

EPIDEMIOLOGY OF *CRYPTOSPORIDIUM* INFECTION IN OSTRICHES (*STRUTHIO CAMELUS*) IN IRAN

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Summary

Behzadi, M. A., S. M. Razavi, H. Yazdanpoor, A. Mirzaei, A. Tamadon & M. Javdani Gandomani, 2009. Epidemiology of *Cryptosporidium* infection in ostriches (*Struthio camelus*) in Iran. *Bulg. J. Vet. Med.*, 12, No 1, 55–61.

Avian cryptosporidiosis has been reported in more than 30 species of birds. To date, the species infecting birds are *C. baileyi*, *C. galli* and *C. meleagridis*. In this study, the prevalence of *Cryptosporidium* in southern Iran and the morphological characteristics of oocysts are described. *Cryptosporidium* oocysts were found in the faeces of 21 of 75 examined ostriches (28%) from 3 farms in southern Iran. The genus identity of the oocysts was confirmed by morphology. The mean ($\pm$ SD) size of 102 oocysts was $4.4 \pm 1.14 \times 3.9 \pm 0.96 \mu\text{m}$ (range $3.8\text{--}8.6 \times 2.9\text{--}7.6 \mu\text{m}$) with a shape index (length/width) of 1.13 ± 0.13 (range 1.0–1.33). Data analyses indicated no significant effect of ostrich population on the prevalence of *Cryptosporidium* infection. Based on the odds ratio, an increase of 1 m² space for each ostrich decreased the prevalence of disease by a factor of 0.99. Stressing conditions leading to immunosuppression or poor husbandry practices related to feed, water or hygiene, were apparently acting as predisposing factors to the onset of cloacal prolapse or other pathology related to the *Cryptosporidium* spp. found in this experiment, since the improvement in husbandry practices has stopped the mortality and clinical signs, even in the presence of the parasite. To our knowledge, this is the first report of *Cryptosporidium* spp. occurrence in ostriches in Iran.

Key words: *Cryptosporidium* spp., Iran, morphology, ostriches

INTRODUCTION

Protozoa of the genus *Cryptosporidium* are apicomplexan parasites that complete their biological cycle in the surface of epithelial cells of the digestive and respiratory systems of a wide variety of vertebrates (Xiao *et al.*, 2004). Once considered rare and irrelevant, *Cryptosporidium* spp. are now known to be important pathogens, with a widespread distribution in livestock, wildlife, and humans (Fayer, 2004). Transmission is through the faecal-oral route, following direct or indirect contact with *Cryptosporidium* oocysts via

animal-to-animal, waterborne, foodborne or airborne contact (Fayer, 2004). Cryptosporidiosis has been reported in more than 30 species of birds in many countries (Lindsay & Blagburn, 1990a; Sréter & Varga, 2000; Morgan *et al.*, 2001). The parasite has been found in gastrointestinal, respiratory, urinary, pancreatic, and biliary tracts of hosts, and has been associated with disease (Gajadhar, 1993). Cryptosporidiosis is a significant cause of intestinal and respiratory tract disease in domestic chickens and turkeys (Fletcher

et al., 1975; Hoerr *et al.*, 1978). Other avian species raised in confinement have also experienced severe disease caused by cryptosporidial infections of kidneys, nasal tissues, conjunctiva, intestine, and trachea (Goodwin, 1989; Palkovic & Pecka, 1989; Lindsay & Blagburn, 1990b). The infection in ostriches may be subclinical (Gajadhar, 1993) or associated to prolapse of phallus and cloaca (Allwright & Wessels, 1993; Bezuidenhout *et al.*, 1993; Penrith & Burger, 1993; Penrith *et al.*, 1994; Santos *et al.*, 2005) and pancreatic necrosis (Jardine & Verwoerd, 1997).

In Europe, *Cryptosporidium* oocysts have been found in ostriches from Portugal, Netherlands, Belgium, France, Spain (Ponce Gordo *et al.*, 2002), and Greece (Sotiraki *et al.*, 2001). Iran is third only to South Africa and China in the world for ostrich breeding. The ostriches in Iran are mostly imported from Spain, Belgium, South Africa and Australia.

So far, there is no reports on the occurrence of *Cryptosporidium* in ostriches (*Struthio camelus*) in Iran. This paper presents evidence of cryptosporidial infection in this species, the prevalence and morphological characteristics of *Cryptosporidium* in ostriches, reared in southern Iran.

MATERIALS AND METHODS

Farms evaluation and sampling

Out of 6 ostrich farms in Shiraz, southern Iran (29°50'N, 52°46'E and an altitude of 1630 m), 3 farms that had high morbidity rates of diarrhoea of unknown origin, recorded from 1 month to 4 years ago, were selected.

At least one freshly passed individual faecal sample was obtained from 75 of 350 ostriches (25 samples of each farm)

(Table 1). The entire stool samples were examined as wet smears for detection of helminthic ova and larvae; then precipitated and stained by Giemsa and modified Zielh-Neelsen technique for detection of *Cryptosporidium* oocysts.

Case history

A female 2.5-month-old ostrich (*Struthio camelus*) from an ostrich rearing farm with cloacal prolapse was examined. The bird had a history of poor nutrition, diarrhoea, repeated antibiotic therapy, overcrowding and a contaminated environment. Faecal sample was collected aseptically, and submitted for parasitological and bacterial screening. No pathogenic bacteria were detected in the culture. The faecal sample was stained by Giemsa and modified Ziehl-Neelsen technique. Oocysts of *Cryptosporidium* were found. The bird died after 2 hours because of severe dehydration.

Morphological analysis

The size and morphology of the oocysts were determined by evaluating 102 oocysts with an Olympus BX-45 microscope equipped with a calibrated ocular eyepiece micrometre.

Statistical analysis

The data were analyzed with the chi-square test. Values of $P < 0.05$ were considered significant. The variables possibly affecting the prevalence of *Cryptosporidium* infection are listed in Table 1. The population of ostriches in the third herd (250) was used as reference. The data from each herd were compared by logistic regression analysis using the prevalence rates as the dependent variable and population and density as independent factors. The factor population of ostriches was coded as a class variable. Density of os-

triches was considered as continuous variables.

Regression analyses were conducted according to the method of Hosmer & Lemeshow (1989) by the logistic procedure of the SPSS package (SPSS for Windows, version 11.5, SPSS Inc, Chicago, Illinois). Basically, this method involves five steps as follows: preliminary screening of all variables for univariate associations; construction of a full model using all the variables found to be significant in the univariate analysis; stepwise removal of non-significant variables from the full model and comparison of the reduced model with the previous model for model fit and confounding; evaluation of plausible two-way interactions among variables and assessment of model fit using Hosmer–Lemeshow statistics. Variables with univariate associations showing $P < 0.25$ were included in the initial model. We continued modeling until all main effects or interaction terms were significant according to the Wald statistic at $P < 0.05$.

RESULTS

No helminthic ova and larvae were found in wet smears. Oocysts were found in the faeces from 21 of 75 ostriches (28%), out of which 10 (40%) cases were in farm 1, 4 (16%) in farm 2 and 7 (28%) in farm 3 (Table 2).

102 oocysts of *Cryptosporidium* in ostriched measured $3.8\text{--}8.6 \times 2.9\text{--}7.6 \mu\text{m}$ (mean $\pm$ SD $4.4 \pm 1.14 \times 3.9 \pm 0.96 \mu\text{m}$) with a shape index (length/width) of 1.0–1.33 (mean $\pm$ SD 1.13 ± 0.13) (Table 3). Oocysts contained four sporozoites that were seen by Nomarski interference (Fig. 1).

Logistic regression analyses indicated no significant effect of ostrich population size on the prevalence of *Cryptosporidium* infection. Table 4 shows the odds ratio of the variable included in the final logistic regression model. Based on the odds ratio, the increase of 1 m² space for each ostrich decreases the prevalence of disease in ostriches by a factor of 0.99.

Table 1. Risk factors assessed for possible effects on the prevalence of *Cryptosporidium* infection in ostriches (n=350)

Risk factor	N classes	Class description (N per class)	Mean $\pm$ SD (range)
Density ^a	Continuous		157.1 $\pm$ 41.7 (100–250)
Population	3	1 (50), 2 (50), 3 (250)	

^a Farm space/ostrich (m²).

Table 2. Data about the prevalence of *Cryptosporidium* infection in three sampled ostrich farms in Shiraz, Iran.

Farm	Density ^a	Population	Faecal samples	Positive faecal samples
1	100	50	25	10 (40%)
2	250	50	25	4 (16%)
3	150	250	25	7 (28%)
Total	-	350	75	21 (28%)

^a Farm space/ostrich (m²)

Table 3. Size and frequency of isolated *Cryptosporidium* sp. oocytes from faecal samples of ostriches in Shiraz, southern Iran

Dimensions (µm)	Shape index	Frequency of isolation, %
3.8 × 2.9	1.31	15
3.8 × 3.8	1.0	49
4.8 × 3.8	1.26	19
5.7 × 4.8	1.18	12
8.6 × 7.6	1.13	5

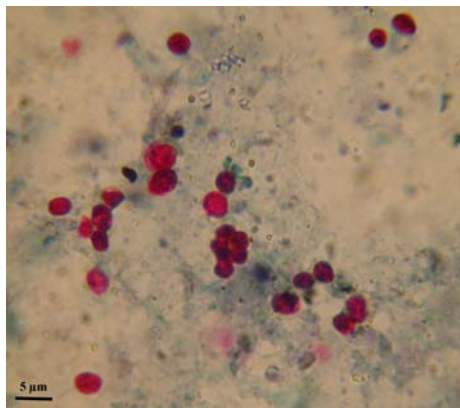


Fig. 1. Different sizes of *Cryptosporidium* sp. oocysts from faecal samples of ostriches in Shiraz, southern Iran; Zielh-Neelsen staining, bar = 5 µm.

Table 4. Odds ratio of the variable included in the final logistic regression model for the prevalence of *Cryptosporidium* infection in ostriches

Variable	Farm space/ostrich (m ²)
Class	Continuous
Odds ratio	0.992
95% confidence interval	0.985–0.998
P-value	0.011

Likelihood-ratio test = 407.9, 1 d.f., $P = 0.02$; Hosmer and Lemeshow goodness-of-fit test = 0.20, 1 d.f., $P = 0.65$ (the model fits).

DISCUSSION

To our knowledge, this is the first report of *Cryptosporidium* in ostriches in Iran. Natural infections of *Cryptosporidia* are widespread among birds and have been associated with disease (Goodwin, 1989; Lindsay & Blagburn, 1990b). The number of distinct species of *Cryptosporidia* that can infect birds is unknown. This is due to uncertainty about the extent of host range of *Cryptosporidia* found in a variety of birds.

The *Cryptosporidia* are important from economic and sanitary points of view. There are some references to *Cryptosporidium* spp. prevalence in ratites; all of them are from ostriches only, although this parasite has been found by Ponce Gordo *et al.* (2002) in rheas as well. In ostrich chicks, it has been indicated as the cause of phallus and cloacal prolapse (Bezuidenhout *et al.*, 1993; Penrith & Burger, 1993; Penrith *et al.*, 1994), enteritis (Huchzermeyer, 1999) and pancreatic necrosis (Jardine & Verwoerd, 1997). Clinical illness was not observed in any of the adult ostriches where cryptosporidial oocysts were isolated. Gajadhar (1994) described the presence of oocysts related to the occurrence of subclinical cryptosporidiosis in adult ostriches. However, as with *C. parvum*, *C. meleagridis*, and *C. baileyi* (Goodwin, 1989; Fayer *et al.*, 1990; Lindsay & Blagburn, 1990b), it is possible that the present *Cryptosporidium* spp. of ostriches causes disease in young ostriches and birds that are immunocompromised or infected with other agents. The occurrence of fatal disease when cryptosporidiosis was associated with secondary bacterial or viral infection has been reported (Current, 1985). It appears that adult ostriches are susceptible to patent cryptosporidial infection, and

may be a source of infection for young and immunocompromised birds.

Stressing conditions leading to immunosuppression or poor husbandry practices related to feed, water or hygiene could appear as predisposing factors to the onset of cloacal prolapse or other pathology related to the *Cryptosporidium* infection observed in this study. Based on the odds ratio, the increase of 1 m² space for each ostrich decreased the prevalence of disease in ostriches by a factor of 0.99, since the improvement in husbandry practices has stopped the mortality and clinical signs, even in the presence of the parasite. Therefore, this implies that high population density (overcrowding) could have been the most important predisposing factor for development of disease in ostrich farms with the group size used in the present study.

The species of *Cryptosporidium* that infect ostriches are still undetermined. Some authors consider it could be a new species, whose oocysts are similar in size to those of *C. meleagridis* (6–8 µm) (De Graaf *et al.*, 1999, Morgan *et al.*, 2001). Besides this species, and according to the size of the oocysts we have found, a second species whose oocysts are of the same size of those of *C. parvum* (3–5 µm) was also involved and its prevalence was higher.

Some oocysts of the Iranian isolate are similar to those of *Cryptosporidium* found in ostriches by Gajadhar (Gajadhar, 1993; 1994) with spherical to subspherical shape and dimensions (mean±SD) 4.4±1.14× 3.9±0.96 µm; shape index (length/width; mean±SD) 1.13±0.13.

Probably, the present ostrich isolate is the same species that caused cloacal prolapse described in other reports (Allwright & Wessels, 1993; Penrith *et al.*, 1994; Jardine & Verwoerd, 1997). The morphological data of the isolate

described by Gajadhar (1994) are related to *C. meleagridis*, which is spherical and measures 5.2×4.6 µm; and has a shape index 1.13 (Lindsay *et al.*, 1989), and not to *C. baileyi* whose oocysts have an ovoid shape and measure 6.0×4.6 µm; shape index 1.31 (Meireles & Figueiredo, 1992) or 6.3×5.2 µm; shape index 1.4 (Current *et al.*, 1986) and that is similar to the morphological data of the Brazilian ostrich isolate, 6×4.8 µm; shape index 1.31 (Santos *et al.*, 2005).

For a definitive classification of this isolate as *C. baileyi* or as a new species infecting ostriches, a comparison of the sequenced fragments would be necessary, followed by phylogenetic analysis. Besides, further studies on its biological and morphological characteristics would also be necessary. Avian genotype II isolates were identical to a novel *Cryptosporidium* genotype recently identified in ostriches (Meireles *et al.*, 2006, Ng *et al.*, 2006).

The adequate identification of these organisms should be achieved after application of biochemical and molecular techniques. A detailed description of the parasite and further taxonomic studies will be reported separately.

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- Paper received 21.07.2008; accepted for publication 10.11.2008

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