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First detection of microsporidia in raised pigeons in Poland

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Abstract

Microsporidia are single-celled intracellular parasites which occur in a number of animals, both vertebrates and invertebrates. Several species of microsporidia can cause disease in humans in both immunocompromised and immunocompetent individuals. However, the sources of human infection and the routes of transmission of microsporidia have not yet been fully determined, although more and more researchers are of the opinion that microsporidia in humans is zoonotic. The aim of the presented study was to identify the most common microsporidial species in the droppings of raised and wild pigeons in Poland. A total of 139 collective samples of droppings (33 samples of droppings from feral pigeons and 106 samples from raised pigeons) were examined using conventional staining and molecular techniques. Using chromotrope staining and multiplex FISH techniques, the microsporidial spores were found in 12 (8.6%) of the 139 samples of droppings. The spores of *Encephalitozoon hellem* were detected in five samples of pigeon droppings (3.6%), spores of *E. intestinalis* in four samples of droppings (2.9%), while spores of *E. cuniculi* and *E. bieneusi* were only detected in two samples (1.4%). Furthermore, a mixed infection of *E. bieneusi* and *E. cuniculi* was found in a single sample of droppings (0.7%). The presence of microsporidial spores in droppings collected from raised pigeons indicates a risk of infection to humans, mainly pigeon fanciers.

Key words

microsporidia, *Encephalitozoon* spp., *Enterocytozoon bieneusi*, multiplex FISH technique, pigeons

INTRODUCTION

Microsporidia are a highly diversified and specialised group of single-celled intracellular parasites which occur in a number of animals, both vertebrates and invertebrates. It was found only quite recently that microsporidiosis affecting humans involves a serious life risk for immunocompromised individuals and a health hazard to those with a normally functioning immunological system [1, 2, 3]. However, to date, neither the microsporidia transmission routes nor sources of infection in humans have been fully investigated, although according to an increasingly prevailing opinion, microsporidiosis affecting humans may be zoonotic in nature [4].

Recently, in various parts of the world, there has been a growing number of reports on the occurrence in urban feral pigeons of microsporidial spores that are infectious to humans [5, 6, 7, 8]. It has been stated that pigeons living in urban parks may be a source of zoonotic microsporidiosis, affecting especially paediatric and geriatric populations [7]. The risk of infection by microsporidia is considerable, given the fact that infection may occur by inhalation [6].

Studies on the presence in pigeons of human-virulent microsporidia have not been previously conducted in Poland. It was the objective of this work to study samples of droppings from wild pigeons and from raised pigeons for the presence of *E. bieneusi*, *E. intestinalis*, *E. hellem* and *E. cuniculi* spores, using the multiplex FISH technique.

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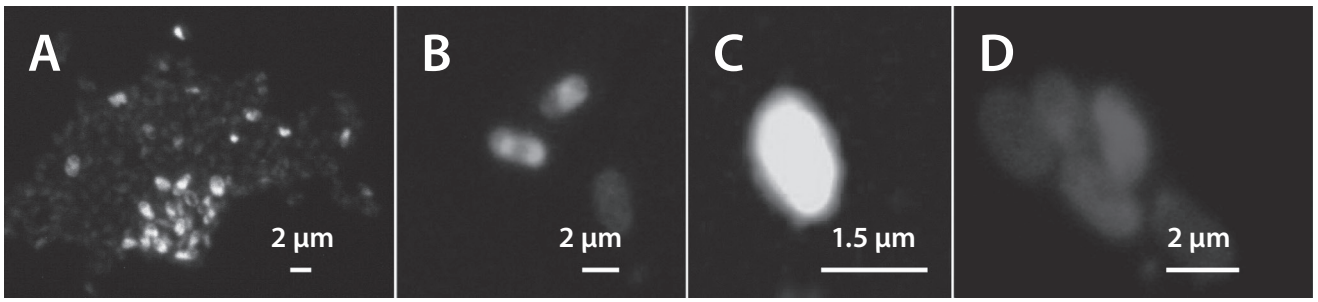
MATERIALS AND METHODS

A total of 139 collective samples of droppings (each weighing approx. 30 g) were tested, that included 33 samples of droppings from feral pigeons and 106 samples from raised pigeons. Specimens from feral pigeons were collected at four locations in the centre of Poznan, and those from raised pigeons were obtained from 32 pigeon lofts in 17 localities in five provinces in Poland (Tab. 1). All samples were collected between 2008-2010.

Smears of droppings were stained with chromotrope-2R, the method developed by Weber et al. [9]. The preparations were examined microscopically using an immersion lens (1,000 × magnification). The fluorescent *in situ* hybridization technique, reported earlier, was used to confirm the microscopic observation of the spores in a fixed stained preparation and to identify the microsporidium species [10, 11]. Fluorochrome-tagged oligonucleotides that hybridize with those 16S rRNA fragments of microsporidia which are specific for given species were used in the multiplex FISH technique. The preparations were examined using a microscope with epifluorescent capability, and equipped with a combination of filters which let in the light at different wavelengths in the range of 450-490 nm (Olympus BH2-RFL). The respective species were identified based on the spore sizes and their specific fluorescence colours.

RESULTS

Among the 139 collected samples of pigeon droppings, microsporidial spores were detected in 12 samples (8.6%). The presence of microsporidia was detected only in droppings



Figures 1A, B, C, D. Fluorescent *in situ* hybridization (FISH) images of spores of *E. hellem* (A), *E. intestinalis* (B), *E. bieneusi* (C), and *E. cuniculi* (D).

from raised pigeons. Microsporidial spores were detected in the same samples of droppings, irrespective of the diagnostic technique used. The use in the multiplex FISH technique of oligonucleotides which are specific for given species enabled identification of four microsporidial species: *E. hellem*, *E. intestinalis*, *E. cuniculi*, and *E. bieneusi* (Tab. 1). *E. hellem* spores were found in five samples of droppings (3.6%), *E. intestinalis* spores in four samples of droppings (2.9%) and those of *E. cuniculi* and *E. bieneusi* in two samples of droppings (1.4%). Furthermore, a mixed infection of *E. bieneusi* and *E. cuniculi* (0.7%) was found in a single sample of droppings. As observed under a microscope with epifluorescent capability, *E. hellem*, *E. intestinalis*, *E. bieneusi* and *E. cuniculi* spores fluoresced green, red, yellow, and blue, respectively (Fig. 1). Positive results in the test detecting the presence of microsporidia were obtained in samples from 10 pigeon lofts in five localities.

Table 1. Results of testing 106 raised pigeons dropping samples for microsporidian spores using Chromotrope-2R and multiplex FISH

Province	Location	Total No. of samples/ No. positive samples	Result of testing by:				
			Chromo-trope-2R	Multiplex FISH			
				E. hellem	*E. intestinalis*	*E. cuniculi*	*E. bieneusi*
Greater Poland Province	Antoninek	1/0	-	-	-	-	-
	Czerwonak 1	3/0	-	-	-	-	-
	Czerwonak 2	2/0	-	-	-	-	-
	Czerwonak 3	2/0	-	-	-	-	-
	Gorsko	4/0	-	-	-	-	-
	Kicin 1	4/0	-	-	-	-	-
	Kicin 2	2/0	-	-	-	-	-
	Kicin 3	1/0	-	-	-	-	-
	Kicin 4	3/0	-	-	-	-	-
	Kicin 5	1/0	-	-	-	-	-
	Kicin 6	1/0	-	-	-	-	-
	Kobylnica	2/0	-	-	-	-	-
	Koziegłowy 1	6/1	+	-	-	+	-
	Koziegłowy 2	4/0	-	-	-	-	-
	Mielno	1/0	-	-	-	-	-
	Murowana Goslina	2/0	-	-	-	-	-
	Owinska	4/0	-	-	-	-	-
	Poznan 1	8/1	+	+	-	-	-
	Poznan 2	2/1	+	-	+	-	-
	Poznan 3	4/1	+	-	-	-	+
	Poznan 4	1/0	-	-	-	-	-
	Poznan 5	2/0	-	-	-	-	-
	Przebedowo	3/0	-	-	-	-	-
Lower Silesian Province	Lwowek 1	8/2	+	+	-	-	-
	Lwowek 2	5/1	+	-	-	+	+
	Lwowek 3	6/2	+	+	+	-	-
	Mojesz 1	6/1	+	+	-	-	-
	Mojesz 2	5/1	+	-	+	-	-
	Siedlecin	4/0	-	-	-	-	-
Masovian Province	Jozefow	2/0	-	-	-	-	-
West Pomeranian Province	Nakielno	3/0	-	-	-	-	-
Lodzkie Province	Piotrkow Trybunalski	4/1	+	-	+	-	-

DISCUSSION

The presented study on the presence of microsporidial spores in pigeon droppings is the first to be conducted in Poland. The findings indicate the presence in droppings of raised pigeons of *E. hellem*, *E. intestinalis*, *E. cuniculi* and *E. bieneusi* spores. The same species of microsporidia were detected in droppings from urban feral pigeons in Amsterdam [5].

The present findings indicate that *E. hellem* is the dominant species of microsporidia in pigeons, which is not surprising as this particular species is reported to have been the cause of the majority of cases of microsporidiosis in birds raised in aviaries or at home [12, 13, 14], as well as in feral birds [11, 15]. Haro et al. [7] reported the presence of *E. hellem* in 6.5% of samples of droppings from 124 urban pigeons after live pigeon were caught in seven urban parks in Spain. Therefore, the general prevalence of *E. hellem* in urban pigeons in Spain was comparable to that of the general prevalence of *E. hellem* in raised pigeons (4.7%), as found in the presented study.

E. intestinalis is the second most frequent species of microsporidia detected in the droppings of raised pigeons in Poland. The spores of this species have been identified in four samples of droppings (2.9%) from raised pigeons. So far, *E. intestinalis* have been detected only in urban feral pigeons in Spain and in the Netherlands [5, 7], and in samples of droppings from ostriches [16].

In the presented study, the presence of *E. bieneusi* was only detected in two samples of pigeon droppings (1.4%). During the last decade, this particular species has been more and more often identified in various raised or feral birds. So far, *E. bieneusi* has been found mainly in urban pigeons [5, 6, 7, 8, 17], but has also been detected in birds of the order Psittaciformes (African grey parrot, cockatoo, and budgerigar) and Passeriformes (*Bathilda ruficauda*), as well as in chickens and ostriches [8, 16, 18]. Furthermore, there have been reports of an *E. bieneusi* outbreak in falcons in the United Arab Emirates, which has led to major losses in the breeding of falcons being trained for hawking [19].

E. cuniculi spores were detected in 2 samples (1.4%) of pigeon droppings in the presented study. Literature data

indicate that *E. cuniculi* has been detected in three bird species so far, namely in pigeons, hens, and cockatoos [5, 20, 21, 22].

In these studies, a mixed infection caused by *E. bieneusi* and *E. cuniculi* was detected in one sample only (0.7%). Also Haro et al. [7] detected, in nearly 5% of pigeons, a mixed infection mainly attributed to *E. bieneusi* and *E. hellem*.

Since the microsporidial spores are resistant to dryness, pigeons may play an important role in spreading microsporidiosis. It was recently demonstrated that humans may become infected with microsporidia by inhalation [6]. The authors reported that contact with pigeon droppings for 30 minutes, for instance, when cleaning the droppings, is sufficient time to admit 3.5×10^3 infectious microsporidial spores into the human body. Furthermore, it was found that even humans who are not in direct contact with pigeon droppings but happen to be near a place where pigeon droppings, are found are able to inhale with the air as many as 1.3×10^3 infectious spores of *E. bieneusi* [6]. It is therefore safe to assume that the detection in the presented study of *E. hellem*, *E. intestinalis*, *E. cuniculi* and *E. bieneusi* spores in raised pigeons may have a major epidemiological impact on pigeon fanciers.

Detection in droppings from urban feral pigeons in Portugal, Spain, the Netherlands and USA of microsporidial spores which are infectious to humans indicates that pigeons living in urban parks may be a source of zoonotic microsporidiosis, affecting especially paediatric and geriatric populations [5, 6, 7, 8]. No microsporidial spores were found in droppings from urban feral pigeons in the presented study, which probably indicates that too few samples were tested. In view of the above findings, further studies seem to be necessary as the number of pigeons is very large in some parts of Poland, which means that contact with their droppings is inevitable.

As demonstrated in the presented study, the sensitivity and specificity of FISH analysis of fecal samples for microsporidian spores were similar to the sensitivity and specificity of conventional staining (Chromotrope-2R). However, identification of the species of microsporidian spores is possible only by FISH techniques [6, 10, 11].

The present findings and literature reports indicate that human contact with birds infected with microsporidia, for instance with pigeons, or with droppings from such birds, poses a risk of infection with these dangerous pathogens. The present findings are important from the epidemiological aspect. Both feral and raised pigeons contribute to environmental pollution with microsporidial spores and may be potentially infectious to any person in direct contact with them, for instance to pigeon fanciers or ornithologists.

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