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Original article

Endoparasitism of Greek ostriches: First report of *Entamoeba struthionis* and *Balantioides coli*

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ABSTRACT

Ostrich farming is a worldwide practice and an internationally developing industry. Among challenges in livestock production are the parasitic infections. The present study aimed to the investigation of the gastrointestinal parasites biota and prevalence in ostriches raised in different areas of Greece. A total of 141 clinically healthy ostriches originating from four different localities of Greece were coprologically examined for parasites of the gastrointestinal tract. Coprological examination revealed a considerably high rate of infection (65.9%) with protozoa; however, no helminths (trematodes, cestodes and nematodes) were detected. In detail, cysts of *Entamoeba struthionis* have been found in 57.4% of the examined birds. Moreover, 39.0% of sampled ostriches harboured cysts of *Balantioides coli* (syn. *Balantidium coli*), while oocysts of *Cryptosporidium* sp. were detected at a low percentage (2.1%). Partial sequences of the small subunit rRNA (16S rRNA) gene and the ITS region were amplified from pooled *Entamoeba* and *Balantioides* positive samples, respectively, confirming for the first time the presence of *Entamoeba struthionis* and *Balantioides coli* in ostriches in Greece. Some of these parasitoses require attention as they may affect productivity performance of the animals in commercial ostrich farming and possibly pose disease risk for livestock and humans.

1. Introduction

Ostriches (*Struthio camelus*) belong to the order Struthioniformes together with the cassowaries, emus, kiwis, rheas and tinamous. For the last two decades, ostriches have been commercially farmed mainly in hot and dry climates (Gillespie and Schupp, 1998). Ostrich farming is a profitable business due to the various high quality products obtained, such as meat, leather and feathers (Gillespie and Schupp, 1998; Al-Khalifa and Al-Naser, 2014; Bonato et al., 2015). In Greece commercial ostrich farming became popular twenty years ago and many farms spread all over the country (Theodoropoulou et al., 2001). Nowadays the number of farms has dropped significantly because of a bird flu outbreak in the 2000 decade, and the industry has managed to survive on a much smaller scale.

Semi-intensive farming is the most commonly used husbandry method for ostriches and although it provides profit, it constitutes a predisposing factor for parasitism (Black and Glatz, 2011). Ostriches may harbour a variety of parasites, most of which have been reported in the gastrointestinal tract (Sotiraki et al., 2001; Ponce-Gordo et al., 2002; Eslami et al., 2007; Němejc and Lukešová, 2012). Gastrointestinal parasitic infections in ostriches are often subclinical, but in some cases, especially in chicks, the animals may exhibit weakness, anorexia, diarrhoea, anaemia, impaction of the proventriculus and sometimes, even death (Craig and Diamond, 1996; Black and Glatz, 2011). In any case, parasitism impairs the general health status of the flock, which in turn affects productivity causing reduction in growth and poor reproductive outcomes, thus threatening the viability of production (Tully and Shane, 1996; Shanawany and Dingle, 1999; Cooper, 2005).

Interestingly, some of the parasites infecting ostriches (especially, protozoa like *Entamoeba struthionis* and *Balantioides coli* [syn. *Balantidium coli*, Chistyakova et al., 2014]) could infect other wild and domestic animals, as well as humans (Ponce-Gordo et al., 2002; Yoshikawa et al., 2004; Smith and Nichols, 2006; Ponce-Gordo et al., 2008; Ponce-Gordo and Martínez-Díaz, 2010; Ponce-Gordo et al., 2011). In addition, parasitism may have implications for wildlife populations. Ostriches are reared in open-air facilities with free access for other birds, and in some countries, farmed ostriches are reintroduced

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into the wild. Therefore, identification of parasites infecting farmed ostriches helps assess the risk of transmission of their pathogens to endangered and threatened wildlife species (Pedersen et al., 2007; Pedersen and Fenton, 2007; Thompson et al., 2010).

Some previous studies have investigated the occurrence of ostrich gastrointestinal parasitism throughout Europe and reported a variety of parasites (Jansson and Christensson, 2000; Ponce-Gordo et al., 2002; Němejc and Lukešová, 2012). In Greece little research has been done on parasitism of these birds and scarce data exist from only one publication over the last twenty years (Sotiraki et al., 2001). The objective of the present study was to determine the current gastrointestinal parasite biota of ostriches from different farms in Greece and to assess the prevalence of identified species so that proper husbandry practices and control strategies can be applied.

2. Materials and methods

2.1. Study area, ostrich population and faecal sample collection

The four farms that are registered in Greece for ostrich farming were included in the study; two of these farms are located in mainland Greece (one in Northern and the other in Central Greece) while the other two are located in the islands of Rhodes and Crete. In all farms, the bedrock is limestone, which permits water penetration (no moisture retention) and neutralizes acid soil. The water for the animals is supplied from the pipe network of the water supply system except the farm in Rhodes where the water comes from a drilling. Following veterinarian advises, balanced nutrient feed (in terms of trace elements, protein, fiber, energy) are used in all farms; in the case of Northern Greece, oregano is added to the feed as the farmer considered his animals' meat will taste better. Also, in all farms, ostrich chicks are kept in small groups after incubation and hatching, with no contact with other groups of chicks or with adults.

The sampled animals (141 in total) represented half of the size of herds from each farm: 45 ostriches were sampled in Northern Greece, 31 in Central Greece, 40 in Rhodes and 25 in Crete. The majority of ostriches were adult, i.e. > 6 months (87.2%, n = 123 ostriches), while young animals represented 12.8% of the studied population (n = 18 ostriches). Sex of ostriches was almost evenly distributed with 77 (54.6%) male and 64 (45.4%) female birds. None of the examined animals received any antiparasitic treatment at least 3 months prior to sample collection and all of them were clinically healthy.

From each individual ostrich, a faecal sample was collected under supervision, either immediately after spontaneous elimination or fresh from farm ground avoiding contamination with soil debris. Samples were labeled with consecutive numbers, placed individually in plastic containers, stored at 2–6 °C, transferred to the Laboratory of Parasitology and Parasitic Diseases of the School of Veterinary Medicine in Aristotle University of Thessaloniki and processed within 48 h.

2.2. Coprological methods used

For each sample the flotation technique was applied as follows (Faust et al., 1938). Approximately 1 g of faeces was diluted with tap water, passed through a sieve (No. 150) into a centrifuge tube and centrifuged at 200 ×*g* for 3 min. After discard of the supernatant, zinc sulfate solution (33.2% w/v, specific weight 1.3) was added to the sediment, which was thoroughly diluted. Thereafter, zinc sulfate solution was added to just over the top of the tube and a coverslip was placed on the top of the formed meniscus. After centrifugation at 150 ×*g* for 1 min in a swing out rotor centrifuge, the coverslip was removed and placed on a microscope slide. Parasitic elements were identified based on morphological characteristics (Taylor et al., 2007).

Since not all parasitic elements are detectable with the flotation method and in order to increase the sensitivity of coprological examination, the sedimentation method was also performed after minor modifications as follows (Thienpont et al., 1986; Foreyt, 2001). Approximately 1 g of faeces was diluted in 16% hydrogen chloride and passed through a sieve (No. 150) in a centrifuge tube followed by addition of 5 ml diethyl ether. The content of the tube was homogenized by vigorous shaking and centrifuged at 200 ×*g* for 3 min. After centrifugation, all supernatant was discharged. Drops of the sediment were placed on slides, stained with Lugol and examined under the optical microscope at ×100 and ×400 magnification. For detection of *Cryptosporidium* oocysts, faecal smears were prepared and stained with the Ziehl-Neelsen method (Henriksen and Pohlenz, 1981).

In those cases, in which protozoan structures were detected, their identification to the species level was made after genetic analysis. For this purpose, for each parasite, the content of the positive temporal slides in which a minimum of 15 cysts were observed, were washed with 70% alcohol; the eluates from slides made from samples from the same farms were pooled and centrifuged at 200 ×*g* for 3 min, and the sediments were transferred to 1.5 ml Eppedorf tubes and stored at −20 °C until use. Thereafter, the sediments were sent to the Department of Microbiology and Parasitology, Faculty of Pharmacy, Complutense University, Madrid, Spain for further molecular analyses. *Cryptosporidium* oocysts were not further investigated due to the very low number of oocysts observed in the stained slides (see Results).

2.3. Parasite DNA isolation

Eppendorf tubes containing the sediments were centrifuged at 300 ×*g* for 5 min in a tabletop centrifuge; the 70% alcohol was discarded and the sediments were washed in sterile phosphate buffered saline (PBS, pH 7) and centrifuged at 300 ×*g* for 5 min in a tabletop centrifuge (3 times), followed by an overnight incubation at 37 °C in a thermoblock in order to evaporate the remaining alcohol. For *Entamoeba*, total DNA was obtained by using the Speedtools Tissue DNA Extraction Kit (Biotools), following the manufacturer's instructions. For the *Balantioides* cysts, a small amount of the concentrates was transferred to a watch glass containing 1 ml of PBS; individual cysts were collected with the aid of a modified Pasteur pipette under an Olympus stereoscopic microscope and transferred (in ~ 10 µl) to 1.5 ml Eppendorf tubes. Then the Speedtools Tissue DNA Extraction Kit was used to obtain the DNA.

2.4. PCR, sequencing and analyses

For the identification of the *Entamoeba* species, a fragment of about 770 bp of the small subunit rRNA (SSU rRNA) gene was PCR-amplified with forward primer EG12D (5′-CACGGGAAACTTACCAAGACC) and reverse primer EF2 (5-TGATCCTTCCGCAGGTTCAC). For *Balantioides*, the ITS1-5.8S rRNA-ITS fragment was PCR-amplified with forward primer B5D (5′-GCTCCTACCGATACCGGGT) and reverse primer B5RC (5′-GCGGGTCATCTTACTTGATTTC) (Ponce-Gordo et al., 2011). In all cases, PCR reactions were made by using the illustra PureTaq Ready-To-Go kit (GE Healthcare) in a total volume of 25 µl, including two microliters of each of 5 µM primer solutions and 10 µl of DNA template solutions. The reactions were performed in a Mastercycler Gradient 5331 thermocycler (Eppendorf) programmed as follows: 10 min at 94 °C, 30 cycles of 1 min at 94 °C, 1 min at 60 °C and 1 min at 72 °C, and a final extension of 5 min at 72 °C. The existence of PCR products, their purification and sequencing (in both senses, with the same primers used for PCR amplification) was as indicated in Ponce-Gordo et al. (2008). Chromatograms were processed with the ChromasPro software, version 1.5 (Technelyssium Pty Ltd). Sequences were compared with those available in the GenBank/EMBL/DDBJ databases by using the blastn algorithm available in the National Center for Biotechnology Information (NCBI) website (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.5. Data handling - statistical analyses

For each farm, data about their characteristics (soil type, water supply and feeding) and on the sampled animals (number of ostriches examined, their sex and age), as well as the results obtained for each sample, were recorded and processed in IBM SPSS Statistics, ver. 24 (IBM Corporation). The Pearson's chi-square test was used to determine if there were significant differences in parasites' prevalence estimates for any of the variables considered. A probability value $p \leq .05$ was considered significant.

3. Results

In the present study only *Entamoeba* and *Balantioides* cysts, and *Cryptosporidium* oocysts, were detected in the samples; no helminth eggs or larvae have been found. *Entamoeba* was the most prevalent parasite in the studied population (57.4%, n = 81 ostriches), followed by *Balantioides* (39.0%, n = 55 ostriches) and *Cryptosporidium* (2.1%, n = 3 ostriches). Infected animals were present in all four farms. Overall, 65.9% (93/141) of the studied ostriches were found infected with at least one parasitic species. Among examined animals, 42 (29.8%) and 1 (0.7%) ostriches had mixed infections with two and three different parasite genera, respectively.

Clear and statistically significant differences (except for *Cryptosporidium*) were found between chicks and adult birds, as no parasites were detected in chicks (Table 1); with *Cryptosporidium*, the non-existence of statistically significant differences (p = .613) is due to the very low percentage of positive adult samples (2.13% in average, ranging 0.00–4.44%). The infection rate was similar in male and female ostriches within each farm (Table 2). No significant differences were found among farms for the different parasites found except for the prevalence of *E. struthionis* in Crete, which was clearly significant (p = .004) if all animals (chicks and adults) were considered together; however, if only the results from adult birds are compared, the p-value is significant but borderline (p = .036) (Table 3). Regarding other farm characteristics (soil type, water supply and feeding) there were no significant differences in parasites' prevalence estimates for any of the variables considered.

Entamoeba cysts were usually presented in moderate (2 to 10 cysts per field) to large (> 10 cysts per field) quantities in each positive sample. Morphological and morphometric data from these cysts were recorded from Lugol-stained slides. Cysts were almost always spherical rather than ovoid. All cysts had a thin refractive wall and measured 14–17 μm in diameter. An eccentric vesicular-type nucleus was observed in the majority of cysts. Bi-nucleated cysts were detected with a very low frequency (< 5% of the cysts) (Fig. 1). PCR amplification of the SSU rRNA represented a fragment of about 770 bp, consisted with the expected size, without nonspecific bands (Fig. 2). The SSU rDNA sequences from all *Entamoeba* isolates were identical (Genbank Accession number MN192186) and corresponded to *E. struthionis*, although there are 7 base differences respect to the published sequence (AJ566411), all of them located together in a highly variable fragment located near the 3′ side of the sequence.

Balantioides cysts were observed in moderate (2 to 3 cysts per field) quantities in the positive samples. They were spherical (40 to 65 μm) and with a macronucleus comparably large, generally ovoid (occasionally irregular) and usually eccentric (Fig. 3). PCR amplification of the ITS sequence represented a fragment of 550 bp, consisted with the expected size, without nonspecific bands (Fig. 4). The ITS sequences obtained from these isolates corresponded in all cases to *B. coli*, genetic variant A (Genbank Accession number MN194595).

Cryptosporidium oocysts were detected in only 3 samples, and only 1–2 oocysts were observed in the whole stained smears. Oocysts were spherical to subspherical and measured 4–5 μm in diameter (Fig. 5).

4. Discussion

In the present study three protozoan parasites, i.e. *Entamoeba* and *Balantioides* cysts, and *Cryptosporidium* oocysts were detected in ostriches in Greece. Ostrich farming has been drastically reduced in the country from about 30 farms in the beginning of the 2000s decade (Theodoropoulou et al., 2001) to only 4 farms nowadays, and our study demonstrated the actual situation of intestinal protozoan infections in the birds of the existing farms.

The only *Entamoeba* species described so far in ostriches is *E. struthionis* (Ponce-Gordo et al., 2004), which forms mature one-nucleated cysts. The morphology and the genetic analysis of the Greek samples analyzed in this study allow their identification as this species. The SSU rDNA fragment amplified corresponds to ~600 bases in the 3′ end, including several highly variable (expansion segment, ES) regions, one of which (that corresponding to the ES10) has been proposed as a key for species identification in *Entamoeba* (Alfonso et al., 2012). According to this, the *Entamoeba* species found in the Greek ostriches undoubtedly correspond to *E. struthionis*. The base differences between the present sequences and that of *E. struthionis* AJ566411 (the only published one including the 3′ end of the gene) are located in other variable region (ES12); these differences have been also found in other isolates from ostriches (Ponce-Gordo, unpublished) and they could indicate the existence of sequence variants of this species.

Entamoeba struthionis were the most common protozoa in ostriches in the present study with a high prevalence (overall, 57.45%), which is in accordance with previously recorded findings in Greece (Sotiraki

Table 1
Parasitic infection was statistically compared to age of ostriches in each farm.

	Age	*Entamoeba struthionis*			*Balantioides coli*			*Cryptosporidium* sp.		
		Positive	%	Pearson X^2	Positive	%	Pearson X^2	Positive	%	Pearson X^2
Rhodes	Chick (n = 8)	0	0.00	0.000	0	0.00	0.014	0	0.00	0.613
	Adult (n = 32)	23	71.88		15	46.88		1	3.13	
	Total (n = 40)	23	57.50		15	37.50		1	2.50	
Central Greece	Chick (n = 5)	0	0.00	0.012	0	0.00	0.070	0	0.00	–
	Adult (*n* = 26)	16	61.54		11	42.31		0	0.00	
	Total (n = 31)	16	51.61		11	35.48		0	0.00	
Northern Greece	Chick (n = 0)			–	–	–	–	–	–	–
	Adult (n = 45)	34	75.56		18	40.00		2	4.44	
	Total (n = 45)	34	75.56		18	40.00		2	4.44	
Crete	Chick (n = 5)	0	0.00	0.086	0	0.00	0.027	0	0.00	–
	Adult (n = 20)	8	40.00		11	55.00		0	0.00	
	Total (*N* = 25)	8	32.00		11	44.00		0	0.00	
Total	Chick (n = 18)	0	0.00	0.000	0	0.00	0.000	0	0.00	0.503
	Adult (n = 123)	81	65.85		55	44.72		3	2.44	
	Total (n = 141)	81	57.45		55	39.01		3	2.13	

Table 2
Parasitic infection was statistically compared to sex of ostriches in each farm.

	Sex	*Entamoeba struthionis*			*Balantioides coli*			*Cryptosporidium* sp.		
		Positive	%	Pearson X^2	Positive	%	Pearson X^2	Positive	%	Pearson X^2
Rhodes	Male (n = 22)	12	54.55	0.676	8	36.36	0.870	1	4.55	0.360
	Female (n = 18)	11	61.11		7	38.89		0	0.00	
	Total (n = 40)	23	57.50		15	37.50		1	2.50	
Central Greece	Male (n = 17)	9	52.94	0.870	5	29.41	0.436	0	0.00	–
	Female (n = 14)	7	50.00		6	42.86		0	0.00	
	Total (n = 31)	16	51.61		11	35.48		0	0.00	
Northern Greece	Male (n = 25)	20	80.00	0.438	11	44.00	0.540	1	4.00	0.872
	Female (n = 20)	14	70.00		7	35.00		1	5.00	
	Total (n = 45)	34	75.56		18	40.00		2	4.44	
Crete	Male (n = 13)	4	30.77	0.891	6	46.15	0.821	0	0.00	–
	Female (n = 12)	4	33.33		5	41.67		0	0.00	
	Total (n = 25)	8	32.00		11	44.00		0	0.00	
Total	Male (n = 77)	45	58.44	0.793	30	38.96	0.990	2	2.60	0.672
	Female (n = 64)	36	56.25		25	39.06		1	1.56	
	Total (n = 141)	81	57.45		55	39.01		3	2.13	

et al., 2001). Similarly, these species have been observed in nearly 90% of farmed ostriches in Spain (Martínez Díaz et al., 2000). *Entamoeba* species dissemination patterns can be attributed to their low infective dose in combination with a direct life cycle and a short prepatent period (Berrilli et al., 2011; Morf and Singh, 2012). Additionally, the lack of adequate knowledge on control strategies among ratite farmers enhances the rapid dispersion of these parasites (Martínez Díaz et al., 2000). Up to date, there is no evidence that these organisms cause clinical symptoms, as damage of the intestinal tract or tissue invasion have not been reported in ostriches (Craig and Diamond, 1996). Interestingly, the presence of such commensal protozoa as *Entamoeba* sp. in humans has been shown to interact with gut bacterial microbiota (Morton et al., 2015). Since composition and diversity of gut microbiota may have consequences on metabolic and immune conditions, any factor influencing them is of major health importance (Cho and Blaser, 2012). It should also be noted that this species has been also identified in humans (Ponce-Gordo et al., 2004; Ponce-Gordo and Martínez-Díaz, 2010) and thus it might pose a health risk especially for the personnel at the farms.

Another protozoon detected in relatively high percentage (39.0%) was *Balantioides*. Based on morphological features (large macronucleus with a deep depression on one side) and host range, the species *Balantioides struthionis* was proposed as ostrich specific (Hegner, 1934). However, the validity of the fore mentioned criteria has been questioned since protozoa identification requires comparison of genes, which are related taxonomically (Fried et al., 2002; Stoeck et al., 2003). Following revision of the morphological key features of the ciliates from ostriches and comparison of the DNA sequences corresponding to the ITS1 and ITS2 regions, *B. struthionis* has been considered a synonym of *B. coli* (Ponce-Gordo et al., 2008; Ponce-Gordo et al., 2011). Also, in these studies, two main sequence variants (named as A and B) have been identified. The genetic analysis of the Greek isolates has shown that they correspond to *B. coli*¸ sequence variant A.

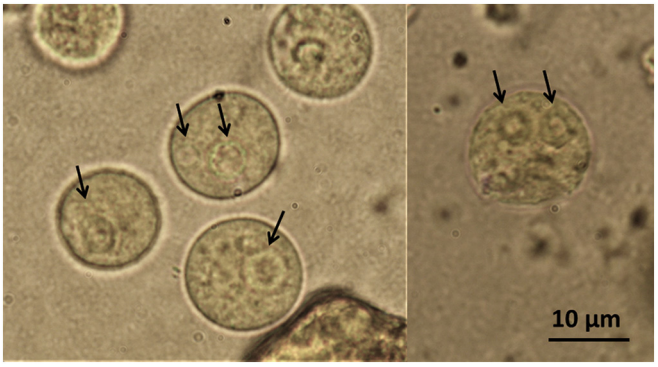

Fig. 1. Single nucleus (one arrow) and two nuclei (two arrows) cysts of *Entamoeba struthionis* found in the faeces of ostriches in Greece (scale 10 µm).

The prevalence of *B. coli* observed in this study was lower than the reported prevalence in other surveys carried out in Greece and Spain, which was 74.1% (Sotiraki et al., 2001) and 80% (Ponce-Gordo et al., 2002), respectively. It has been documented from experimental infections (Schumaker, 1930) and in vitro cultures (Barbosa et al., 2015) that carbohydrates are necessary for the onset of *B. coli* infection. Therefore, this discrepancy might be attributed to a different diet with a ration that includes a lower proportion of vegetable fiber, which is transformed into small carbohydrates by the intestinal flora (Aganga et al., 2003). *Balantioides coli* is commonly identified in the caeca and

Table 3
Parasitic infection was statistically compared to different ostrich farms.

	Entamoeba struthionis			*Balantioides coli*			*Cryptosporidium* sp.		
	Positive	%	Pearson X^2	Positive	%	Pearson X^2	Positive	%	Pearson X^2
Chicks and adult birds									
Rhodes (n = 40)	23	57.50	0.004	15	37.50	0.917	1	2.00	0.636
Central Greece (n = 31)	16	51.61		11	35.48		0	0.00	
Northern Greece (n = 45)	34	75.56		18	40.00		2	4.44	
Crete (n = 25)	8	32.00		11	44.00		0	0.00	
Only adult birds									
Rhodes (n = 32)	23	71.88	0.036	15	46.88	0.717	1	3.13	0.649
Central Greece (n = 26)	16	61.54		11	42.31		0	0.00	
Northern Greece (n = 45)	34	75.56		18	40.00		2	4.44	
Crete (n = 20)	8	40.00		11	55.00		0	0.00	

Fig. 2. PCR products analyzed by electrophoresis on agarose gel. The band of 770 bp represents the amplified PCR product of the SSU rRNA sequence of *Entamoeba struthionis*.

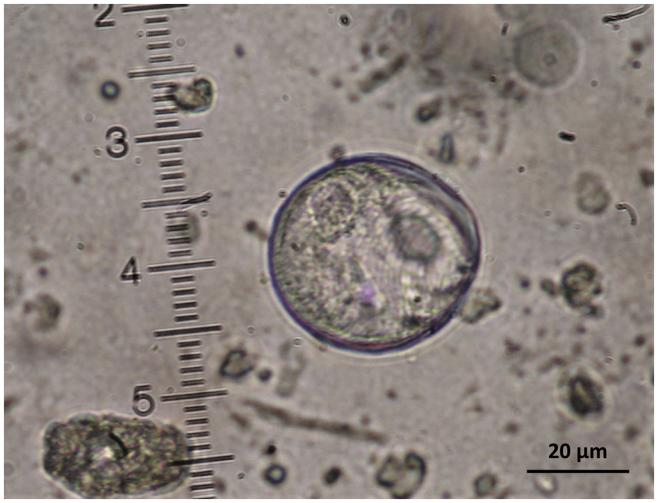

Fig. 3. *Balantioides coli* cysts detected in ostriches in Greece (scale 20 µm).

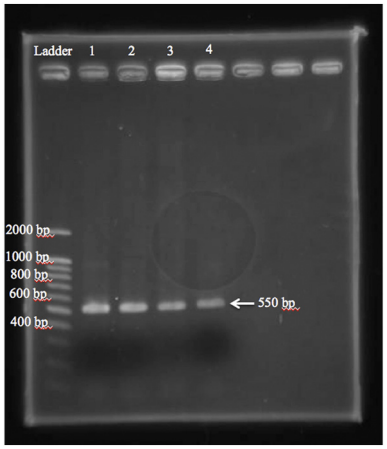

Fig. 4. PCR products analyzed by electrophoresis on agarose gel. The band of 550 bp represents the amplified PCR product of the ITS sequence of *Balantioides coli*.

proximal large intestine of a wide range of mammals (including bovines, swine, humans, non-human primates) and also in birds, ostriches and rheas (Ponce-Gordo and Jirků-Pomajbíková, 2017). This protozoon is considered as non- or low-pathogenic to the host (Huchzermeyer,

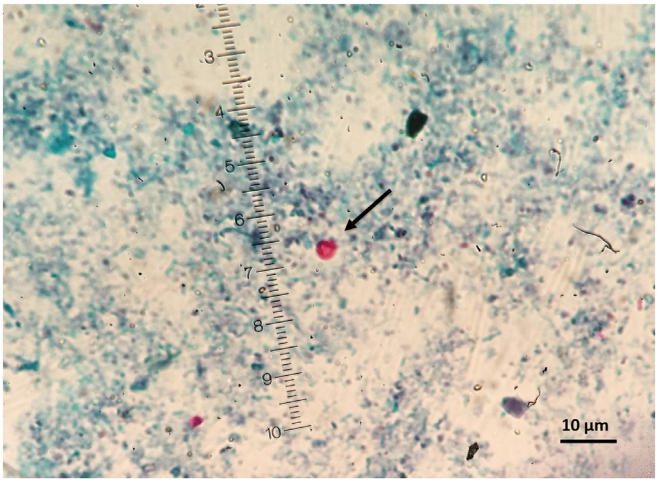

Fig. 5. *Cryptosporidium* sp. oocyst (arrow) observed in ostriches in Greece (scale 10 µm).

1999a; Ponce-Gordo et al., 2002; Ponce-Gordo and Jirků-Pomajbíková, 2017), although some surveys correlate it with intestinal inflammation (Huchzermeyer, 1999b; Craig and Diamond, 1996), and consequently it also might pose a risk for the personnel at the farms.

Finally, oocysts of *Cryptosporidium* sp. were found at a low prevalence (2.1%) in the present study. While in Greece, *Cryptosporidium* oocysts have been also previously identified only in 0.6% of sampled ostriches (Sotiraki et al., 2001), results from a European survey indicated that these protozoa are widely disseminated in Spain and Portugal and also present in the Netherlands, Belgium and France (Ponce-Gordo et al., 2002). Nowadays at least two unidentified species of *Cryptosporidium* have been reported in ostriches; one with oocysts similar in size to those of *Cryptosporidium meleagridis* (6–8 µm) (De Graaf et al., 1999; Morgan et al., 2001; Ponce-Gordo et al., 2002) and a second one, whose oocysts are approximately the size of those of *Cryptosporidium parvum* (3–5 µm) (Ponce-Gordo et al., 2002). The oocysts detected in the present study measured 4–5 µm in diameter and may belong to *C. parvum*. However, since the definitive characterization of positive isolates requires genetic analysis (Morgan et al., 2001), which was not feasible in this study, the species of the parasite was not determined. Protozoa of this genus complete their life cycle on the surface of epithelial cells of the digestive and respiratory track of birds and infect the bursa, the rectum and the pancreas of ostriches (Ponce-Gordo et al., 2002; Santos et al., 2005). Clinical signs, such as phallus and cloacal prolapse (Bezuidenhout et al., 1993; Penrith et al., 1994), pancreatitis (Jardine and Verwoerd, 1997) and enteritis (Huchzermeyer, 1999a), manifest mainly in ostrich chicks.

Overall, a high prevalence (65.9%) of gastrointestinal parasitism was recorded in reared ostriches in Greece. In the same frame, previous surveys conducted in Sweden (Jansson and Christensson, 2000) and Greece (Sotiraki et al., 2001) have demonstrated that 62% and 78.8% of farmed ostriches, respectively, were infected with parasites. Although the presence of metazoan parasites (strongylids, most likely *Libyostrongylus*) has been confirmed in the aforementioned survey in Greece (Sotiraki et al., 2001), only protozoa were detected in the present study. The follow-up of the owners on their animals and the common use of anthelmintics (mainly imidazothiazoles and ivermectin) under veterinary advice by the farmers during the 2000s decade (Theodoropoulou et al., 2001) would have led to the nematodes' eradication among farmed ostriches in Greece, while other mostly non-pathogenic, direct life cycle parasites have persisted. Gastrointestinal protozoa, such as amoebae and ciliates are frequently observed parasites in ostriches in Europe as demonstrated in previous surveys and confirmed by the present results (Sotiraki et al., 2001; Ponce-Gordo et al., 2002).

It should be emphasized that these parasites are rarely pathogenic

and commonly recorded in clinically healthy ostriches (Black and Glatz, 2011), as is the case of our study. However, when certain circumstances, such as environmental stress, immunosuppression or concurrent infections with other pathogens occur in infected birds, these protozoa may provoke disease (Craig and Diamond, 1996; Cooper, 2005). Noteworthy, it has been demonstrated that the protozoa detected in this survey downgrade gut health and parasitism has a negative impact on feed conversion and rate of growth while it also results in higher rates of condemned carcasses (Urquhart et al., 1990; Tan, 2004; Smith and Nichols, 2006). Taken into account that ostrich farming is a comparatively new field of livestock production and thus other control strategies, such as vaccination, are not yet developed, improving gut health is crucial for maintaining high standards of management (Waite and Taylor, 2015).

The protozoa reported in this study have a direct life cycle, where transmission occurs through the faecal–oral route mainly by consumption of contaminated water and food. Protozoan infections were absent in ostrich chicks. This may be attributed to certain practices of commercial ostrich farming, like the artificial incubation of eggs and the raising of chicks in segregated flocks, which result in interruption of the life cycle of these parasites (Shanawany and Dingle, 1999). Another crucial aspect of these protozoa that requires attention is their zoonotic implication due to the risk of infection they may pose to professional workers, e.g., veterinarians and farm workers (Tully and Shane, 1996; Ponce-Gordo et al., 2002). Some authors have highlighted the involvement of ostriches in the epidemiology cycle of *Entamoeba* or *Balantioides* and have establish that these protozoa are also pathogenic for other hosts (birds and mammals) and even man (Ponce-Gordo et al., 2004; Yoshikawa et al., 2004; Smith and Nichols, 2006; Marietto-Goncalves et al., 2008). Whether *Cryptosporidium* species affecting ostriches are ratite-specific remains to be resolved by credible molecular tools.

In conclusion, a considerably high prevalence of protozoa was recorded in ostriches in Greece and *E. struthionis* and *B. coli* were identified for the first time. Under favourable conditions these parasites may cause gastrointestinal disorders and have an impact on the viability of a commercial ostrich farm. Our findings underline the need for further analyses to assess the economic impact of protozoa on ratite production as well as the subsequent risk of infection for other animals and humans. In addition, increasing awareness on parasitism among farmers constitutes a first step towards a prosperous ostrich farm management.

Ethics statement

The study was conducted in compliance with the national animal welfare regulations. The applied diagnostic procedures are not within the context of relevant EU legislation for animal experimentations (Directive 86/609/EC) and may be performed in order to diagnose diseases and improve animal welfare. No suffering was caused during sample collection and consent was obtained from the owners of each farm.

Declaration of Competing Interest

The authors declare no competing interests.

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