Occurrence of parasitic protozoa in wild waterfowl in southern coastal Caspian sea lagoons

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Abstract:

BACKGROUND: Protozoa are common in poultry and other birds and can cause mild to severe disease. **OBJECTIVES:** This study was concerned with the prevalence of parasitic protozoa in wild waterfowl in Caspian sea lagoons in the North of Iran. **METHODS:** A total of 293 waterfowl belonging to various genera including *Tadorna tadorna* (common shelduck), *Aythya fuligula* (Tufted duck), *Aythya ferina* (Common puchard), *Spatula clypeata* (Shoveler), *Anser anser* (greylag goose), *Cygnus Cygnus* (Whooper Swan), *Anas strepera* (Gadwall), *Anas Penelope* (Eurasian Widgeon), *Anas crecca* (common teal), and *Anas platyrhynchos* (mallard) were sampled and tested for intestinal, tracheal, and blood protozoa between winter 2010 and spring 2011. **RESULTS:** The results showed that 184 birds of 293 (62.8%) harbored protozoan parasites. The highest prevalence of protozoan contamination belonged to *Giardia* spp (24.2%) and the lowest belonged to *Haemoproteus* spp. (6.1%). *Thricomunas gallinea*, *Plasmodium* spp., and *Cryptosporidium* spp. were found in 7.2%, 8.2%, and 17% of waterfowl, respectively. Moreover, statistical analyses showed that the highest rate of giardiosis was in female *Aythya fuligula* (43.75%). However, tricomuniasis belonged to *Anas crecca* (30.5%). On the other hand, female *Cygnus cygnus* had the highest rate of cryptosporidiosis (44.7%), and more infection to *Haemoproteus* and *Plasmodium* spp. were observed in *Anas platyrhynchos*, concurrently. **CONCLUSIONS:** Migration of various species of waterfowl toward the northern parts of Iran has an important impact on parasitic diseases in birds and human in these regions.

Introduction

Waterfowl are hosts to a wide variety of internal and external parasites, such as protozoa, that infect many of vertebrate hosts, including mammals and birds (Bennett et al., 1977). Most of these parasites are common pathogenic species in humans. Protozoa are common parasite in a wide variety of birds and can cause mild to severe disease. Some enteric protozoa, such as *Giardia* and *Cryptosporidium*, are pathogenic and have been associated with drinking water related outbreaks (Current et al., 1986; O'Donoghue, 1995; Hsu et al., 1999). They may be found in water following direct or indirect contamination by the feces of humans or other animals such as waterfowl. Coccidian are found in birds, also *Histomonas* species cause a disorder of the ceca and liver in many birds (Silvanose et al., 1998), Trichomonas species affect the upper gastrointestinal tract of turkeys and

chickens (Cooper and Petty, 1988). *Haemoproteus* spp. is common among many bird species, and causing severe myositis in avian hosts (Atkinson, 2009; Zabransky et al., 2008).

There are nearly 520 species of birds in Iran and Passeriformes order includes a big population of birds in this fauna (Mansoori, 2008). These birds are all in close contact with human residential areas as well as native and industrial poultry and other domestic fowls; as a result, there is a potential risk to contaminant other birds and human (Halajian et al., 2011).

Some of waterfowl nest in Northern provinces, while the rest are migratory birds that spend the winter in Iran or flyover the country during migration. Mazandaran and Gilan are two of the 31 provinces of Iran, along the Caspian sea. Population estimates of waterfowl, waders, and water birds in these provinces have been reported about 1.6 million in Gilan and 1.2 in Mazandaran. However, within the last five years, some significant changes in population and diversity have taken place (Barati & Khalilipoor, 2006).

Because of a lack of confident information and very few publications concerning the protozoan infection of waterfowl such as duck, goose, and swan in lagoons of Mazandaran and Gilan Provinces, northern Iran, this study was conducted to evaluate parasitic protozoa in wild waterfowl in Caspian sea lagoons.

Materials and Methods

Sample collection: A total of 293 waterfowl including twenty *Tadorna tadorna*, sixteen Aythya fuligula, thirteen *Aythya ferina*, twenty-three *Spatula clypeata*, seventeen *Anser anser*, forty-four *Cygnus Cygnus*, thirty-four *Anas strepera*, twenty-seven *Anas Penelope*, thirty-six *Anas crecca*, and sixty-three *Anas platyrhynchos* were live trapped or killed with shotguns from 4 localities from 2 provinces in Southern coastal Caspian sea lagoons between winter 2010 and spring 2011.

Each bird was sexed by cloacal examination in the field, and it was confirmed by gonad examination. Then, intestinal, tracheal, and blood protozoa were diagnosed in the laboratory.

After taking blood samples, the intestines from below the pancreas to above the anus and sections of the upper respiratory tract were removed from the abdominal cavity of each bird. Each sample was placed in an individual container and labeled.

Intestinal content examination: Immediately after the arrival of a sample in the lab, 1cm was cut from each end of the intestine with a sterile scalpel to eliminate any cross-contamination during processing. The fecal material was then forced into a sterile 50mL centrifuge tube containing 10 mL of a 2.5% (wt/vol) K2Cr2O7 to maintain the (oo) cysts during storage. Fecal samples were then kept at 4°C less than 4 weeks before processing (Kuhn, 2002).

Fecal specimens were tested for *Giardia* spp. by examining trichrome-stained direct smears of fecal pellets (Spaulding et al., 1983). Slides were screened at × 400 magnifications, and cysts of *Giardia* spp. were confirmed at × 1,000 magnification. Internal characteristics that were used to identify the cysts were included two to four nuclei, median bodies, and axonemes.

The modified zeilnelson staining technique was used to identify *Cryptosporidium*, the oocysts appear as pink to red, spherical to ovoid, bodies on a green background. According to Henriksen and Pohlenz's (1981) instruction, fecal and tracheal smears were prepared on a microscope slide, air dried and fixed with methanol for 5 min. Fixed smears were stained with dilute carbol fuchsin (1:10) for 3 to 5 min and washed with tap water. Smears were decolorized using acid alcohol, then counterstained with 0.5% Malachite Green solution for 1 min. Smear slides were dried in air and examined under the microscope at 400 × magnification.

Intestinal contents of samples were tested by saline wet mount preparation to survey the presence of intestinal protozoa such as trichomonas (Silvanose et al., 1998).

Tracheal examination: Swabs were taken from tracheal mucosa, the buccal cavity and pharynx, were spread onto cover slips that were fixed in schadin and stained with trichorom for examination of tracheal Trichomonas contamination.

As described previously, tracheal smears were examined based on Henriksen and Pohlenz' (1981) instruction for *Cryptosporidium* spp.

Blood examination: Blood samples were collected from the brachial vein. Then, they were placed in EDTA for hematological investigations.

Blood smears were fixed with 90% methanol and stained with Giemsa. The blood smear was examined at least for 20 min, which included examination of the periphery of the smear for diagnose of large hematozoans such as *Plasmodium*, *Haemoproteus* and *Leucocytozoon*. Medium (400×); high (1000×) magnification were used to scan further until at least 50,000 red blood cells were viewed. Quantification of parasite intensity followed recommendations by Godfrey et al. (1987).

Statistical analysis: Data analysis of the relationship between prevalence rates and different areas were evaluated by the SPSS and Chi-square test. Statistical significance was defined as $p<0.05$.

Results

In this study, out of the 293 examined waterfowl, 184 birds (62.8%) harbored protozoan parasites. Result comparison showed that the highest prevalence of protozoan contamination belonged to *Giardia* lamblia (24.2%), also giardiosis was observed in all examined species; however, the infection rate was different between 43.75% in *Aythya fuligula* and 8.3% in *Anser anser* (Figure 1).

Thricomunas gallinea was found in 7.2% of samples, the highest rate of tricomuniasis belonged to *Anas crecca* (30.5%). Intestinal form of tricomuniasis was not found in samples, however, tracheal form was found only in *Anas strepera* and *Anas crecca* without significant difference between males and females.

The rate of contamination of the examined waterfowl to intestinal *Cryptosporidium* spp. oocyst was 17%, and tracheal form was not observed in this study. *Cygnus cygnus* had the highest rate of contamination and *Spatula clypeata* was in the second place, meanwhile *Cryptosporidium* was not found in *Tadorna tadorna*, *Aythya ferina* and *Anser anser* (Figure 2).

Plasmodium spp. and *Haemoproteus* spp were observed in blood samples of the studied waterfowl. The total rate of contamination to *Plasmodium* spp. (8.2%) was higher than *Haemoproteus* spp. (6.1%), but the prevalence rate was not different significantly ($p>0.05$). *Haemoproteus* spp. was not found In *Spatula clypeata* and *Tadorna tadorna* species (Figure 3), while *Aythya ferina* and *Spatula clypeata* samples were free from *Plasmodium* spp. contamination (Figure 4).

The highest rate of infection to *Haemoproteus* and *Plasmodium* spp. was observed in *Anas platyrhynchos* concurrently. The prevalence of *Haemoproteus* was differente in male (10.6%) and female (8.2%) ($p<0.05$), but there was not any significantly difference between male (13.6%) and female in *Plasmodium* infection (14.7%), ($p>0.05$).

Leucocytozoon spp. and microfilariae were not observed in examined blood samples.

Discussion

Waterfowl can act as a main source of different types of parasites; they can pick up infection from their habitat, carry and spread them in the environment, including drinking water supplies and also domestic animals (Graczyk et al., 1998). Moreover, it is estimated that 80 to 96% of surface waters in the United States are contaminated with *Cryptosporidium* and *Giardia* (Hansen, 1991).

The contributions of *Giardia* cysts and *Cryptosporidium* oocysts from avian species to the concentrations of cysts and oocysts in water samples are largely unknown, as are the extent of transmission of bird-vectored organisms to mammalian hosts and the importance of these parasites in avian species (Erlandsen, 1990).

Migration of various species of waterfowl toward the northern parts of Iran has important impact on parasitic diseases in birds and human in Northern part of Iran.

Giardia spp. commonly was reported from various birds including budgerigars (*Melopsittacus undulatus*), cockatiels (*Nymphicus hollandicus*), love birds (*Agapornis* spp.), grey-cheeked parakeets (*Brotogeris pyrrhopterus*) and other psittacines (Greiner & Ritchie, 1994). *Giardia* cysts are commonly found in sewage and surface waters and occasionally in drinking water. In Canada, a cross-sectional survey in 72 municipalities performed between 1991 and 1995, Wallis et al. (1996) found that 72.6%, 21% and 18.2% of raw sewage, raw water, and treated water samples, respectively, contained *Giardia* cysts. In a similar study, fecal samples were taken from wild ducks on the lower Rio Grande River around Las Cruces, from 2000 to 2001.

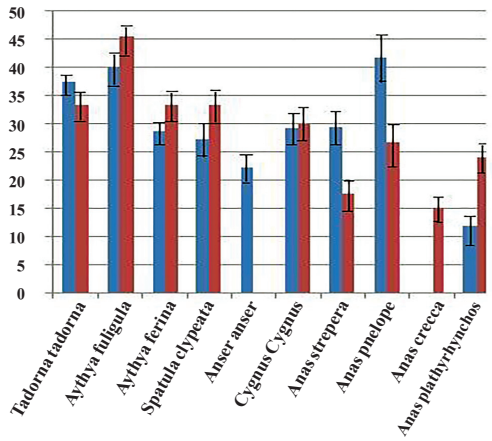

Figure 1. Prevalence rate of waterfowl with *Giardia* spp contamination. Male Female

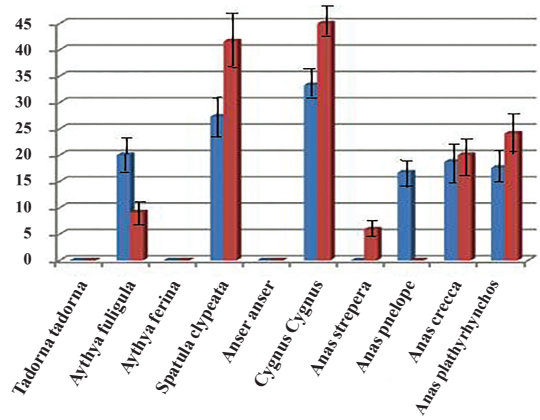

Figure 2. Prevalence rate of waterfowl with intestinal *Cryptosporidium* spp. contamination. Male Female

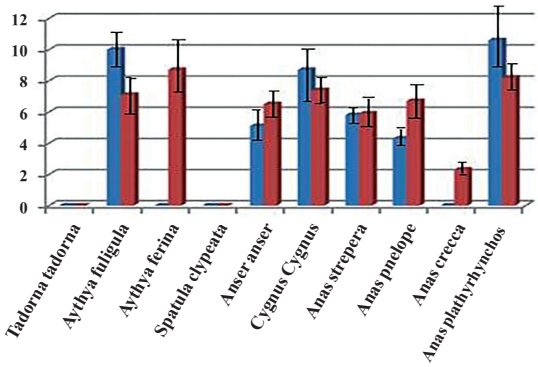

Figure 3. Prevalence rate of waterfowl with *Haemoproteus* spp. contamination. Male Female

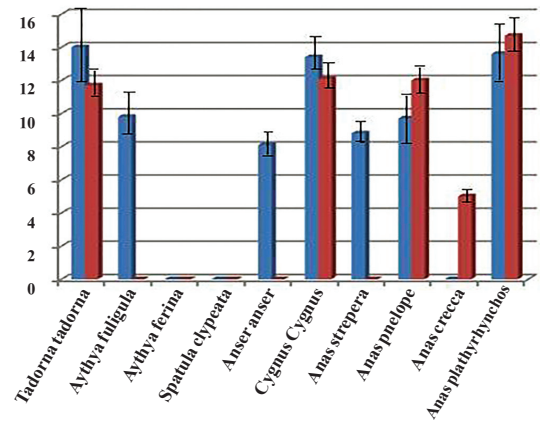

Figure 4. Prevalence rate of waterfowl with *Plasmodium* spp. contamination. Male Female

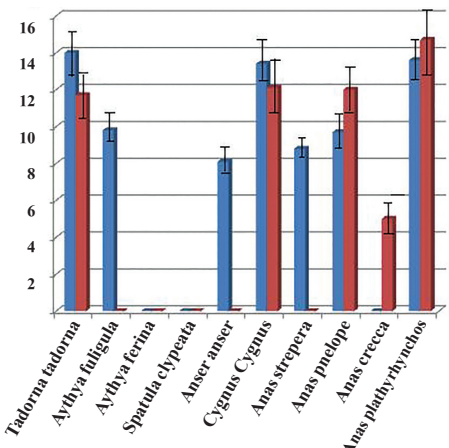

Figure 5. Prevalence rate of waterfowl with *Plasmodium* spp. Contamination. Male Female

The results of this study indicated that 49% of the ducks were carriers of *Cryptosporidium*, also 28% of the ducks were positive for *Giardia* (Kuhn et al., 2002). In our study, prevalence of giardiosis was 24.2%, which is very close to the result of Kuhn et al.'s (2002). In the present study, the infection rate of giardiosis in females was 25.3% and in male ducks was 23.1%. There was no significant difference between giardiosis rate in male and female ducks in most species ($p<0.05$), excluding *Anser anser* for which the infection rate in males was higher than in females, and *Anas crecca* for which the infection rate in females was higher than in males ($p>0.05$).

Trichomonas gallinarum has been documented from the lower digestive tract and caeca of chickens and other gallinaceous birds (McDougald, 1997). Silvanose (1998) studied captive houbara bustards, 49 (43%) were found positive to protozoa including 34 (29.8%) *Trichomonas gallinarum*; 12 (10.5%) *Chilomastix gallinarum*; one (0.9%) *Lophomonas* spp.; one (0.9%) *Giardia* spp., all the birds infected

with *T. gallinarum* and *C. gallinarum* appeared clinically normal. In the current study, 21(7.2%) of the examined waterfowl were infected; the rate of infection was higher than houbara bustards study. In addition, *Anas strepera* and *Anas crecca* were the only species that *Trichomonas gallinarum* were found in them. The prevalence rate of *Thricomunas gallinea* in waterfowl trachea was 7.2%, which was considerably less than that reported by Silvanose (1998). However, intestinal trichomoniasis was not found in our study. The highest rate of infection was found in *Anas crecca* males which was significantly higher than in females ($p<0.05$).

Humans and animals are important reservoirs for *Cryptosporidium*. Human cryptosporidiosis has been reported in more than 90 countries (Fayer et al., 2000). Contaminated drinking water, recreational water, and food are very important and are a major pathway for transmission. *Cryptosporidium* oocysts are commonly found in sewage and surface waters and occasionally in treated water (Ranjbar-Bahadori et al., 2013). To date, only two valid species of *Cryptosporidium* (*Cryptosporidium baileyi* and *Cryptosporidium meleagridis*) have been proven to cause infections in birds (Current, 1986; Slavin, 1955), and *Cryptosporidium* has been detected in more than 30 species of birds including geese (*Anser anser*), tundra swans (*Cygnus* sp.), black-headed gulls (Larus ridibundus), chickens (Gallus gallus) (Shemshadi et al., 2011), turkeys (Meleagris gallopavo), mallards (*Anas platyrhynchos*), and (Cairina moschata) Muscovy ducks (O'Donoghue, 1995). In this study, intestinal *Cryptosporidium* infection rate were in *Cygnus Cygnus* 36.0%, *Spatula clypeata* 32.3%, *Anas platyrhynchos* 22.1%, *Anas crecca* 11.8%, *Anas penelope* 11.2% and *Anas strepera* 6.2%, which were similar in most species to the results of a study carried out in Canada on drinking water. However, in *Anas strepera* and *Anas crecca*,the difference was considerable, no infection was found in *Tadorna tadorna* and *Aythya ferina* species.

Thul et al. (1980) studied 213 wood ducks (Aix sponsa) in the Atlantic Flyway for blood parasites from 1976 to 1977. They found *Haemoproteus* nettionis was the most common parasite, occurring in 56% of the northern wood ducks; *Leucocytozoon* simondi (20%), *Plasmodium* circumflexum (6%), and also they found (18%) microfilariae. Fakhar et al. (2012) . Respectively It has shown the prevalence rate of infection by *Plasmodium* spp., and *Haemoproteus* spp. (2.3%), and (6.6%), in Iranian duck, goose, turkey, poultry, and pigeon and also they found only ducks and turkeys were infected with *Plasmodium* spp. In this study, it was determined that *Plasmodium* spp. exists in blood smear of 24 (8.2%) of the examined waterfowl. The results of *plasmodium* infection rate of the current study is more similar to Thul et al.'s (1980) than the rate has been reported by Fakhar et al. (2012). *Anas platyrhynchos* had the highest infection rate (14.2%) of *Plasmodium* spp. in this study, while *Aythya ferina* and *Spatula clypeata* blood examination were negative.

The prevalence rate of H. columbae in North of Iran has been reported 17.47%. Also, the prevalence of *Haemoproteus* in birds in Costa Rica, Alaska, and Japan revealed rates lower than 10%. In the United states, Colombia, Bulgaria and Queensland the prevalence rate ranged from 20-30% (Yousefi et al., 2010). Prevalence of *H. crumenium* from 42 nestling wood storks during 2003 that was reported by Cody et al. was (7.2%). Also, the rate of infection showed by Fedynich et al. (1998) in Georgia was (3 of 75; 4%) and in this study the prevalence of *Haemoproteus* spp. (6.1%) was completely lower than the rate of infection has reported by Yousefi et al. (2010) in the North of Iran, but slightly higher than that published by Fedynich et al. (1998) in Georgia (3 of 75; 4%) and published by Forrester and Spalding (2003) collected in Florida (4 of 98; 4%), but it was close to the results demonstrated by Zabransky et al. (2008).

In this study, *Leucocytozoon* spp., and microfilariae were not observed in the examined blood samples, also in Zabransky study in 2008, *Leucocytozoon* sp., *Plasmodium* sp. and microfilariae were not found.

Therefore, with regard to our findings, migration of various species of waterfowl toward the northern parts of Iran can be an important source for transmission of parasitic diseases to other birds and even human.

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وقوع تک یاخته‌های انگلی در پرندگان آبزی وحشی ساکن مرداب های سواحل جنوبی دریای خزر، شمال ایران

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چکیده

زمینه مطالعه: تک یاخته‌های انگلی می توانند در انواع پرندگان سبب ایجاد اشکال خفیف تا شدید بیماریزایی گردند. **هدف:** مطالعه حاضر جهت بررسی شیوع تک یاخته های انگلی در پرندگان آبزی وحشی در مرداب های سواحل جنوبی دریای خزر در شمال ایران انجام گردید. **روش‌کار:** بدین منظور نمونه گیری در فاصله زمانی زمستان ۲۰۱۰ تا بهار ۲۰۱۱، از ۲۹۳ قطعه پرنده‌آبزی متعلق به جنس‌های مختلف شامل: تادورنا تادورنا(اردک اهلی)، ایتیا فولیگولا(مرغابی پرزدار)، ایتیا فرینا(مرغابی وحشی)، اسپاچولا کلیپیتا(مرغابی بیل زن)، انسر انسر(غازوحشی اروپایی)، سیگنوس سیگنوس(قو)، اناس استره پرا(اردک قهوه ای)، اناس پنه لوپه(مرغابی دورگه)، اناس کرکا(مرغابی جره)، و اناس پلتی رینکوس(اردک وحشی) انجام گردید و نمونه های اخذشده به لحاظ تک یاخته های خونی، ریوی و روده ای مورد بررسی قرار گرفتند. **نتایج:** از مجموع ۲۹۳ پرنده مورد مطالعه، ۱۸۴ قطعه (۶۲/۸٪) آلوده به تک یاخته‌های انگلی بودند. بیشترین میزان شیوع مربوط به گونه‌های ژیاردیا (۲۴/۲٪) کمترین آن متعلق به گونه های هموپروتئوس (۶/۱٪) بود. تریکوموناس گالینه، گونه های پلاسمودیوم، و گونه‌های کریپتوسپوریدیوم به ترتیب در ۷/۲٪، ۸/۲٪، و ۱۷٪ پرندگان مورد بررسی یافت گردید. همچنین مطالعات آماری انجام‌شده نشان داد که بیشترین میزان ژیاردیوزیس در ایتیا فولیگولا ماده (۴۳/۷۵٪) و در مورد تریکومونیازیس در اناس کرکا (۳۰/۵٪) مشاهده شد. در ضمن، سیگنوس سیگنوس ماده بیشترین میزان آلودگی به کریپتوسپوریدیوزیس (۴۴/۷٪) را داشته و بیشترین موارد آلودگی به هموپروتئوس و پلاسمودیوم در اناس پلتی رینکوس دیده شد. **نتیجه‌گیری نهایی:** بنابراین مهاجرت گونه های متنوعی از پرندگان آبزی به نواحی شمالی ایران سبب تأثیرات وسیعی در آلودگی های انگلی پرندگان و حتی انسان در مناطق یادشده می گردد.

واژه‌های کلیدی: دریاچه خزر، تک یاخته‌های انگلی، پرندگان آبزی

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