Prevalence of bacteria and parasites in White Ibis in Egypt

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Summary

A field survey was conducted to evaluate the prevalence of bacterial infections among free-living White Ibis (*Nipponia nippon*) in which 92 bacterial isolates were recovered from 193 different internal organs of 55 apparently healthy Ibis. *Escherichia coli* and *Salmonella* spp. were isolated at rates of 43.6% and 14.5%, respectively. The other bacterial pathogens isolated were *Shigella* spp. (34.5%), *Enterobacter* spp. (21.8%) *Citrobacter* spp. (18.1%), *Klebsiella* pneumonia (16.3), *Staphylococcus aureus* (10.9%) and *Proteus mirabilis* (7.2%). The antibiogram indicated that all isolates were highly sensitive to ciprofloxacin, enrofloxacin, trimethoprim and penicillin. Penicillin was most effective against *S. aureus*. An examination of the gastrointestinal tract revealed the presence of a nematode, *Ascaris* (*Porroceacum ensicaudatum*), and three trematodes (*Echinochasmus perfoliatus*, *Apatemon aracilis* and *Patagifer bilobus*). Other trematodes were detected in enlarged gall bladder and kidney lesions. Histopathological examination showed signs of hepatitis. The gall bladder had cholangitis, cholicystitis which may have been caused by trematode infestation. The kidneys also showed multiple parasitic cysts of trematodes and non-suppurative interstitial nephritis. This study suggests the possible role of the White Ibis, when living near poultry populations, in transmitting certain pathogens to poultry.

Keywords

Bacterium, Egypt, Ibis, *Nipponia nippon*, Parasite, Pathogen, Poultry, White Ibis.

Prevalenza di batteri e parassiti nell'ibis bianco in Egitto

Riassunto

*E' stata condotta un'indagine di campo su 55 bis bianchi (*Nipponia nippon*), allo stato libero e apparentemente sani, per valutare la prevalenza di infezioni batteriche. Sono stati effettuati 92 solati batterici da 193 rgani interni. Sono stati rilevati* Escherichia coli *e* Salmonella *spp., rispettivamente, nel 43,6% e 14,5% degli isolati. Sono stati rilevati altri agenti patogeni batterici:* Shigella *spp. (34,5%),* Enterobacter *spp. (21,8%),* Citrobacter *spp. (18,1%),* Klebsiella pneumonia *(16,3),* Staphylococcus aureus *(10,9%) e* Proteus mirabilis *(7,2%). L'antibiogramma ha permesso di rilevare l'alta sensibilità di tutti gli isolati a ciprofloxacina, enrofloxacina, trimetoprim e penicillina. In particolare, la penicillina è risultata il farmaco, tra quelli impiegati, più efficace contro* S. aureus*. L'esame del tratto gastro-intestinale ha dimostrato casi con presenza di un nematode* Ascaris *(*Porrocaecum ensicaudatum*) e 3 trematodi (*Echinochasmus perfoliatus, Apatemon gracilis *e* Patagifer bilobus*). Altri trematodi sono stati individuati in cistifellea dilatata e lesioni renali. L'esame istopatologico ha mostrato casi con segni di epatite e casi di colangite e colecistite della cistifellea determinati da trematodi. I reni hanno mostrato molteplici cisti parassitarie di trematodi, è stato evidenziato un*

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caso di nefrite interstiziale non suppurativa. Questo studio ribadisce il possibile ruolo dell'Ibis bianco, presente nelle aree limitrofe agli allevamenti avicoli, nella trasmissione di determinati agenti patogeni al pollame.

Parole chiave
Agente patogeno, Batterio, Egitto, Ibis bianco, *Nipponia nippon*, Parassita, Pollame.

Introduction

The spread of certain bacterial pathogens and their persistence in the environment may be facilitated by wild birds which, in view of their mobility and possible carrier state, have been identified as a possible reservoirs or sources of bacterial infections to domestic poultry (3, 4).

The world population of White Ibis (*Nipponia nippon*) has increased significantly since 1983 and these birds are frequently observed in close contact with people (22). This has led to concern that Ibis may transmit pathogens that threaten not only the poultry industry, but also public health. The prevalence of different bacterial isolates in White Ibis was documented by the isolation of *Pseudomonas*, *Escherichia coli*, *Salmonella*, *Proteus* and *Pasteurella haemolytica* (23). In another study, the same pathogens were isolated in addition to *Streptococcus faecalis*, *Arizona hydrophila* and *Staphylococcus aureus*. (5). *E. coli* was isolated from different internal organs in six septicaemic cases of young Crested Ibis (*Bubulcus ibis*) (28). *E. coli* and *Salmonella* spp. were isolated from free-living passerines (25). *Salmonella* spp. has also been reported in free-living wild birds (3, 4, 6, 20, 24, 27).

Salmonella spp. has commonly been observed in the intestines of wild birds which appear to be relatively resistant to salmonellosis but may serve as effective carriers of *Salmonella* by shedding the organism in their faeces and could to be a source of infection for domestic poultry (26).

The upper respiratory tract of healthy birds can harbour the *Klebsiella* micro-organism, which acts as an opportunistic pathogen and causes localised or systemic infection in poultry and other birds (1).

Little is known about the incidence of these enteropathogens in wild birds that live near poultry facilities and their possible transmission to domestic poultry. The objective of this survey was to determine the prevalence of common avian pathogens in apparently healthy free-living White Ibis and to test drug susceptibilities.

Materials and methods

Birds

For the survey, 55 apparently healthy White Ibis were hunted in different areas of the Sharkia Province of Egypt.

Necropsy and sampling

Birds were examined clinically and subjected to post-mortem examination. The specimens (heart blood, lung, liver, spleen, kidney and ovaries) were taken using aseptic techniques for bacteriological and histopathological investigations.

Bacteriological examination

Samples were inoculated in nutrient broth and brilliant green bile broth and incubated at 25°C and 37°C for 24 h. They were subsequently subcultured into differential and specific media, such as eosin-methylene blue (EMB) agar, xylose-xysine-deoxy-cholate (XLD) agar, MacConkey agar, brilliant green bile agar, Mannitol salt agar and nutrient agar. The inoculated plates were incubated at 37°C for 24 h. Colonies with characteristic growth of any bacteria were phenotypically identified by using Gram stain and standard biochemical tests (18). The tests included lactose fermentation, indol production, the methyl red test, use of citrate, presence of urease, hydrogen sulphide gas production, the Voges-Proskauer test for the production of acetoin and the motility test.

Antimicrobial sensitivity test

All isolates were subjected to disc sensitivity tests according to the procedure given by the National Committee for Clinical Laboratory Standards (NCCLS) (16) using available commercial antibiotic discs (Oxoid Laboratory, Oxoid, Unipath Ltd, Basingstoke). The diameter

of inhibition zones were measured in millimetres after 24 h of growth and the interpretation chart provided by the manufacturer was used to classify isolates into 'sensitive' or 'resistant' groups.

Parasitological examination

Examination of the gastrointestinal tract of Ibis was performed to detect different enteric parasites. Faecal samples were collected in clean sterile containers. Some of the sample was fixed in 10% formalin, followed by direct concentration and centrifugation in saturated salt solution. Gross helminths passed in faeces were identified after staining with borax carmine in the case of trematodes and cestodes. Nematodes were studied after clearing them in lactophenol according to standard procedures (17).

Histopathological examination

Specimens showing characteristic lesions were collected from the liver, gall bladder and kidneys; they were fixed in 10% neutral buffered formalin solution and embedded in paraffin wax. Then 5 µm sections were prepared and stained with haematoxylin and eosin (H&E) for microscopic examination (2).

Results and discussion

Although the hunted white Ibis appeared apparently healthy, post-mortem examination revealed signs of septicaemia in 55% of birds examined. Liver samples showed subcapsular haemorrhages with necrosis, in addition to greenish discoloration; gall bladders were severely enlarged and distended with bile. Kidneys were greatly enlarged and had a nodular appearance and the ureters were filled with urate. Proventriculi were enlarged and thickened in many cases. Gastrointestinal tracts showed congestion, with haemorrhagic spots on intestinal walls and some cases showed mucosal thickening. Testis were enlarged with haemorrhagic spots. Few ovaries were misshaped and had greenish coloured ovum. These observations are consistent with previous studies (5, 28).

Records of bacterial isolations from 55 White Ibis and the recovery from different organs are presented in Tables I and II.

Table I
Frequency* of different bacterial pathogens isolated from 55 wild Ibis

Bacterial isolates	Number positive	Percentage (%)
Escherichia coli	24	43.6
Salmonella	8	14.5
Shigella	19	34.5
Proteus	4	7.2
Citrobacter	10	18.1
Enterobacter	12	21.8
Klebsiella	9	16.3
Staphylococcus	6	10.9
Total	92	

* mixed infection was observed in certain cases

The highest percentage of bacterial isolation was recorded for *E. coli* (43.6%) followed by *Shigella* (34.5%), *Enterobacter* (21.8%), *Citrobacter* spp. (18.1%), *Klebsiella* pneumonia (16.3%), *Salmonella* (14.5%), *S. aureus* (10.9%) and *Proteus mirabilis* (7.2%). A high rate of recovery was from lungs, heart blood and liver. *E. coli* 078 (33.3%), *Salmonella* enterica Typimurium (20.8%) and *Proteus vulgaris* (0.83%) were isolated from White Ibis (23). Similar isolation percentages were observed for *E. coli* (35%), *Salmonella* spp. (5%), *Proteus* spp. (10%) and *S. aureus* (10%) (5). *E. coli* was isolated from six cases of young septicaemic Crested Ibises (28). Several authors have documented the presence of *E. coli* and *Salmonella* spp. from wild birds found near broiler chicken houses (3, 4, 9, 10, 13, 21). *Klebsiella* spp. was isolated from different species of wild birds, such as the house crow (*Corvus splendens*), hoopoe (*Upupa epops major*), Egyptian house sparrow (*Passer domesticus niloticus*), Egyptian laughing dove (*Streptopelia senegalensis aegyptiaca*) and quail (*Coturnix coturnix*), but not from African Sacred or Crested Ibis (*Bubulcus ibis*) (5).

Table II
Incidence of bacteria isolated from blood, liver, lung, kidney and ovaries

Isolates	Heart blood		Liver		Lung		Kidney		Ovary		Total
	No.	%	No.	%	No.	%	No.	%	No.	%	
Escherichia coli	11	23.4	6	12.8	15	32	7	14.8	8	17	47
Salmonella	9	30	8	26.7	7	23.3	1	3.3	5	16.7	30
Shigella	3	14.3	6	28.6	9	42.8	3	14.3	–	–	21
Proteus	2	25	3	37.5	2	25	1	12.5	–	–	8
Citrobacter	11	32.4	10	29.4	9	26.5	3	8.8	1	2.9	34
Enterobacter	9	22.5	17	42.5	11	27.5	2	5	1	2.5	40
Klebsiella	7	30.4	5	21.7	6	26.1	2	8.7	3	13.1	23
Staphylococcus	1	16.7	2	33.3	3	50	–	–	–	–	6
Total											209

Results of biochemical identification of the different bacterial isolates are summarised in Table III.

Salmonella spp. were isolated on XLD agar. This method is extremely sensitive for the detection of *Salmonella* spp., even for samples that have a high contamination level of other *Enterobacteriacae* (11). At the same time, it has been suggested that the natural occurrence of *Salmonella* in healthy birds during migration in Sweden may be low (8). Therefore, *Salmonella* incidence is probably low for most wild birds. These results support the low number of *Salmonella* spp. isolates in our samples in comparison to the other bacterial isolates.

The pattern of antibiogram susceptibility of the different bacterial isolates is shown in Table IV.

Results of susceptibility testing showed that most of these isolates were highly sensitive to ciprofloxacin, enrofloxacin, trimethoprim, norfloxacin and amoxycillin. At the same time, *Salmonella* spp., *E. coli* and *Proteus* spp. isolates were also sensitive to neomycin, oxalinic acid

Table III
Biochemical identification of the different bacterial isolates

Biochemical test	Bacterial isolates								
	E. coli	Citro	*Shigella* spp.	Salmo	Proteus	Pseudo	Staph	Kleb	Entero
Lactose	+	–	–	–	–	X	X	+	+
Indol	+	–	–	–	+	–	X	+	–
Citrate	–	–	–	+	X	+	X	+	+
Urea	–	+	–	–	+	–	X	+	–
H_2S	–	+	–	+	–	X	X	X	X
Methyl red	+	+	+	+	X	+	X	–	–
Voges–Proskauer	–	–	–	–	X	–	X	+	+
Motility	+	+	–	+	+	+	–		–
Coagulase	X	X	X	X	X	X	+	X	X

E. coli *Escherichia coli*
Citro *Citrobacter* spp.
Salmo *Salmonella* spp.
Pseudo *Pseudomonas* spp.
Staph *Staphylococcus* aureus
Kleb *Klebsiella* spp.
Entero *Enterobacter* spp.

\+ positive
– negative
X not done

Table IV
Antimicrobial sensitivity of bacterial isolates using disc diffusion method

Antibiotic disc concentration	Sensitivity of bacteria to each respective compound (%)							
	E. coli	Salmo	Proteus	Shig	Entero	Kleb	Citro	Staph
Ciprofloxacin 10 µg	67% +	55%+	40%+	60%+	70%+	–	60%+	–
Enrofloxacin 5 µg	75% +	85% +	60%+	–	50%+	55%+	40%+	40%+
Trimethoprim 25 µg	70% +	50% +	50%+	–	–	40%+	–	–
Norfloxacin 10 µg	70% +	75%+	–	60%+	–	60%+	–	60%+
Amoxycillin 10 µg	80%+	25% +	60%+	40%+	–	–	60%+	70%+
Gentamycin 10 µg	58% +	–	–	20%+	–	–	40%+	–
Pencillin 10 µg	–	+	–	–	–	–	–	85%+
Streptomycin 10 µg	50%+	45%+	20%+	–	–	–	–	40%+
Oxalinic acid 30 µg	67%+	25%+	–	–	–	–	–	45%+
Flumoquine 10 µg	70%+	75%+	–	75%+	–	–	–	–
Oxytetracyclin 30 µg	–	–	30%+	30%+	–	70 +	60%+	50%+
Kitasamycin 70 µg	–	60%+	–	–	–	–	–	–
Neomycin 30 µg	50%+	75%+	65%+	–	–	–	40%+	–

E. coli *Escherichia coli*
Salmo *Salmonella* spp.
Shig *Shigella* spp.
Entero *Enterobacter* spp.
Kleb *Klebsiella* spp.
Citro *Citrobacter* spp.
Staph *Staphylococcus* aureus
\+ positive
– negative

and streptomycin. To some extent, our results resembled those of a previous study (5) with the exception of amoxicillin in which the reported isolates were resistant. *Klebsiella* spp. isolates were highly sensitive to gentamycin and less sensitive to penicillin (1). These findings were in disagreement with our results in which *Klebsiella* spp. was resistant.

A microscopic examination of gastrointestinal tract lavage revealed the presence of three species of trematodes (*Echinochasmus* spp., *Apatemon* spp. and *Patagifer* spp.), in addition to a nematode (*Porroceacum* spp.). These findings explain the presence of severe congestion in the gastrointestinal tract with a thickening of the mucosa and haemorrhagic spots in some instances.

Histopathological examinations of liver showed massive areas of fatty change (Fig. 1), coagulative necrosis of hepatocytes, congestion, hyperplasia of the bile duct, infiltration with macrophages, lymphocytes and heterophils (Fig. 2). Multifocal areas of mononuclear cell infiltration, mainly in macrophages and lymphocytes, with necrotic changes and degeneration of hepatocytes were also observed (Figs 3 and 4).These lesions may be associated with bacterial infection (principally *Salmonella* and *E. coli*). Our results concurred with the results of several other studies (7, 28, 29).

Severe cholangitis and cholecystitis were observed in close association with a trematode (*Echinostomatidae* spp.) in the gall bladder and bile duct (Figs 5 and 6). This parasite has previously been detected in the gall bladder of Ibis (15). Biliary necrosis, hyperplasia, eosinophilic and mononuclear cell infiltrations and fibrosis were also detected. Multifocal areas of eosinophilic cell infiltration and lymphocytes with degeneration and necrosis of hepatocytes were observed in some cases (Figs 7 and 8). These aggregations of eosinophils may be due to larva migration through the hepatic tissue. The presence of parasites within the gall bladder and bile duct is most likely due to a trematode *(Echinostomatidae*); this has also been documented by Murata *et al.* (15) who found *Echinostomatidae* (*Pegosomum* spp.) in the lumen of gall bladder and bile ducts of cattle egret (*Bubulcus ibis*). A similar histopathological picture was observed by other authors (14, 19).

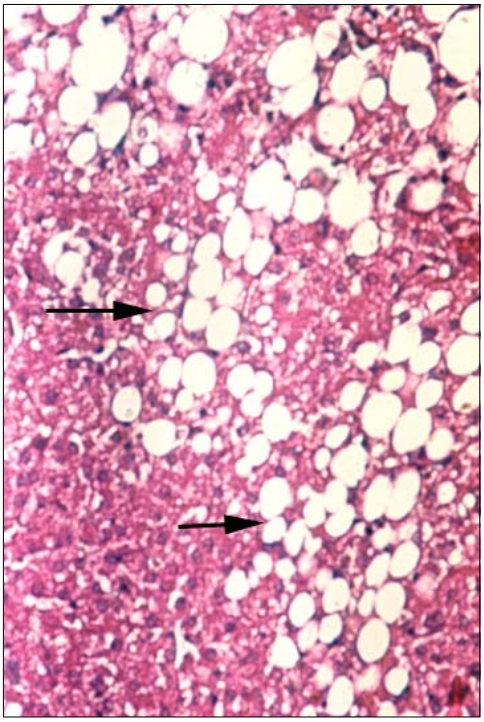

Figure 1
Liver showing fatty build up among hepatocytes (arrows)
(H&E ×400)

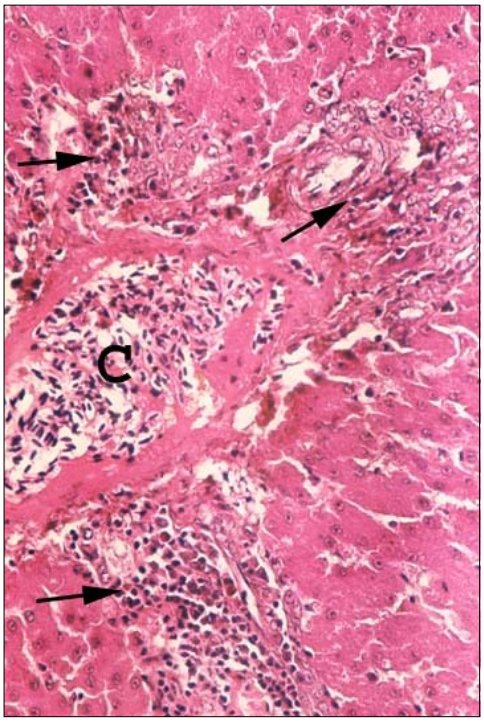

Figure 2
Photomicrograph of the liver showing hyperplasia of bile duct along with leukocytic infiltrations, mainly lymphocytes and macrophages (arrows)
C: congestion of blood vessels
(H&E ×400)

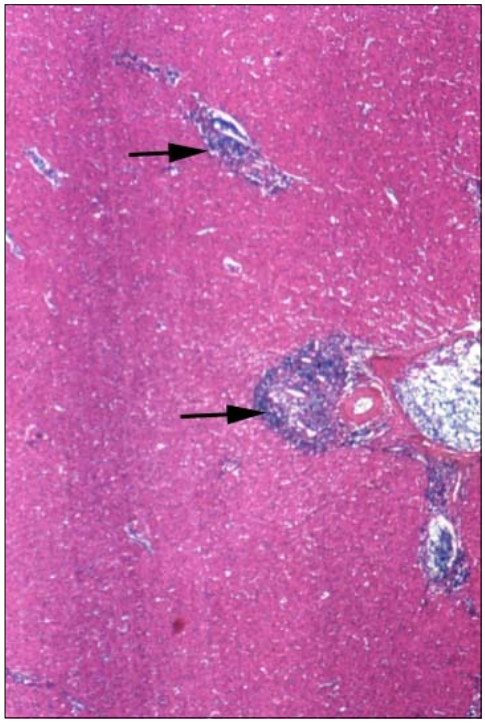

Figure 3
Photomicrograph of the liver showing multifocal areas of leukocytic infiltrations (arrows), along with focal necrosis
(H&E ×100)

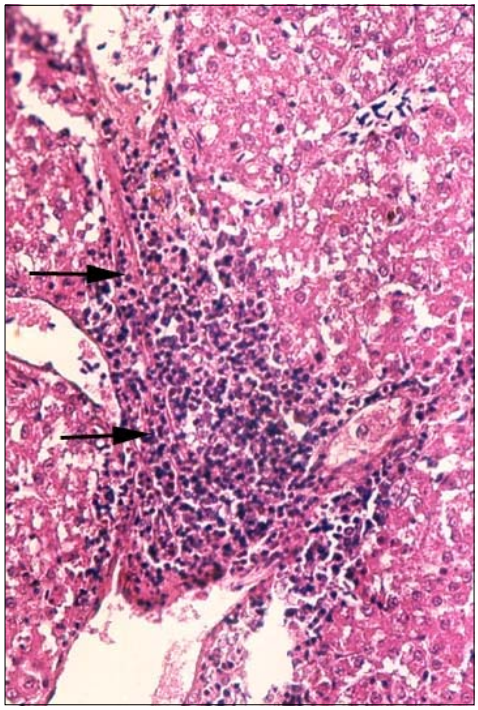

Figure 4
Photomicrograph of the liver
Magnification of Figure 3, showing vacuolar degeneration of hepatocytes, necrotic changes of hepatocytes and infiltrations with lymphocytes and few heterophils
(H &E ×400)

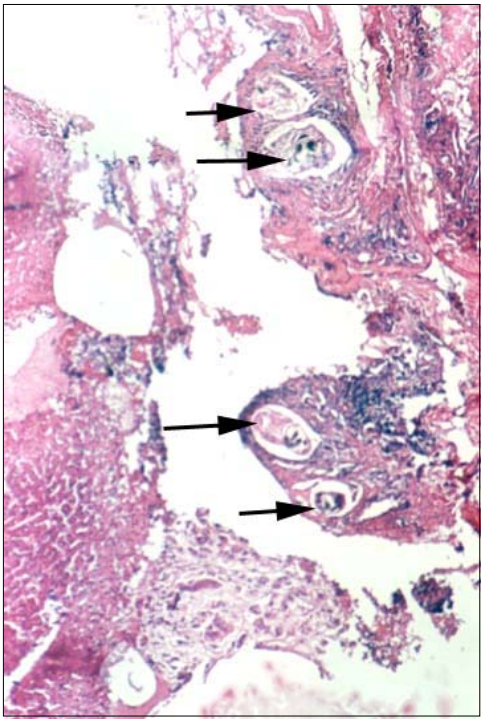

Figure 5
Photomicrograph of the gall bladder, showing severe cholangitis and cholecystitis with association of the parasites in the bile duct (arrows)
(H&E ×100)

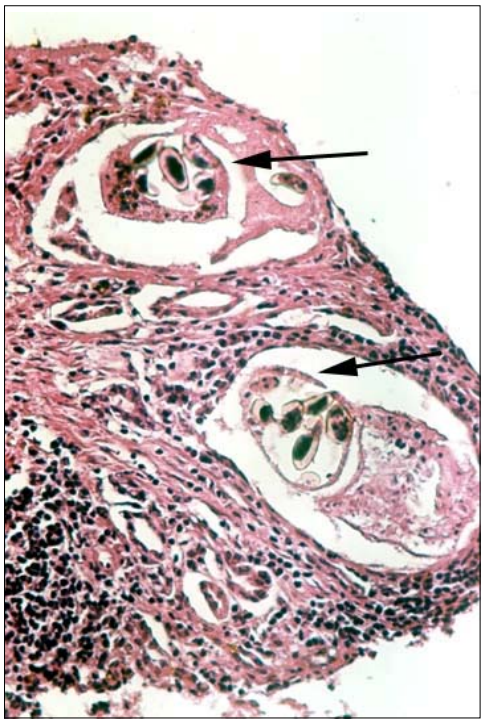

Figure 6
Cross sections of the parasites with massive lymphocytic infiltrations
Magnification of Figure 5
(H&E ×400)

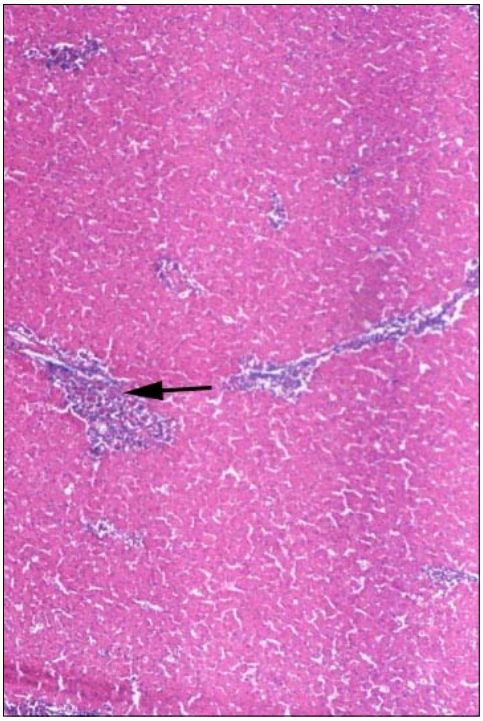

Figure 7
Photomicrograph of the liver showing multifocal areas of leukocytic infiltrations with eosinophils
(H&E ×100)

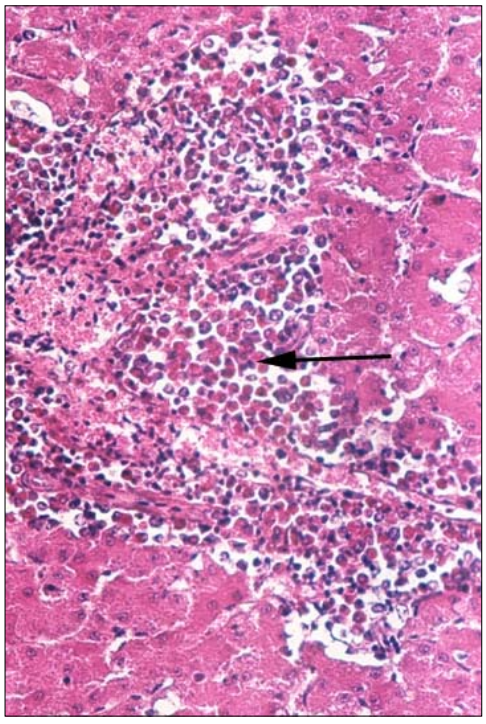

Figure 8
Photomicrograph of the liver showing massive infiltrations with eosinophils and some lymphocytes
Magnification of Figure 7
(H&E ×100)

A histological examination of the kidney revealed multiple cross-sections of trematodes. Most often, the trematodes were present within cystic spaces lined by cuboidal cells. In addition, they showed a typical picture of nephritis with multiple and large gravid flukes in distended ducts that were most likely related to a trematodiosis. Adjacent kidney parenchyma were compressed with obliteration of tubule lumen and focally infiltrated with few lymphocytes (Figs 9, 10 and 11). The histological lesions attributed to the trematodes were areas of mononuclear cell infiltration, principally in lymphocytes and a few macrophages and degenerative changes. The internal organs of the parasite and its oval nucleated bodies were also seen (Fig. 12). These histological lesions were similar to those described previously (12, 19). Interstitial nephritis represented by mononuclear cell infiltration of lymphocytes and macrophages could be attributed to bacterial infection.

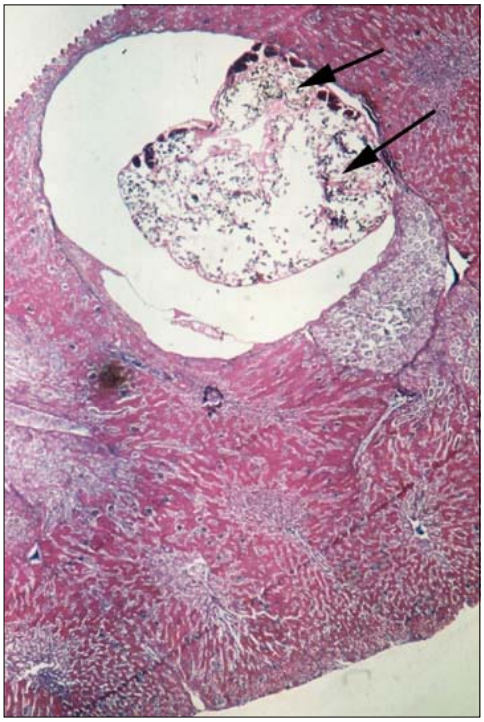

Figure 9
Photomicrograph of the kidney showing large cyst embedded in the parenchyma of the renal tissues
This resembles a section of a parasite with minimal tissue reaction
(H&E ×100)

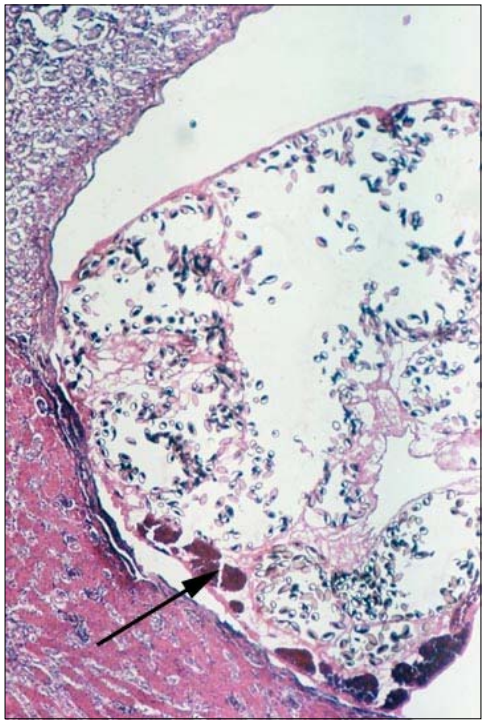

Figure 10
Magnification of the parasitic cyst in the kidney showing sections of parasite internal organs and oval nucleated bodies
(H&E ×200)

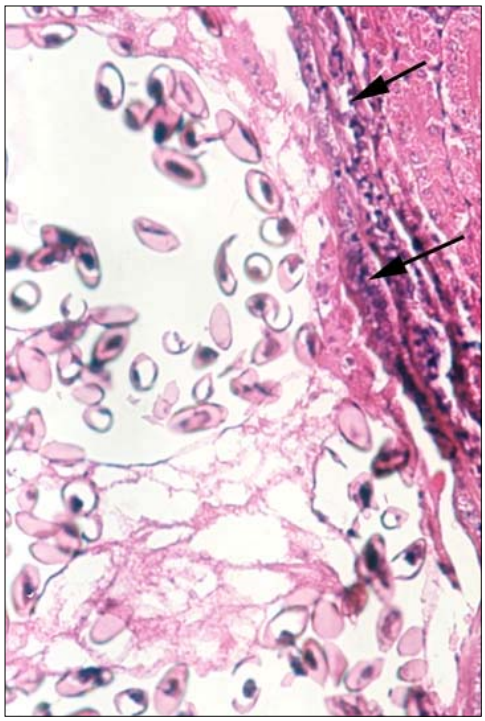

Figure 11
Minimal tissue reaction consisting of few lymphocytic infiltrations
Magnification of Figure 10
(H&E ×400)

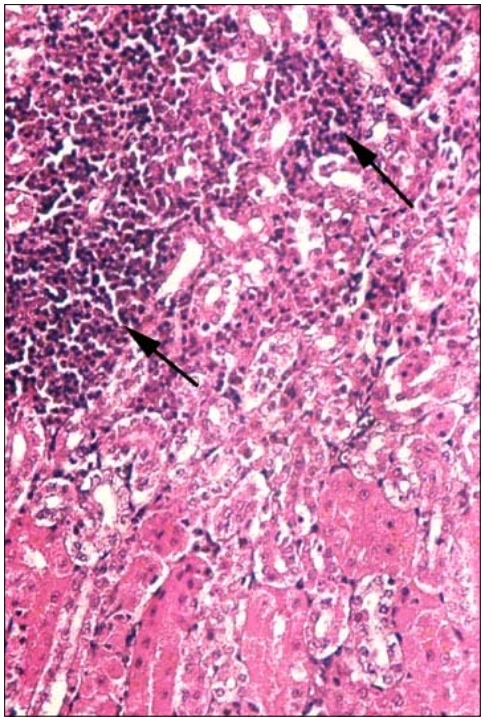

Figure 12
Photomicrograph of the kidney showing focal interstitial nephritis mainly with lymphocytic infiltrations and few lymphocytes
(H&E ×400)

Conclusions

Our results reflected the possible potential role of White Ibis in the transmission of certain pathogens to domestic poultry and also to human populations.

The continuous growth and geographic expansion of Ibis populations in rural and urbanised settings provides a greater opportunity for Ibis to interact with humans and livestock which may represent an increased risk of pathogen transmission. Further investigations are required to provide additional information on the role of other wild birds in the transmission of pathogens to humans and to domestic birds.

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