First detection of microsporidia in raised pigeons in Poland

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INTRODUCTION

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.

RESULTS

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.

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 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.
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.

**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 rufigenada), 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 $3.5 \times 10^3$ 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 $1.3 \times 10^3$ 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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**REFERENCES**