ECOLOGICAL STUDIES ON CYATHOCOTYLE BUSHIENSIS (DIGENEA) AND SPHAERIDIOTREMA GLOBULUS (DIGENEA), POSSIBLE PATHOGENS OF DABBLING DUCKS IN SOUTHERN QUÉBEC

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ECOLOGICAL STUDIES ON *CYATHOCOTYLE BUSHIENSIS* (DIGENEA) AND *SPHAERIDIOTREMA GLOBULUS* (DIGENEA), POSSIBLE PATHOGENS OF DABBLING DUCKS IN SOUTHERN QUÉBEC

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ABSTRACT: Over the past 20 yr, recurrent late-summer mortality of dabbling ducks in southern Québec has been attributed to the digenean parasite *Cyathocotyle bushiensis*. This study attempted to determine whether this parasite was still implicated in the epizootics, and examined aspects of the ecology of the parasite in the definitive host. Comparison of prevalence and mean intensity of infection of all species of Digenea between salvaged carcasses and hunter-shot ducks revealed that *C. bushiensis* and a second digenean *Sphaeridiotrema globulus* were both significantly more prevalent in salvaged ducks. Mean intensity of infection was consistently higher for *S. globulus* in the salvaged carcasses than in hunter-shot ducks. These data provide strong circumstantial support for the hypothesis that *C. bushiensis* continues to contribute to the annual mortality, but also strongly suggest that *S. globulus* may be involved. Comparison of the relative numbers of each species of duck between the salvaged carcass sample and the hunter-shot sample revealed that blue-winged teal (*Anas discors*) occurred significantly more frequently in the salvaged carcass sample whereas wood ducks (*Aix sponsa*) were common in the hunter-shot sample but were never found in the salvaged carcass sample. A comparison of susceptibility to experimental infection with *C. bushiensis* revealed that mallards (*Anas platyrhynchos*), black ducks (*Anas rubripes*), blue-winged teal, pintail (*Anas acuta*) and lesser scaup (*Aythya affinis*) had similar susceptibilities but that wood ducks were significantly less susceptible to infection. Experimental infections also showed that age of mallards significantly influenced the number of parasites obtained 7 days postinfection; ducklings were most heavily infected. Significant differences were detected in the gross pathology caused by *C. bushiensis* among duck species. Data obtained from sentinel ducks placed in the field for 24 hr revealed that transmission of both *C. bushiensis* and *S. globulus* occurs at least between mid-May and mid-September and that large, potentially lethal, infections can be acquired in as little as 24 hr. Finally evidence is presented to suggest that ducks acquire resistance to challenge infections of *C. bushiensis*.
Key words: Ducks, *Cyathocotyle bushiensis, Sphaeridiotrema globulus*, Digenea, ecology, parasite-induced mortality, survey, experimental infection.

INTRODUCTION

Massive, late-summer mortality of dabbling ducks on the St. Lawrence River in southern Québec during the 1960's was attributed to the digenean *Cyathocotyle bushiensis* because this parasite was consistently recovered from the ceca of ducks found dead (Gibson et al., 1972). Late-summer mortality of dabbling ducks has continued to occur in southern Québec (R. Parent, pers. comm.; Demers, 1985).

Although recent reseach has contributed to knowledge of the ecology of larval stages of *C. bushiensis* (Ménard and Scott, 1987a, b), nothing is known about the ecology of this parasite in its definitive host. The objectives of this study were to determine whether *C. bushiensis* is still implicated in the recurring annual duck mortality, and to investigate aspects of the ecology of *C. bushiensis* in the definitive host. The effects of host age and species on susceptibility to infection, and gross cecal pathology were assessed. In addition, preliminary experiments were undertaken to determine whether ducks exposed to *C. bushiensis* acquire resistance to subsequent challenge infections and to investigate acquisition of *C. bushiensis* under field conditions.

MATERIALS AND METHODS

Field data

Ducks collected from the field were classified as "salvaged carcasses" or "hunter-shot ducks." Biologists who spent the summers in the field collected all moribund or dead duck carcasses seen during the period from August 1983 to September 1985 from five areas in southern Québec: Gentilly (46°24′N, 72°20′W), Lac St. Pierre (46°5′N, 73°10′W), Lac St. François (45°2′N, 74°30′W), Rivière du Sud (45°6′N, 73°14′W) and around the island of Montréal (45°22′N, 73°52′W and 45°25′N, 73°38′W). Those ducks with no obvious external cause of death were necropsied. The species, age and sex of each carcass was determined by cloacal and plumage characteristics (Bellrose, 1976; Larson and Taber, 1980) and the gastrointestinal tract was removed.

Gastrointestinal tracts of "hunter-shot ducks" were collected from three of the areas in southern Québec where duck mortality had been reported: Lac St. Pierre, Lac St. François and Rivière du Sud. These samples were collected on the opening weekend of hunting season (mid-September) from 1983 to 1985. Species, age and sex of each duck was recorded, and the gastrointestinal tracts were frozen for later examination.

Intestines were examined for large helminths using a dissecting microscope (5×). Smaller helminths were found by washing the intestinal contents through a sieve (180 μm) and examining the residue with a dissecting microscope (10×). In addition, the underside of the koilin layer of the gizzard was examined for nematodes with a dissecting microscope (5×); because this was a time-consuming process, only blue-winged teal (*Anas discors*) were examined.

A subsampling technique was used to estimate intensity of infection in ducks heavily infected (>500 worms) with *Sphaeridiotrema globulus* (Digenea). The washed intestinal contents were suspended in 50 ml of water and thoroughly mixed. A 2-ml aliquot was removed, the worms counted and the number used to calculate the total number of worms. The accuracy of this technique was evaluated by estimating the intensity of infection in 12 ducks through subsampling and then counting all worms individually. Spearman rank correlation analysis yielded a significant positive relationship between the two estimates ($r = 0.811$, $P < 0.01$, mean % difference = 21.4), indicating that this technique provided a reliable estimate for intensity in heavily infected ducks.

Helminths were fixed in 10% buffered formalin. Large helminths were stored in 70% alcohol prior to identification while smaller helminths were stained with acetocarmine, cleared in xylene and mounted in Permount. Because of the reasonably good condition of Digenea, emphasis was placed on quantifying digenean infections. Digeneans were identified using McDonald (1981); representative specimens have been deposited in the Parasite Collection of the National Museums of Canada (accession numbers NMCP 1986-0012 to 1986-0022).

Experimental infections—effect of age and species of duck

All ducks were obtained from the suppliers as ducklings (see Acknowledgments) and were reared in the laboratory at the Institute of Parasitology, Macdonald College, Ste-Anne de Bellevue, Québec, Canada H9X 1C0. They were assumed to be free from helminth infections. Water and commercial, non-medicated 18% poultry laying mash (Cooperative du Québec, Ste-Rosalie, Québec, Canada J0H 1X0) were supplied ad libitum. Ducks were classified into three age groups for this study. Ducklings were infected at <6 wk of age. Juveniles were approximately 3 mo old at the time of infection. Adults were in or beyond the postjuvenile molt (Karstad and Sileo, 1971). Adult mallards (*Anas platyrhynchos*) were approximately 6 mo old and adult blue-winged teal were approximately 14 mo old at infection. The effect of duck age on susceptibility to infection was investigated using 20 mallards from each age class and using seven juvenile and eight adult blue-winged teal. Interspecific comparisons were done using data from juvenile ducks: 20 black ducks (*Anas rubripes*), 20 pintails (*Anas acuta*), 18 wood ducks (*Aix sponsa*) and 10 lesser scaup (*Aythya affinis*) and the 20 juvenile mallards and seven juvenile blue-winged teal which had been used in the study of age effects. Both male and female ducks were used, but no attempt was made to investigate differences between sexes.

Metacercariae were obtained throughout the summers of 1984 and 1985 from naturally infected *Bithynia tentaculata* (Gastropoda: Prosobranchia) collected at the Rivière du Sud (45°6′N, 73°14′W) and at Lac St. François (45°2′N, 74°30′W) in southern Québec. Prior to each infection, a group of snails was crushed in mass, washed through a course sieve (425 μm) and *C. bushiensis* metacercariae were collected on a fine sieve (125 μm). Ducks were infected by intubation with 25 metacercariae. Only thick-walled, supposedly "mature" metacercariae (Gibson et al., 1972) were used. After infection, ducks were housed as a group in an indoor pen with ad libitum food and water. When 24-hr feces collections were made, ducks were individually housed in 63- × 46- × 38-cm rabbit cages.

Ducks were killed on Day 7 postinfection (PI). Food was removed 2 hr before sacrifice to allow intestinal tracts to empty, thus facilitating examination for worms. The ceca, colon and terminal section of the small intestine (equal in length to the ceca) were examined for *C. bushiensis* as described above.

Gross pathology was also quantified for juvenile ducks. However, because quantification of pathology was initiated part way through the study, data are only available for 10 mallards, nine black ducks, 20 pintails, seven blue-winged teal, 17 wood ducks and 10 lesser scaup. Three measures of gross pathology of the ceca were recorded. "Hemorrhagic spots" were the sites where *C. bushiensis* had been recently attached and feeding (Erasmus and Ohman, 1963). "Plaque formations" were caseous secretions attached to the wall of the ceca (Gibson et al., 1972). "Cores," apparently of fibrin and blood (Gibson et al., 1972), were secretions occupying part or all of the cecal lumen. To quantify the extent of hemorrhagic spots and plaque formations, affected ceca were gently pressed under a plexiglass plate and a dotted acetate sheet (7.8 dots/cm^2) was superimposed over the plate. Numbers of dots covering hemorrhagic spots, plaque formations and unaffected cecal wall were counted. Cores were weighed to the nearest 0.1 g.

Experimental infections—effect of primary infection on resistance to challenge infection

Eight juvenile call ducks (a domesticated mallard) were used for experimental infections to examine whether acquired resistance to *C. bushiensis* infection occurs. Ducks were housed as a group and were given ad libitum food and water. Five ducks were intubated on Day 0 with 25 *C. bushiensis* metacercariae as described above. To estimate worm establishment, feces were collected for 24 hr on Day 7 and washed through sieves. *Cyathocotyle bushiensis* eggs were trapped on a 45-μm sieve. These eggs were resuspended in 400 ml of water and eggs present in 10 aliquots were counted using a McMaster Worm Egg Slide (Hawksley and Sons Ltd., Marlborough Rd., Lancing, Sussex, United Kingdom BN15 8TN). The mean value was used to calculate eggs shed per 24 hr as an estimate of number of *C. bushiensis* (see next paragraph). On Day 15, when *C. bushiensis* from the initial infection should have even eliminated (Khan, 1962), the five ducks were reinfected with 25 metacercariae. Three additional ducks infected at this time served as controls for the challenge infection. Seven days later, all ducks were killed and examined as described above.

An additional 19 call ducks were infected with 25 metacercariae in order to quantify the per capita egg production of *C. bushiensis*. A 24-hr fecal sample was collected from each duck on Day 7. Each duck was then killed, and the number of *C. bushiensis* recorded.

Sentinel ducks

The experiments were conducted at Rivière du Sud (45°6′N, 73°14′W), located approximately 50 km southeast of Montréal, Canada in the St. Lawrence Lowlands (see Ménard and Scott, 1987b, fig. 1). This region supports breeding populations of blue-winged teal, mallards, black ducks and wood ducks (CTARS, 1984). Two cages were built in a part of the river intensively used by these species for feeding during late summer. Each cage covered approximately 3 × 3 m of water and was constructed from standard chicken wire mesh supported on 5-cm × 5-cm × 2.4-m wooden stakes. Cage sites were selected where late-summer water depth was expected to be about 0.5 m. When the cages were built in early May 1985, water depth was slightly <1 m. Water levels dropped more than anticipated and the area covered by Cage 1 became dry between the July and August sampling periods. Therefore, Cage 1 was moved into water (approximately 20 m towards midstream) prior to the August experiment.

Blue-winged teal were used as sentinel ducks because they are the species most commonly involved in the late-summer mortality (Gibson et al., 1972). Adult ducks, overwintered in the laboratory, were used in May, June and July; juvenile ducks were used in August and September.

Three wing-clipped ducks were placed together in each of the two cages for a period of 24 hr once during the middle of each month from May through September. Ducks were placed in the cages at midday and were removed the following midday, thereby allowing uninterrupted feeding during the evening and early morning feeding periods (Swanson and Sargeant, 1972; Stoddart, 1985). After removal from the cages, ducks were returned to the laboratory and maintained as described above. Ducks were killed after 5 to 7 days and the intestinal tracts were examined for helminths.

Statistical procedures

Definitions for prevalence, intensity and abundance are those of Margolis et al. (1982). Log-likelihood (*G*-test) and Fisher's exact tests were used to compare prevalences between "sal-

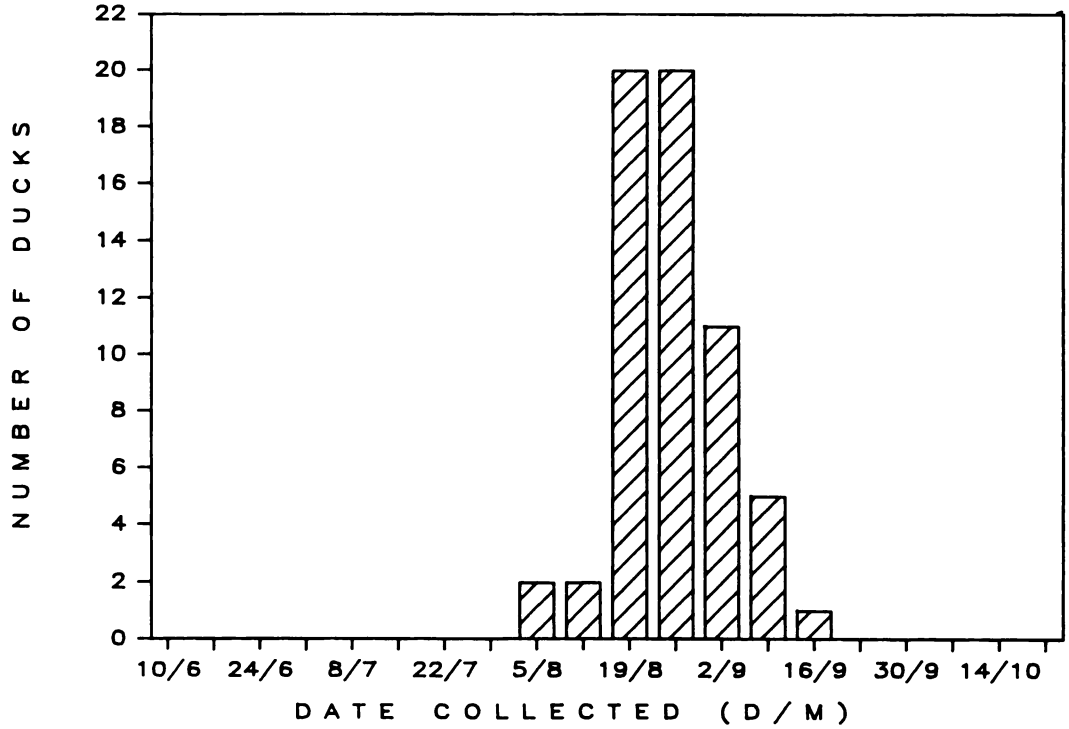

FIGURE 1. Frequency distribution of numbers of salvaged carcasses of ducks obtained per week. Data from 1983 to 1985 are pooled. (D, day; M, month.)

vaged carcasses" and "hunter-shot duck" samples. Mean intensities of infection and gross pathology measurements were compared using Kruskal-Wallis test (Rohlf, 1982). A non-parametric multiple comparisons test for unequal sample sizes (Zar, 1984) was used to locate non-significant subsets among means. Analysis of covariance was used on gross pathology measures (SAS, 1985). Abundance data were overdispersed ($s^2/\bar{x} > 1$); therefore parametric analyses were performed on transformed data. Abundance data on *C. bushiensis* and *S. globulus* were transformed ($\log_{10}(Y + 1)$) to normalize the data prior to Pearson product-moment correlation analyses (SAS, 1985). Simple linear regression (SAS, 1985) was used to determine whether cecal pathology was related to number of *C. bushiensis* per cecum. The level of significance was set at $\alpha = 0.05$.

RESULTS

Field data

Sixty-one salvaged carcasses were collected: Lac St. Pierre ($n = 25$), Gentilly (28), Rivière du Sud (3), Lac St. François (3) and around the island of Montréal (2). Salvaged carcasses were first found in early August but two-thirds (40) of all carcasses were collected during the last 2 weeks of August (Fig. 1); the numbers declined until mid-September. Three green-winged teal (*Anas crecca*) and one each of pintail, gadwall (*Anas strepera*) and shoveler (*Anas clypeata*) were collected but comparison of prevalence and mean intensity of infection were restricted to the three species most frequently collected; blue-winged teal ($n = 30$), black duck (14) and mallard (11).

In 1983, 132 hunter-shot ducks were collected from Lac St. Pierre; prevalence and intensity data were collected for *C. bushiensis*. Beginning in 1984, the prevalence of all helminths and intensity data for both *C. bushiensis* and *S. globulus* were recorded. A total of 135 ducks were collected from Lac St. Pierre, 78 from Lac St. François and 155 from Rivière du Sud in 1984. In 1985, 62 ducks were collected from Lac St. Pierre.

Comparison of the representation of

species in the two samples reveals two statistically significant differences. Blue-winged teal comprised 49% of the salvaged carcass sample (30/61) whereas they only comprised 8% of the hunter-shot sample (43/562). Wood ducks comprised 11% of the hunter-shot sample (60/562) but there were no salvaged wood ducks.

This study was not intended to be a complete survey of helminths of ducks in southern Québec. The objective was to determine if *C. bushiensis* or other Digenea might be associated with the observed annual mortality. Among the cestodes, nematodes and acanthocephalans, thoroughness of parasite identification was influenced by condition of specimens, and ease of identification. Examination of a few specimens indicated the presence of three cestodes (*Hymenolepis* spp., *Diorchis* spp. and *Fimbriaria fasciolaris*), an acanthocephalan (*Corynosoma* spp.), a cecal nematode (*Capillaria* sp.) and a gizzard nematode (*Epomidiostomum* sp.). The many specimens that were not carefully examined cannot be assigned with confidence to genera. Data are only presented for the three most common species in the salvaged carcass samples; blue-winged teal, mallards and black ducks. The general results are presented in Table 1. Only data on Digenea were analysed statistically.

Generally the digenean fauna of the salvaged carcass and the hunter-shot samples was similar (Table 1), but statistical comparison of the prevalences produced two noteworthy results. First, prevalences of *C. bushiensis* were significantly higher in salvaged carcasses than in hunter-shot ducks for all three host species. Second, prevalences of *S. globulus* were also significantly higher in the salvaged carcasses than in the hunter-shot ducks.

Mean intensity of *S. globulus* (Table 2) was consistently higher in salvaged carcasses than in hunter-shot ducks. Although mean intensity of *C. bushiensis* tended to be higher in salvaged carcasses, the differences were not always statistically different (Table 2). Both of these parasites were found also in the salvaged carcasses of the three green-winged teal (5, 22 and 1 *C. bushiensis*; 1,206, 793 and positive but not counted *S. globulus*), the pintail (101 *C. bushiensis* and 17,500 *S. globulus*), the gadwall (73 and 10, respectively) and the shoveler (114 and 11,500, respectively). Pearson product-moment correlation analyses (SAS, 1985) of log-transformed ($\log_{10}(Y + 1)$) abundance data revealed significant positive correlations between *C. bushiensis* and *S. globulus* in hunter-shot samples of blue-winged teal, green-winged teal and black ducks (Table 3).

The prevalence and intensity of *C. bushiensis* and *S. globulus* in all species of hunter-shot ducks are presented in Table 4. Both prevalence and intensity varied significantly among duck species for *C. bushiensis* ($G = 46.917$, df = 14, $P < 0.001$; $H = 23.4881$, df = 5, $P < 0.001$) and for *S. globulus* ($G = 54.140$, df = 14, $P < 0.001$; $H = 25.9526$, df = 8, $P < 0.001$).

Analyses of the effects of duck age and sex on infection prevalence and intensity were not attempted due to the small numbers of ducks in each subcategory.

Experimental infections—effect of age and species of duck

All ducks survived experimental infection with 25 *C. bushiensis* metacercariae and were killed 7 days PI. Gross signs of morbidity were not detected during the 7-day period of infection. Due to small sample sizes, the effect of host sex was not investigated.

In mallards, mean abundance of *C. bushiensis* on Day 7 PI (Table 5) differed significantly with age ($H = 9.745$, df = 2, $0.005 < P < 0.01$). Ducklings were most heavily infected. Mean abundance was not significantly different between juvenile and adult blue-winged teal ($H = 2.697$, df = 1, $0.10 < P < 0.25$).

Juvenile ducks were used for comparison of abundance among species (Table 5). Mean abundance on Day 7 PI differed significantly among species ($H = 21.075$, df = 5, $P < 0.001$). Wood ducks were lightly

TABLE 1. Prevalence of helminth infections in salvaged carcasses and hunter-shot ducks collected between 1983 and 1985.

	Blue-winged teal					Mallards					Black ducks				
	Salvaged			Hunter-shot		Salvaged			Hunter-shot		Salvaged			Hunter-shot	
Parasite	*n*	Prevalence %		*n*	Prevalence %	*n*	Prevalence %		*n*	Prevalence %	*n*	Prevalence %		*n*	Prevalence %
Digenea															
Cyathocotyle bushiensis	30	100	***[c]	43	33	11	73	***	194	8	14	86	***	110	19
Sphaeridiotrema globulus[a]	30	100	***	35	31	11	100	***	147	6	14	100	***	91	33
Notocotylus attenuatus[b]	23	39	NS	32	25	6	50	NS	134	15	12	17	NS	85	19
Zygocotyle lunata[b]	23	13	NS	32	6	6	33	NS	134	43	12	0	NS	85	37
Maritrema sp.[b]	23	30	NS	32	19	6	33	NS	134	16	12	0	NS	85	22
Psilostomatid[b]	23	70	**	32	25	6	33	NS	134	34	12	25	NS	85	34
Echinostoma revolutum[b]	23	78	***	32	13	6	83	NS	134	73	12	92	NS	85	75
Echinoparyphium recurvatum[b]	23	9	NS	32	47	6	33	NS	134	50	12	33	NS	85	66
Hypoderaeum conoideum[b]	23	4	NS	32	3	6	0	NS	134	7	12	0	NS	85	8
Levinseniella sp.[b]	23	9	NS	32	19	6	0	NS	134	32	12	0	NS	85	38
Cotylurids[b]	23	39	NS	32	59	6	50	NS	134	78	12	42	NS	85	74
Cestoda (intestinal)[b]	23	57	+	32	69	6	50	+	134	88	12	42	+	85	93
Acanthocephala[b]	23	48	+	32	63	6	50	+	134	68	12	50	+	85	58
Nematoda															
Cecal nematodes[b]	23	57	+	32	59	6	67	+	134	55	12	50	+	85	62
Gizzard nematodes[b]	23	100	+	18	100	—			—		—			—	

[a] Data on *S. globulus* were obtained from all ducks examined in 1984 and 1985.
[b] Parasites were not noted in ducks collected in 1983 or in the first 50 ducks examined in 1984.
[c] ** $P < 0.01$, *** $P < 0.001$, NS $P > 0.05$, + statistical comparison not made.

TABLE 2. Comparison of mean intensities of *Cyathocotyle bushiensis* and *Sphaeridiotrema globulus* infections between salvaged carcasses and hunter-shot samples of blue-winged teal, mallards and black ducks.

		C. bushiensis							*S. globulus*						
		Salvaged			Hunter-shot				Salvaged			Hunter-shot			
Duck species	Year	n	$\bar{x}$	SD	n	$\bar{x}$	SD	P	n	$\bar{x}$	SD	n	$\bar{x}$	SD	P
Blue-winged teal	1983	7	88.3	83.8	1	7.0	—		—	—	—	—	—	—	
	1984	9	59.3	55.1	13	39.0	55.7		9	12,417.3	9,977.3	11	2,770.2	2,611.7	
	1985	14	19.2	13.5	0	—	—		13	4,955.9	2,330.7	0	—	—	
	Total	30	47.4	56.6	14	36.7	54.2	>0.05	22[a]	8,008.3	7,424.7	11	2,770.2	2,611.7	<0.01
Mallard	1983	3	27.0	26.2	5	2.4	1.7		—	—	—	—	—	—	
	1984	3	29.3	26.5	7	1.7	1.5		4	5,084.0	4,896.7	8	250.0	368.2	
	1985	2	4.5	2.1	4	4.3	4.6		2	4,699.0	1,907.8	1	412.0	—	
	Total	8	22.3	22.8	16	2.6	2.6	<0.001	6	4,955.7	3,892.9	9	268.0	348.7	<0.01
Black duck	1983	2	97.0	84.9	1	8.0	—		—	—	—	—	—	—	
	1984	4	31.3	39.6	16	11.0	14.4		5	4,850.0	3,102.1	23	282.5	559.4	
	1985	6	7.5	6.6	4	9.8	8.6		7	5,245.6	1,687.0	7	224.1	432.7	
	Total	12	30.3	46.8	21	10.6	12.9	>0.05	12	5,080.8	2,256.8	30	268.9	526.1	<0.001

[a] Intensity data not available for one duck due to poor condition of carcass.

TABLE 3. Pearson product-moment correlation analyses between abundance[a] of *Cyathocotyle bushiensis* and *Sphaeridiotrema globulus* infection in hunter-shot ducks collected in 1984 and 1985.

Duck species	*n*	Correlation coefficient	$P > r$
Blue-winged teal	35	0.729	<0.01
Green-winged teal	43	0.483	<0.01
Mallard	147	0.147	>0.05
Black duck	91	0.621	<0.01

[a] Analyses done of $\log_{10}(Y + 1)$ transformation.

infected; mean abundance was higher and similar among all other species (Table 5).

In order to determine whether the pathology in a cecum was a function of the number of *C. bushiensis* present, regression analyses were performed using measurements of area covered by hemorrhagic spots, by plaque formation and weight of core as a function of worm numbers per cecum. In all species of ducks at least one of the parameters was significantly affected by the number of *C. bushiensis* per cecum (Table 6). Therefore, each infected cecum was treated as an individual sampling unit for subsequent analyses of gross pathology. The data are summarized in Table 7. Analysis of covariance correcting for parasite numbers revealed significant differences among duck species with respect to area of the cecum covered by hemorrhagic spots ($F_{6,139} = 12.12$, $P < 0.0001$), covered by plaques ($F_{6,139} = 23.54$, $P < 0.0001$) and with respect to the weight of the core ($F_{6,139} = 7.19$, $P < 0.0001$). Of particular interest was the high average core weight in blue-winged teal, despite the fact that blue-winged teal had the lowest average number of parasites per cecum and the smallest ceca (Table 7).

Experimental infections—effect of primary infection on resistance to challenge infection

Eggs of *C. bushiensis* were detected in feces of all five juvenile call ducks 7 days after the primary infection. In call ducks, *C. bushiensis* produce an average of 1,595 eggs/worm/day ($n = 19$, SD = 940.0) on Day 7 PI with no evidence of density-dependence over the range of intensities used (slope = −75.33, $t_s = 1.941$, df = 17, $0.05 < P < 0.10$). Therefore, Day 7 egg production was used to estimate the number of *C. bushiensis* present on Day 7 of the primary infection in each duck (Table 8). On

TABLE 4. Prevalence and intensity of *Cyathocotyle bushiensis* and *Sphaeridiotrema globulus* in hunter-shot ducks from southern Québec.

	C. bushiensis					*S. globulus*				
Duck species	*n*	Prevalence %	*n*	Intensity $\bar{x}$	SD	*n*	Prevalence %	*n*	Intensity $\bar{x}$	SD
Blue-winged teal	43	33	14	36.7	54.2	35	31	11	2,770	2,612
Green-winged teal	67	5	3	2.7	2.9	43	35	15	92	195
Mallard	194	8	16	2.6	2.6	147	6	9	268	349
Black duck	110	19	21	10.6	12.9	91	33	30	269	526
Mallard/black duck	6	0	0	—	—	6	17	1	39	—
Wood duck	60	3	2	2.0	0.0	60	8	5	4	6
Pintail	19	16	3	1.3	0.6	9	33	3	148	124
Gadwall	33	0	0	—	—	10	30	3	119	189
Widgeon	8	0	0	—	—	8	0	0	—	—
Shoveler	6	0	0	—	—	4	0	0	—	—
Ring-necked duck	12	0	0	—	—	12	8	1	1	—
Lesser scaup	1	0	0	—	—	1	0	0	—	—
Redhead	1	0	0	—	—	1	0	0	—	—
Common goldeneye	1	0	0	—	—	1	0	0	—	—
Hooded merganser	1	0	0	—	—	1	0	0	—	—

TABLE 5. Effect of age and species of duck on numbers of *Cyathocotyle bushiensis* recovered 7 days after experimental infection with 25 metacercariae.

Duck species	Age	n	Number of *C. bushiensis* $\bar{x}$[a]	SD	Range
Effect of age					
Mallard	D	20	9.35[c]	6.13	0–20
Mallard	J	20	5.15[bc]	5.35	0–18
Mallard	A	20	3.25[b]	4.13	0–12
Blue-winged teal	J	7	1.57[b]	1.62	0–4
Blue-winged teal	A	8	6.50[b]	6.07	0–17
Effect of species					
Wood duck	J	18	1.05[b]	1.95	0–7
Blue-winged teal	J	7	1.57[bc]	1.62	0–4
Black duck	J	20	3.90[bc]	4.42	0–14
Pintail	J	20	3.95[bc]	4.93	0–19
Mallard	J	20	5.15[c]	5.35	0–18
Lesser scaup	J	10	6.40[c]	3.84	1–13

[a] Column values with same superscripts (b, c) are not significantly different ($P > 0.05$).

Day 15, these five ducks were reinfected and three additional ducks were given a primary infection. Digenean intensity in the three control ducks on Day 22 was not significantly different from estimated intensities of the primary infection on Day 7 ($H = 2.721$, df $= 1$, $0.05 < P < 0.10$). On Day 22, no digeneans were found in any of the five challenged ducks (Table 8). Median *C. bushiensis* intensity of the challenge infection was significantly lower than in control infections and in the estimates of the primary infection ($H = 9.096$, df $=$ 1, $0.001 < P < 0.005$).

Sentinel ducks

In June and July three ducks escaped from sentinel Cage 2. Sentinel ducks acquired both *C. bushiensis* and *S. globulus* infections during the 24-hr exposure period (Table 9) in each month except June, when no *C. bushiensis* were acquired. Maximum daily acquisition was 14 *C. bushiensis* and 2,757 *S. globulus*. In addition, one immature *Zygocotyle lunata* (Digenea) was found in one duck in August and small numbers (≤6) of immature *Echinostoma revolutum* (Digenea) were found in several ducks in August and September.

Three ducks died after 5 to 7 days in the laboratory. They were among the most heavily infected with *C. bushiensis* and *S. globulus* (Table 9).

DISCUSSION

Gibson et al. (1972) attributed the late summer epizootics of dabbling ducks in southern Québec to the digenean *C. bushiensis* on the basis of consistently high numbers of parasites in the dead ducks. Since their study, waterfowl biologists in southern Québec have continued to record late-summer mortality and to attribute the cause of death to *C. bushiensis*. As in the study by Gibson et al. (1972), most salvaged carcasses obtained in the present study were collected in late summer.

The first objective of the present study was to obtain further data on the role of

TABLE 6. Relationship between numbers of *Cyathocotyle bushiensis* per cecum and measures of gross cecal pathology in juvenile ducks.

Duck species	n[a]	Total area (cm²) covered by: Hemorrhagic spots t	Hemorrhagic spots $P > t$	Plaques t	Plaques $P > t$	Weight (g) of core t	Weight (g) of core $P > t$
Mallard	20	4.599	<0.001	8.103	<0.001	1.034	>0.05
Blue-winged teal	14	2.305	<0.05	2.116	>0.05	5.686	<0.001
Pintail	40	3.866	<0.001	2.095	<0.05	3.553	<0.01
Black duck	18	1.985	>0.05	4.295	<0.001	3.310	<0.01
Wood duck	34	7.316	<0.001	13.933	<0.001	8.597	<0.001
Lesser scaup	20	0.903	>0.05	3.920	<0.01	0.523	>0.05

[a] Each of the two ceca per duck was analysed as an independent unit.

TABLE 7. Comparison among species of gross cecal pathology in juvenile ducks 7 days after experimental infection with 25 *Cyathocotyle bushiensis* metacercariae.

Duck species	n[a]	Worms/cecum		Cecal area (cm^2)		Hemorrhagic spots (cm^2)		Plaque formations (cm^2)		Core weight	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Blue-winged teal	7	1.6	0.5	4.82	1.08	0.09	0.10	0.59	0.82	0.49	0.25
Wood duck	10	1.9	1.2	10.58	2.59	0.12	0.09	1.28	0.96	0.33	0.51
Black duck	15	2.7	1.9	10.46	1.95	0.14	0.15	1.08	1.25	0.39	0.73
Lesser scaup	19	3.4	2.3	8.94	1.97	0.10	0.08	2.27	2.56	0.38	0.37
Pintail	21	3.8	3.0	9.19	1.95	0.37	0.34	0.69	0.63	0.37	0.63
Mallard	14	4.4	4.0	10.49	1.92	0.20	0.14	2.13	2.68	0.03	0.11

[a] Number of infected ceca.

C. bushiensis in the mortality of dabbling ducks, through a comparison of levels of infection (prevalence and intensity) between salvaged carcasses and hunter-shot ducks. Such a comparison did provide strong circumstantial support for the involvement of *C. bushiensis* as a factor in the late-summer mortality. However, this study also implicated a second digenean, *S. globulus*. Both species were more prevalent among the salvaged carcasses, and the mean intensity of *S. globulus* infection was significantly higher among salvaged carcasses compared to hunter-shot ducks. Although prevalences of both *Maritrema* sp. and a psilostomatid (Digenea) were significantly higher in salvaged blue-winged teal than hunter-shot blue-winged teal, this difference was not seen in mallards or black ducks (Table 1). Neither prevalence nor intensity of infection with other digeneans differed between the salvaged carcass and the hunter-shot samples. Data on cestode, acanthocephalan, and nematode infections were not compared because of the lack of species specific identifications. Gross signs of viral or bacterial pathology or of lead poisoning were not observed in the salvaged carcasses.

TABLE 8. Results from experiment designed to determine whether a primary infection with *Cyathocotyle bushiensis* has an effect on success of a challenge infection.

Duck number	Primary infection: EPD (day 7)	Primary infection: Estimated number of worms (7 DPI)[a]	Challenge infection: Number of worms day 22 (7 DPC)[b]	Control on challenge: Number of worms day 22 (7 DPI)
1	19,476	12	0	—
2	1,868	1	0	—
3	5,336	3	0	—
4	4,536	3	0	—
5	7,204	5	0	—
6	—	—	—	4
7	—	—	—	17
8	—	—	—	14

[a] DPI = days post first infection.
[b] DPC = days post challenge infection.

In comparing parasite infections between salvaged carcasses and hunter-shot ducks, it is important to realize that salvaged carcasses were collected between 1 and 6 wk earlier in the year (average 3.1 wk) than the hunter-shot ducks which were collected in mid-September. This presents a possible bias in the data, because the profile of transmission may have changed. However, two observations suggest that transmission of both parasites continues through September. First, immature *C. bushiensis* and *S. globulus* were found in hunter-shot ducks. Second, both parasites were acquired by sentinel ducks in mid-September at rates similar to mid-July and mid-August. Therefore, although not conclusive, the comparison of helminth fauna between the two samples does suggest that *S. globulus* and *C. bushiensis* may be involved in the annual duck mortalities.

Blue-winged teal comprised almost 50% of the salvaged carcass sample, whereas only 8% of the hunter-shot ducks were blue-

TABLE 9. Seasonal acquisition of *Cyathocotyle bushiensis* (*Cb*) and *Sphaeridiotrema globulus* (*Sg*) by blue-winged teal sentinels over 24-hr exposure period.

Individual data

	May		June		July		August		September	
Cage	*Cb*	*Sg*	*Cb*	*Sg*	*Cb*	*Sg*	*Cb*	*Sg*	*Cb*	*Sg*
1	1	0	0	2	14[a]	2,117[a]	13[a]	2,757[a]	7[a]	1,161[a]
	0	0	0	0	0	222	1	1,078	4	843
	3	8	0	3	4	971	3	1,806	2	277
2	0	0	0	10	0	4	2	1,694	1	828
	0	0	—[b]	—	0	32	0	530	1	1,040
	0	0	—	—	—	—	0	135	0	879

Mean values

Month	*C. bushiensis* mean	*S. globulus* mean
May	0.7	1.3
June	0.0	3.8
July	3.6	669.2
August	3.2	1,333.3
September	2.5	838.0

[a] Duck died in laboratory.
[b] — Duck escaped from sentinel cage.

winged teal. This latter value compares well with records of opening day of hunting season waterfowl bag checks from 1984 (Dolan, 1984) suggesting that our sampling of the hunter-shot population was representative. Although blue-winged teal are known to be early fall migrants (Bellrose, 1976) the high number of blue-winged teal found dead in late summer suggests that they may be particularly susceptible to the effects of infection. Gibson et al. (1972) also reported that blue-winged teal and black ducks were the species most frequently found dead in the late 1960's.

Sphaeridiotrema globulus is a reported pathogen of lesser scaup (Price, 1934), canvasbacks (Cornwell and Cowan, 1963), domestic ducks (Campbell and Jackson, 1977), mute swans (Roscoe and Huffman, 1982) and coots (Trainer and Fischer, 1963). The pathogenicity of *S. globulus* also has been demonstrated experimentally in domestic ducklings infected with 1,000 metacercariae (Macy, 1973). Our data strongly suggest that *C. bushiensis* and/or *S. globulus* may be important in the late-summer mortality, but it is not possible to eliminate involvement of other pathogens or factors in the mortality.

Mixed infections of *S. globulus* and *C. bushiensis* were found in 92% of salvaged carcasses. Among hunter-shot ducks, there also were significant positive associations between abundances of each parasite in blue-winged teal, green-winged teal and black ducks. One possible explanation is that the two parasites are acquired simultaneously through ingestion of snails infected with metacercariae. In southern Québec, *B. tentaculata* is the only species of snail known to act as first or second intermediate host for *C. bushiensis* (Gibson et al., 1972; Ménard and Scott, 1987b). *Sphaeridiotrema globulus* uses *B. tentaculata*, as well as other species of snails, as the first and second intermediate hosts (Huffman and Fried, 1983). Therefore, ducks consuming *B. tentaculata* may simultaneously acquire *C. bushiensis* and *S. globulus*, two potentially pathogenic parasites.

Our study has provided evidence to suggest that not all duck species become equally infected with these two parasites, and suggests that the pathological effects of the infections may differ depending on the species. Both prevalence and intensity of *S. globulus* and *C. bushiensis* varied among duck species. Blue-winged teal were in general more heavily infected than mallards, black ducks or green-winged teal. This was true for both salvaged carcass and hunter-shot samples.

Two hypotheses can be presented to account for differences among duck species in levels of infection: differences in innate susceptibility to infection or differences in rates of exposure associated with differences in feeding ecology. Experimental infections were undertaken in order to determine whether mallards, black ducks, blue-winged teal, pintail, wood ducks and lesser scaup were equally susceptible to experimental infection with *C. bushiensis*. The only species found to differ statistically was the wood duck; levels of infection were significantly lower than in the other species. Therefore, we suggest that varying levels of infection in the field among the other duck species may result from different rates of exposure to the metacercariae. Information is not yet available to comment on interspecific susceptibility to *S. globulus* infection.

Although duck age significantly influenced numbers of *C. bushiensis* recovered after a 7-day experimental infection in mallards, age had no significant effect in blue-winged teal. Scott (unpubl. data) has shown that the average life span of *C. bushiensis* in domestic ducks was longer in larger (older) compared to smaller (younger) birds. The effect of host age needs to be examined further because blue-winged teal sample sizes in the present study were small, and because of the apparent difference in results depending on the host species.

Because *C. bushiensis* is believed to be a pathogen in ducks, crude measures of gross pathology were made in the experimentally infected ducks. Hemorrhagic spots are created at the sites of attachment of the *C. bushiensis* where the epithelial cells lining the duck cecum are extracorporeally digested by the parasites (Erasmus and Ohman, 1963). The parasites apparently move from one spot to another and create a "trail" of hemorrhagic spots on the cecal wall. It is believed that plaque and core formations result from a combination of (1) parasite effects which cause leakage of blood and fibrin (Gibson et al., 1972) into the cecum and (2) intensity of the host response to the presence of the parasite. Plaques apparently form as a delayed response at the site of previous hemorrhagic spots.

As shown in Table 6, the degree of pathology associated with an average *C. bushiensis* did vary among species of ducks, but it is difficult to discern a common pattern. Average core weight was highest in blue-winged teal. This supports the earlier suggestion that blue-winged teal may be particularly affected by this parasite. Interestingly, none of the experimentally infected ducks died over the course of a 7-day infection in the laboratory. However, the maximum number of parasites found in these ducks was only 20.

Three of the sentinel ducks did die between 5 and 7 days after exposure to infection for 24 hr in the field. All three ducks were found dead in their cage in the morning, but signs of stress or illness were not seen the previous evening. It is presumed that their deaths were a result of intense infections with *C. bushiensis* and/or *S. globulus*. These three ducks had the highest numbers of *C. bushiensis* among all 27 sentinel ducks (7, 13, 14), and *S. globulus* infections were also high (1,161, 2,117, 2,757). Two points are of particular interest. First, 11 of the ducks experimentally infected with *C. bushiensis* had higher parasite numbers (≤ 20), but none of these died within 7 days. Second, two of the sentinel ducks acquired as many *S. globulus* (1,694, 1,806) as those ducks that died, but these ducks survived for 7 days in the laboratory. These observations may suggest that a synergistic interaction between *C. bushiensis* and *S. globulus* causes the mortality of wild ducks in southern Québec.

The results from the sentinel duck study clearly demonstrate that transmission of both *C. bushiensis* and *S. globulus* can occur at least between mid-May and mid-September. Additionally, these results indicate that large, potentially lethal infections can be acquired in as little as 24 hr. It is not possible, given the small numbers of ducks used, to say with confidence whether or not the seasonality of the duck mortality can be explained by the rate of transmission of *C. bushiensis* and/or *S. globulus*. However, data obtained by Ménard and Scott (1987b) has shown that the abundance of *C. bushiensis* metacercariae in the snail population of both Rivière du Sud and Lac St. François is lowest in late summer, and actually increases in the early fall. These results suggest that seasonality of transmission may not be the predominant cause of the late-summer mortality of ducks.

Macy (1973) demonstrated that small initial infections with *S. globulus* produced acquired resistance to reinfection. This finding has recently been confirmed by Huffman and Roscoe (1986). Using a similar protocol, ducks were examined to determine whether acquired resistance to *C. bushiensis* infections occurred. Of five ducks receiving both primary and challenge infections, all ducks became infected as a result of the primary infection, as shown by detection of *C. bushiensis* eggs in the feces. However, not one of the ducks was infected 7 days after the challenge infection. These data suggest that ducks may develop some degree of acquired resistance to reinfection with *C. bushiensis*.

There are no known previous surveys of intestinal helminths in ducks from southern Québec. *Sphaeridiotrema globulus* was reported from five of 34 wood ducks collected in Maine and Vermont (Thul et al., 1985). Other surveys of waterfowl parasites in Ontario and eastern Canada (Turner and Threlfall, 1975; Mahoney and Threlfall, 1978; McLaughlin and Burt, 1979) have not reported *S. globulus* or *C. bushiensis*, and *C. bushiensis* was not reported in the wood ducks examined by Thul et al. (1985). The distribution of *C. bushiensis* seems to be limited by the distribution of the intermediate host *Bithynia tentaculata*, which is known to occur commonly only in the lower Great Lakes and the St. Lawrence River drainage system, and in the mid-Atlantic United States (Clarke, 1981). Within this distribution, parasitism of *B. tentaculata* by *C. bushiensis* is geographically heterogenous (Ménard and Scott, 1987b). Although *S. globulus* has been reported from across North America (Price, 1934; Trainer and Fischer, 1963; Macy and Ford, 1964) including Ontario (Speckman et al., 1972) and New Jersey (Roscoe and Huffman, 1982), it seems to be a more frequent cause of mortality in diving ducks and swans than in dabbling ducks. Gibson et al. (1972) did not report *S. globulus* infection in the salvaged ducks from southern Québec in the late 1960's. It is probable that *S. globulus* also has a heterogenous geographical distribution associated with the abundance of intermediate hosts.

The following hypothesis is proposed as an explanation for the observed annual late-summer duck mortality. In the spring, behavioral spacing mechanisms act to disperse breeding ducks (Patterson, 1976) so that ducks breeding in southern Québec are not only found in the marshes of the St. Lawrence River, but are distributed far more widely. These areas may have few or no suitable intermediate hosts for *S. globulus* or *C. bushiensis* and therefore ducks breeding outside the St. Lawrenece River system may not be exposed to infection with either parasite over the summer. Later in the summer, the behavioral spacing mechanisms act less strongly and nutrition becomes a more important factor (Patterson, 1976). The marshes of the St. Lawrence River attract large numbers of ducks from surrounding areas (L. M. Soyez, pers. comm.). Also, Bellrose (1976) noted that between 250,000 and 500,000 blue-winged teal breeding in western Canada move eastward to Ontario and Québec during their fall migration. Many of these ducks may be "naive" to infection with *S.*

globulus and *C. bushiensis*. As shown by the experiment using sentinel ducks, "naive" ducks can acquire potentially lethal infections in as little as 24 hr. In contrast those ducks breeding in the highly productive marshes of the St. Lawrence River may be exposed to low numbers of metacercariae in the spring and early summer, and they may develop resistance to further infection.

Further research should examine: (1) the detailed geographic distribution of *S. globulus* and *C. bushiensis* in both duck and snail hosts; (2) the seasonal transmission dynamics of *S. globulus* in the intermediate hosts; and (3) the pathogenesis of mixed *S. globulus* and *C. bushiensis* experimental infections.

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