9	2					1	50
Nycticorax nycticorax	1	1	1	100				
Pernis apivorus	18	3	1	33			1	33
Phalacrocorax carbo	23	3						
Phoenicopterus ruber	11	3	1	33	1	33	2	67
Podiceps cristatus	1	1						
Rallus aquaticus	10	2						
Scolopax rusticola	7	2						
Streptopelia turtur	41	7	1	14			2	29
Tachybaptus ruficollis	3	2					1	100
Tyto alba	22	5	2	40			1	20
Upupa epops	1	1						
Total	557	135	34	25	2	1.5	51	38

Rates of infestation of wild birds similar to what we have described (2%) have already been reported in past surveys [5]. In a recent survey, Špitalská *et al*. [6] confirmed the presence of *Rickettsia* spp. in ixodes ticks (*Ixodes ricinus*) collected from passerine birds.

Although we failed to demonstrate the presence of *Anaplasma* spp. in *I. ventalloi* ticks, the pathogen has been identified in *I. ricinus* ticks [5] in the past. The ectoparasite species that infest migratory and local birds, along with the infestation rate, were determined for the first time in Cyprus. A probable role of wild birds and their ectoparasites in the transmission chain of the above pathogens was recorded, supporting their role in the spread of diseases in humans and/or animals.

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