Waterfowl and the bacteriological quality of amenity ponds

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ABSTRACT

This study investigated the impact of waterfowl on the bacteriological quality of village ponds in East Yorkshire, north-east England. Water and sediment samples were collected from ponds with and without resident ducks and geese; faecal indicator and potentially pathogenic bacteria were assayed by membrane filtration and by selective enrichment. *Escherichia coli*, faecal streptococci and, to a degree, *Clostridium perfringens* were more abundant in ponds with waterfowl; *Salmonella* was isolated in June–August from the sediment of a pond with waterfowl. The results suggested that the bacteriological quality of village ponds might be adversely affected by waterfowl. All water samples from ponds with waterfowl had faecal indicators at higher concentrations than EU requirements for bathing waters. Although these ponds are not bathing waters we suggest skin contact and accidental ingestion of water should be avoided.

Key words | *Escherichia coli*, faecal indicators, faecal streptococci, ponds, *Salmonella*, waterfowl

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INTRODUCTION

Waterfowl are gross excreters of faecal coliforms and faecal streptococci (Ashbolt *et al*. 2001) and their association with the contamination of water bodies that are used for recreation has been described. Thus, a bathing beach on a Wisconsin, USA, lake was closed because of a high concentration of faecal coliforms that was attributed to mallard ducks (Standridge *et al*. 1979). Likewise, in the UK, mallard ducks, and other birds were found to be, in part, responsible for high densities of faecal coliforms at Morecambe Bay, which failed to comply with EU guidelines for bathing water (Jones 2002).

Waterfowl also harbour bacteria in their intestinal tract that are potential human pathogens. *Salmonella* and *Campylobacter* are major causative agents of bacterial gastroenteritis in both the UK and USA (Rusin *et al*. 2000; Timbury *et al*. 2002). Both organisms have been found in the intestinal tract of ducks and geese (Pacha *et al*. 1988; Ridsdale *et al*. 1998; Feare *et al*. 1999; Aydin *et al*. 2001; Dieter *et al*. 2001; Refsum *et al*. 2002). The carriage of these pathogens by ducks and geese suggests that waterfowl may act as a reservoir for their transmission through the contamination of water. Contaminated water is a potential source of *Salmonella* and *Campylobacter* and may be a vehicle for their transmission to domestic animals and humans (Bolton *et al*. 1987; Melloul & Hassani 1999; Thomas *et al*. 1999).

High numbers of waterfowl are often found on village ponds in England. These ponds and their environs are typically recreational and amenity sites. Visitors and residents frequently encourage waterfowl by feeding them, and direct contact with faecal material or contaminated water is likely to occur. The present study aimed to investigate the impact of waterfowl on the bacteriological quality of some amenity village ponds in East Yorkshire, north-east England.

MATERIALS AND METHODS

Sites and sampling

The study sites were seven roadside village ponds. Four of these had resident semi-tame ducks and sometimes geese:

South Dalton (National Grid Reference SE 969 454, pond area 2,410 m^2), Brantingham (SE 941 296, 240 m^2), Garton-on-the-wolds (SE 983 594, 1,800 m^2) and Little Weighton (SE 988 338, 640 m^2). Waterfowl (ducks and geese) numbers were variable but some birds were always present; numbers ranged from 13 to 75 birds; the water was turbid and aquatic vegetation was sparse or absent. Ducks and geese were never observed at the other three ponds. These were at Holme-on-Spalding-Moor (SE 827 390, 1,530 m^2), Bentley (TA 019 359, 220 m^2) and Sancton (SE 901 392, 85 m^2). Their water was clear, and submerged and emergent aquatic plants were abundant.

Water and sediment samples were collected from South Dalton and Holme-on-Spalding-Moor in the late morning at about monthly intervals from June 2001 to January 2002. Water samples were taken from the other five ponds on one day in August, one day in October and one day in November 2001. Surface water was collected from pond margins into sterile polypropylene bottles. Two replicate sediment cores with overlying water were taken in 44 mm internal diameter, sterile glass tubes (Carr & Goulder 1990). The top 1 cm of sediment was siphoned off. That from one core was used for *Salmonella* and *Campylobacter* enrichment culture. That from the other core was diluted ten times with sterile pond water and used for the other sediment assays. This diluted sediment was homogenized by treatment in a stomacher (Colworth 400; A.J. Seward Ltd, London) for 5 min. All samples were packed in ice and kept in darkness during transport; bacteriological assays were begun on the same day as sampling.

Bacteriological assays

Protocols for faecal indicators and potential human pathogens were based on those used by the Public Health Laboratory Service (PHLS 1998*a–e*) and, for *Clostridium*, on HMSO (1983). Media were from Oxoid Ltd, Basingstoke, UK, unless otherwise indicated. *Escherichia coli*, faecal streptococci and *Clostridium perfringens*, in pond water and appropriately diluted sediment slurries, were assayed by colony counts on 0.45 μm membrane filters. At least two replicate filters were counted per sample. The presence of *Salmonella* and *Campylobacter* was tested for by selective enrichment. The enrichment cultures were seeded with membrane filters bearing the residue from filtration of 1 litre of pond water, or with 5 ml of sediment.

Membranes for *E. coli* counts were incubated on absorbent pads with lauryl sulphate broth; plates were initially incubated at 30°C for 4 hours, followed by 14 hours at 44°C. Colonies of faecal streptococci were counted after incubation of membranes on Slanetz and Bartley agar at 37°C for 4 hours, then at 44°C for 40 hours. Assays for *C. perfringens* used pond water and slurry that had been heat-treated at 75°C for 10 min prior to filtration to ensure that only *C. perfringens* spores remained in the samples. Membranes were incubated anaerobically (Anaero*Gen*, Oxoid) on reinforced clostridial agar for 24 hours at 37°C.

Representative colonies, five or six from each membrane, were subjected to confirmatory tests. *E. coli* was confirmed by culture in Fluorocult broth (Merck, Darmstadt, Germany); incubation was at 37°C for 24 hours, to verify production of both β-galactosidase and β-glucuronidase. Confirmation of faecal streptococci was by incubation on bile aesculin agar plates at 44°C for 16 hours. *C. perfringens* was confirmed by anaerobic incubation in Crossley milk medium for 24 hours at 37°C.

Membranes and sediment subsamples for *Salmonella* assay were subjected to pre-enrichment incubation in buffered peptone water for 18 hours at 37°C. Selective enrichment was then done separately in both Rappaport-Vassiliadis soya peptone broth, with incubation at 41.5°C for 20 hours, and in selenite cystine broth, with incubation at 37°C for 20 hours. These cultures were plated on both xylose lysine desoxycholate agar (XLD) and brilliant green agar (BGA) and the plates were incubated at 37°C for 22 hours. Suspected *Salmonella* colonies (red with black centres on XLD, and red with a bright red halo on BGA) were subcultured on to cystine lactose electrolyte deficient (CLED) agar and were incubated at 37°C for 18 to 22 hours. Flat, blue colonies on CLED agar were confirmed by incubation on lysine iron agar slopes and in urea broth; incubation at 37°C for 14–18 hours.

Enrichment for *Campylobacter* utilized Preston *Campylobacter* enrichment broth; cultures were initially

Table 1 | Bacteria in water and sediment from ponds at South Dalton and Holme-on-Spalding-Moor, June 2001–January 2002

	Median (range) *n*		
	South Dalton	**Holme-on-Spalding-Moor**	***P***
In water			
E. coli ($\times 10^3$ cfu 100 ml^{-1})	81 (3–180) 7	4 (0.01–11) 7	<0.01
Faecal streptococci ($\times 10^3$ cfu 100 ml^{-1})	34 (1.6–490) 6	11 (0–24) 7	<0.1
C. perfringens ($\times 10^3$ cfu 100 ml^{-1})	6.4 (0.11–100) 6	3.9 (0.01–60) 6	NS
Heterotrophic plate count ($\times 10^5$ cfu ml^{-1})	2.5 (1.6–3.3) 5	0.83 (0.32–2.4) 5	<0.05
Total bacteria ($\times 10^7$ ml^{-1})	4.7 (3.4–7.4) 6	1.2 (1.1–3.0) 6	<0.05
In sediment			
E. coli ($\times 10^3$ cfu ml^{-1})	24 (0.14–105) 7	4.6 (0–9.25) 7	<0.01
Faecal streptococci ($\times 10^3$ cfu ml^{-1})	9.9 (0.3–113) 7	0.65 (0–50) 7	<0.1
C. perfringens ($\times 10^3$ cfu ml^{-1})	38 (0.65–113) 6	17.3 (0.6–87) 6	<0.05
Heterotrophic plate count ($\times 10^6$ cfu ml^{-1})	1.6 (1.1–2.3) 5	1.7 (1.3–2.8) 5	NS

There were up to about 40 ducks and geese at South Dalton; none was present at Holme-on-Spalding-Moor.
P values are from the χ^2 test which tested agreement between observations and the null hypothesis that when ponds are compared pairwise the numerical abundance of an indicator bacterium should equally often be lower as greater in the pond with waterfowl; *n*=number of samples; NS=*P*>0.1.

incubated at 37°C for 22 hours, followed by 22 hours at 42°C. Microaerobic conditions were achieved by incubation in screw-top bottles with minimal air-space (PHLS 1998*e*). These broth cultures were then subcultured onto *Campylobacter* selective agar (mCCDA) and incubated in a microaerobic atmosphere (Campy*Gen*, Oxoid) at 37°C for 48 hours. Colonies were then subjected to a confirmation route of a positive oxidase test plus growth on blood agar in a microaerobic but not an aerobic atmosphere.

Spread plate counts of culturable heterotrophic bacteria were made on casein peptone starch agar (Jones 1970). Ten replicate plates were inoculated with 0.1 ml subsamples of diluted water or slurry and were incubated at 20°C for 14 days. Direct counts of total bacteria in water were performed using epifluorescence microscopy (Daley 1979). Bacteria were stained with DAPI (Yu *et al*. 1995) and then concentrated on black 0.2 μm polycarbonate membrane filters and at least 600 cells per preparation were counted at × 1,250 magnification.

Mean values of specific bacterial variables in ponds with and ponds without waterfowl were often skewed by extreme values, hence median values are given.

RESULTS AND DISCUSSION

The faecal indicators, *E. coli* and streptococci, were more abundant in water from South Dalton, which had waterfowl (up to about 40 ducks and geese), than in water from Holme-on-Spalding-Moor (Table 1). Plate counts of heterotrophic bacteria and direct counts of total bacteria in pond water were also greater at South Dalton (Table 1). In sediment, *E. coli*, faecal streptococci and *C. perfringens*

Table 2 | Bacteria in water from five additional East Yorkshire ponds, August–November 2001

	Median (range) *n*		
	Ponds with waterfowl*	**Ponds without waterfowl†**	***P***
E. coli ($\times 10^3$ cfu 100 ml^{-1})	21 (8.8–300) 7	0.009 (0.003–1.5) 6	<0.01
Faecal streptococci ($\times 10^3$ cfu 100 ml^{-1})	1.6 (1.3–300) 9	0.009 (0.001–0.36) 6	<0.01
C. perfringens ($\times 10^3$ cfu 100 ml^{-1})	80 (0.16–930) 9	0.01 (0.009–16.6) 6	<0.01

P values are from the Mann-Whitney U-test; *n*=number of samples.
*Ponds at Brantingham, Garton-on-the-wolds and Little Weighton; up to 75 ducks and geese were present.
†Ponds at Sancton and Bentley.

were more abundant at South Dalton (Table 1). Faecal indicators in the water from five additional ponds were also more abundant in those ponds with waterfowl (Table 2). All presumptive colonies of *E. coli* and faecal streptococci that were tested confirmed as positive, but only about 50% of presumptive *C. perfringens* were positive, hence our results for *C. perfringens* represent presumptive isolates.

Values of the Spearman's correlation coefficient showed that there were significant relationships between faecal indicators in pond water and waterfowl number and waterfowl number per unit pond area (Table 3). The closest relationship was between *E. coli* and number of waterfowl.

Table 3 | Relationships between faecal indicators and waterfowl number and waterfowl per unit pond area, June 2001–January 2002

	Relationship with number of waterfowl		**Relationship with waterfowl per unit pond area**	
	r_s	***P***	r_s	***P***
*E. coli**	0.84	<0.01	0.75	<0.01
Faecal streptococci†	0.63	<0.01	0.49	<0.01
*C. perfringens**	0.42	<0.05	0.50	<0.01

Values are Spearman's correlation coefficients; a two-tailed test was used
*n=27 samples from 7 ponds, †n=29 samples from 7 ponds

Salmonella and *Campylobacter* assays were performed on water from all seven ponds and on sediment from South Dalton and Holme-on-Spalding-Moor. *Salmonella* was recovered only from South Dalton sediment during summer (June, July, August); *Campylobacter* was recovered from none of the ponds.

High counts of *E. coli* and faecal streptococci in pond water (Tables 1 and 2) were probably caused by waterfowl; duck faeces contain about 3.3×10^7 faecal coliforms and 5.4×10^7 streptococci per gram wet weight (Ashbolt *et al.* 2001). The lower and very variable background numbers of faecal indicators found in ponds without ducks and geese perhaps originated from small family groups of coot (*Fulica atra*) and moorhen (*Gallinula chloropus*) and from rainfall-related runoff from adjacent roads and soil. Such runoff, especially from land carrying livestock, may be an appreciable source of faecal indicators to natural waters (Alvarez *et al.* 1991; Jones 2002). Of the ponds that were surveyed in the present study, however, those with more faecal indicators were not obviously more liable to contamination by runoff, hence we suggest that the dense populations of waterfowl were the leading cause of high counts of faecal indicators.

There was sometimes a closer relationship between faecal indicators in pond water and number of waterfowl, than there was with waterfowl per unit pond area (Table 3). This was probably due to non-uniform horizontal distribution of both waterfowl and bacteria. Waterfowl gather to be fed, and will defecate at pond margins adjacent to roadsides; i.e. where the samples were taken. The

lack of a significant difference between *C. perfringens* in the water at South Dalton and Holme-on-Spalding-Moor (Table 1) and its relatively weak correlation with waterfowl abundance (Table 3) suggest that waterfowl were not necessarily the principal source of presumptive *C. perfringens*. Contaminated soil may also be a significant source. The high values of heterotrophic plate counts and total counts in the water at South Dalton (Table 1) probably reflect general nutrient enrichment by waterfowl.

High counts of faecal indicators in the sediment at South Dalton were most likely due to sunken faecal material. Concentrations in sediments were much higher than in overlying water (Table 1) thus the sediments contain a reservoir of bacteria that is potentially recyclable into the water column, perhaps by foraging waterfowl or by extreme weather.

The recovery of *Salmonella* from South Dalton sediment in June–August agrees with Hendricks (1971) who observed greater recovery of *Salmonella* from river sediments than from water, and with Pianetti *et al*. (1998) who most readily found *Salmonella* in rivers in summer and autumn. Its presence in South Dalton sediment might be attributed to its concentration through sedimentation of faecal material. The low frequency of *Salmonella* isolation may be because healthy waterfowl do not necessarily harbour enteric pathogens (Damare *et al*. 1979; Hussong *et al*. 1979); although *Salmonella* has been isolated from a wide range of apparently healthy birds (Kapperud & Rosef 1983). The present study utilised a present/absent assay for the enumeration of *Salmonella* hence absolute number was not determined. Infective doses for salmonellosis in humans are as little as between 10^1 and 10^3 bacterial cells (Blaser & Newman 1982); hence the presence of this potential pathogen might indicate a possible health threat.

The apparent absence of *Campylobacter* from the East Yorkshire ponds that had waterfowl was unexpected; post-enrichment colonies on mCCDA were culturable on blood agar under both microaerobic and aerobic conditions and hence were not confirmed as *Campylobacter*. In contrast, clinical isolates of *Campylobacter*, supplied by Hull PHLS, did confirm as *Campylobacter*. The non-isolation of *Campylobacter* in summer, when morning water temperature was up to 19°C, is compatible with seasonal trends for its recovery from the environment; Obiri-Danso & Jones (1999) found this organism to be sparse in river water in summer. The apparent absence of *Campylobacter* in winter, however, is puzzling. The standard PHLS protocol used in the present study required the filtration of 1,000 ml of water sample. The seeding of enrichment cultures with the residue from such a large volume of water, or with 5 ml of microbially rich sediment, may have led to out-competition of *Campylobacter* to the extent that it was unable to grow to detectable levels (Fricker 1987).

Campylobacter has been found in the intestinal tract of many species of waterfowl (Pacha *et al*. 1988; Ridsdale *et al*. 1998; Aydin *et al*. 2001), although Hill & Grimes (1984) failed to isolate it in caecal samples from 50 waterfowl from a Mississippi lake and suggested that *Campylobacter* may exhibit sporadic distribution in response to feeding habits, geographical distribution and mixing of waterfowl with other birds and animals. Feare *et al*. (1999) also did not manage to isolate *Campylobacter* from 600 faecal droppings of ducks and geese that were collected from 12 sites located in London, south-east England, Yorkshire and northern England over a period of 2 years. This variation between studies is perhaps explained by differences in the methods used to assess *Campylobacter*.

CONCLUSIONS

Waterfowl are often regarded as a positive feature of amenity ponds in English villages; adults and children enjoy feeding them. However, faecal indicators in the water of East Yorkshire ponds with ducks and geese (Tables 1 and 2) always exceeded the EU requirements for bathing waters (EU 1994) of <2000 *E. coli* per 100 ml and <400 faecal streptococci per 100 ml. These ponds are not used for bathing, nevertheless with these high concentrations of faecal indicators there is a potential hazard from pathogens – and *Salmonella* was found in some samples. Skin contact and accidental ingestion of water is probably best avoided. Ponds without waterfowl were better than EU requirements for bathing waters or only intermittently infringed them.

ACKNOWLEDGEMENTS

We thank the PHLS in Hull for assistance with methods and Sarah Lythe for help with sampling.

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