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ABSTRACT: The prevalence of *Toxoplasma gondii* in free-ranging chickens is a good indicator of the prevalence of *T. gondii* oocysts in the soil because chickens feed from the ground. The prevalence of *T. gondii* in 225 free-range chickens (*Gallus domesticus*) from Portugal was determined. Antibodies to *T. gondii* were assayed by the modified agglutination test (MAT) and found in 61 chickens with titers of 1:5 in 8, 1:10 in 6, 1:20 in 3, 1:40 in 23, 1:80 in 5, 1:160 in 4, 1:320 in 8, and 1:640 or higher in 4. Hearts, leg muscles, and brains of 15 seropositive (MAT 1:10 or higher) chickens were bioassayed individually in mice. Tissue from 38 chickens with titers of 1:5 or less were pooled and fed to a *T. gondii*–free cat. Feces of the cat were examined for oocysts, but none was found. *Toxoplasma gondii* was isolated from 16 of 19 chickens with MAT titers of 1:10 or higher. Genotyping of 12 of these 16 isolates with polymorphisms at the SAG2 locus indicated that 4 were type III, and 8 were type II. None of the isolates was lethal for mice. Phenotypically, *T. gondii* isolates from chickens from Portugal were different from those of *T. gondii* isolates from chickens from Brazil.

Toxoplasma gondii infections are widely prevalent in human beings and animals worldwide (Dubey and Beattie, 1988). Humans become infected postnatally by ingesting tissue cysts from undercooked meat, consuming food or drink contaminated with oocysts, or accidentally ingesting oocysts from the environment. However, only a small percentage of exposed adult humans develop clinical signs. It is unknown whether the severity of toxoplasmosis in immunocompetent persons is due to the parasite strain, host variability, or other factors.

Toxoplasma gondii isolates have been classified into 3 genetic types (I, II, III) on the basis of restriction fragment length polymorphism (RFLP; Howe and Sibley, 1995; Howe et al., 1997; Mondragon et al., 1998; Owen and Trees, 1999; Feuntes et al., 2001; Grigg et al., 2001; Ajzenberg et al., 2002; Boothroyd and Grigg, 2002; Jungersen et al., 2002; Aspinall et al., 2003; Ajzenberg et al., 2004; da Silva et al., 2005). The parasite used to be considered clonal with very low genetic variability. However, most of the information was derived from isolates from Europe and North America. On the basis of newer markers for genetic characterization and with the use of recently isolated strains from Brazil and French Guiana, a higher genetic variability has been revealed than previously reported (Ajzenberg et al., 2004; Lehmann et al., 2004).

We have initiated a worldwide study of *T. gondii* population structure. For this purpose, we have chosen the free-range chicken as the indicator host for soil contamination with *T. gondii* oocysts because they feed from the ground. Thus far, we have characterized strains from South America (Brazil [Dubey et al., 2002; Dubey, Graham, da Silva et al., 2003; Dubey, Navarro et al., 2003; Dubey, Gennari et al., 2006], Peru [Dubey, Levy et al., 2004], Guatemala [Dubey, Lopez et al., 2005], Venezuela [Dubey, Lenhart et al., 2005], Argentina [Dubey, Venturini et al., 2003; Dubey, Marcet, and Lehmann, in press], Colombia [Dubey, Gomez et al., 2005]), the Caribbean (Grenada, West Indies; Dubey, Bhaiyat et al., 2005), North America (United States [Dubey, Graham, Dahl, Sreekumar et al., 2003; Lehmann et al., 2003], Mexico [Dubey, Morales, Lehmann, 2004]), Africa (Egypt [Dubey, Graham, Dahl, Hilali et al., 2003], Mali, Kenya, Burkina Faso, and Democratic Republic of Congo [Dubey, Karhemere et al., 2005]), Asia (Sri Lanka [Dubey, Rajapakse et al., 2005], India [Sreekumar et al., 2003], Israel [Dubey, Salant et al., 2004]), and Europe (Austria [Dubey, Edelhofer et al., 2005]). These studies are still not complete, and virulence in mice and genetic diversity have been examined in some detail only for the isolates from chickens from Brazil (Dubey et al., 2002; Dubey, Graham, da Silva et al., 2003; Dubey, Navarro et al., 2003; Dubey, Gennari et al., in press). Nevertheless, a pattern is emerging that indicates isolates from South America are genetically distinct (Lehmann et al., 2004).

In this study, we attempted to isolate *T. gondii* from chickens from Portugal. The chickens were from 18 small farms in rural areas of the country. Most properties were at least 500 m apart (Table I). Initially, 43 chickens (batch 1) were purchased, bled, and killed in Porto, Portugal; serum, heart, leg muscles, and brain from each chicken were sent cold by air to Beltsville, Maryland. Subsequently, serum samples from 182 chickens were tested for *T. gondii* antibodies at the Porto laboratory, and tissues from 34 serologically positive chickens (20 in batch 2, 10 in batch 3, and 4 in batch 4) were sent to Beltsville for bioassays. Five to 9 days elapsed between killing of chickens and receipt of samples.

Sera of chickens were tested for *T. gondii* antibodies with 2-fold serum dilutions from 1:5 to 1:640 by the modified agglutination test (MAT) as described by Dubey and Desmonts (1987). Tissues of chickens were bioassayed for *T. gondii* infection. Brains, leg muscles, and hearts of 5 chickens in batch 1 with MAT titers of 1:10 or higher were each bioassayed in out-bred female Swiss Webster mice obtained from Taconic Farms (Germantown, New York), as described by Dubey et al. (2002). Tissues were pooled, homogenized in saline, digested in acidic pepsin, and washed; the homogenate inoculated subcutaneously into 5 mice. Hearts, leg muscles, and brains from chickens in batch 3 were processed individually. Because tissues from batch 2 were spoiled during transit from Portugal to Beltsville, leg muscles were retained at the laboratory in Portugal and bioassayed in mice; pepsin digests were inoculated into 4 mice (2 mice were immunosuppressed with dexamethasone; Canada et al., 2002).

Tissues from 38 chickens with titers <1:5 or less in batch 1 were pooled and fed to a *T. gondii*–free cat (Dubey et al., 2002). Feces of the cat were examined for shedding of *T. gondii* oocysts 3–14 days postingestion of the chicken tissues as previously described (Dubey, 1995). Fecal floats were incubated for 1 wk at room temperature to allow sporulation of oocysts and were bioassayed in mice (Dubey and Beattie, 1988). Tissue imprints of mice that died were examined for *T. gondii* tachyzoites or tissue cysts. Survivors were bled on day 40–42 postinoculation (PI), and a 1:25 dilution of serum from each mouse was tested for *T. gondii* antibodies with the MAT. Mice were killed 45–48 days PI and brains of all mice were examined for tissue cysts as described (Dubey and Beattie, 1988). The inoculated mice were considered infected with *T. gondii* when tachyzoites or tissue cysts were found in tissues.

Toxoplasma gondii DNA was characterized from the tissues of a single infected mouse from each group (Lehmann et al., 2000). The RFLP strain type of *T. gondii* isolates was determined by nested PCR on the SAG2 locus according to Howe et al. (1997).

Antibodies to *T. gondii* were found in 61 chickens with titers of 1:5 in 8, 1:10 in 6, 1:20 in 3, 1:40 in 23, 1:80 in 5, 1:160 in 4, 1:320 in 8, and 1:640 or higher in 4. *Toxoplasma gondii* was isolated from tissues of 16 chickens; from 5 of 5 chickens in batch 1, none of 20 chickens from batch 2, 7 of 10 chickens from batch 3, and 4 of 4 in batch 4. Of the 7 chickens in batch 3, *T. gondii* was isolated from the brain of 1, brain and heart of 1, hearts of 5, and leg muscles of 5 (Table I). In batch 4 *T. gondii* was isolated from the hearts of 2, brain of 1, and brain and heart of 1. None of the mice inoculated from tissues of in-

TABLE I. Isolation of *Toxoplasma gondii* from tissues of seropositive chickens from Portugal.

Chicken no.	Household designation, location	Chicken MAT titer	Isolation in mice from chicken tissues*			Genotype (isolate designation)
			Brain	Heart	Leg muscle	
1	Netos (Figueira da Foz) 40°14′N, 8°45′W	80	5/5†	n/a†	n/a†	III (TgCkPr1)
2	Mosteiró (Vila do Conde) 41°20′N, 8°42′W	80	5/5†	n/a†	n/a†	III (TgCkPr2)
3	Mosteiró (Vila do Conde) 41°20′N, 8°42′W	160	5/5†	n/a†	n/a†	III (TgCkPr3)
4	Mosterió (Vila do Conde) 41°20′N, 8°42′W	20	1/5†	n/a†	n/a†	II (TgCkPr4)
5	Mosterió (Vila do Conde) 41°20′N, 8°42′W	320	5/5†	n/a†	n/a†	III (TgCkPr5)
6	(Albergaria-a-Velha) 40°41′N, 8°28′W	80	0/5	4/5	4/4	II (TgCkPr6)
7	(Albergaria-a-Velha) 40°41′N, 8°28′W	20	5/5	5/5	0/4	II (TgCkPr7)
8	(Albergaria-a-Velha) 40°41′N, 8°28′W	640	0/5	0/5	4/4	II (TgCkPr8)
9	(Albergaria-a-Velha) 40°41′N, 8°28′W	640	0/5	5/5	4/4	II (TgCkPr9)
10	(Albergaria-a-Velha) 40°41′N, 8°28′W	640	0/5	5/5	4/4	II (TgCkPr10)
11	(Albergaria-a-Velha) 40°41′N, 8°28′W	80	5/5	0/5	0/4	II (TgCkPr11)
12	(Albergaria-a-Velha) 40°41′N, 8°28′W	320	0/5	5/5	0/4	II (TgCkPr12)
13	(Barcelos) 41°32′N, 8°37′W	160	5/5	5/5	ND‡	ND (TgCkPr13)
14	(Barcelos) 41°32′N, 8°37′W	320	5/5	5/5	ND	ND (TgCkPr14)
15	(Barcelos) 41°32′N, 8°37′W	40	5/5	5/5	ND	ND (TgCkPr15)
16	(Barcelos) 41°32′N, 8°37′W	160	5/5	5/5	ND	ND (TgCkPr16)

* (No. of *T. gondii*–positive mice)/(No. of mice inoculated).
† Mice were inoculated with pooled brain, heart, and leg muscle homogenates of chickens.
‡ ND = Not done.

fected chickens became ill or died. The cat fed tissues of 38 seronegative chickens did not shed oocysts. Genotyping of the 12 *T. gondii* isolates from chickens indicated that 8 were type II and 4 were type III; type I was not found.

In this study, *T. gondii* was isolated from all 5 seropositive chickens in the first batch, 0 of 20 chickens in batch 2, from 6 of 10 chickens in batch 3, and 4 of 4 chickens in batch 4. Attempts to isolate *T. gondii* from batch 3 of 20 seropositive chickens were not successful because the tissues had been stored at room temperature while in transit from Porto to Beltsville and were badly autolyzed; these data were not considered further.

Results of this limited study indicate that *T. gondii* isolates from Portugal are similar to those obtained from chickens from North America, Asia, and Africa and different from South American chicken isolates. The isolates from chickens from Asia, Africa, and North America were predominantly type II and type III and were not virulent for mice. *Toxoplasma gondii* genetic type prevalence and mouse virulence showed some heterogeneity among isolates from the chickens from South America studied so far. Seventy percent of 73 *T. gondii* isolates from asymptomatic chickens from Brazil were type I, with no type II; type III mouse virulent isolates were present (Dubey et al., 2002; Dubey, Graham, da Silva et al., 2003; Dubey, Navarro et al., 2003). All isolates from chickens from Portugal were avirulent for mice.

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Helminth Assemblages of the Turtle *Emydura macquarii* (Pleurodira: Chelidae) Queensland, Australia

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ABSTRACT: The helminth fauna of 76 *Emydura macquarii* from 3 river systems in central and northern Queensland was examined. Eleven species were found, including 2 nematodes, 6 trematodes, 1 aspidogastrean, 1 cestode, and 1 monogenean. Analysis of helminth diversity showed that the Fitzroy and Ross River turtles had communities of comparable diversity, but the helminth communities in Proserpine River turtles were much less diverse. The helminth communities in all localities were dominated by trematodes. *Polystomoides australiensis* was the most prevalent, being found in 60% of the Ross River turtles, 57% of the Fitzroy River turtles, and 46% of the Proserpine River turtles. *Notopronocephalus peekayi* was the most abundant species, with mean abundances of 5.9 in the Ross River turtles and 9.8 in the Fitzroy River turtles. Species richness, Simpson's Reciprocal Index, was highest, 4.68, for the Ross River helminth community, Sorensen's Qualitative Index showed 95% similarity between the Ross River and Fitzroy River communities, although Sorensen's Quantitative Index indicated only 35% similarity between the 2 sites. Host feeding patterns are likely the most important factor affecting species richness of the helminth infracommunities, as the majority of helminth species are transmitted by food-web interactions involving intermediate hosts.

Emydura macquarii (Gray, 1830) is a member of the Pleurodira, turtles characterised by sideways flexion of the neck vertebrae during head retraction. This group of turtles is limited to freshwater environments in the southern hemisphere and is the remnant of a much larger group with a cosmopolitan distribution. *Emydura* is 1 of 5 genera of chelid turtles currently recognized from Australia. Recent work by Georges and Adams (1996) has placed 2 of the previously accepted species, *E. krefftii* Gray and *E. signata* Ahl from north Queensland and northern New South Wales, as synonyms of *E. macquarii*. Prior to this change, *E. krefftii* was recognized in the north and *E. macquarii* in the southern region below Bundaberg (24°52′S, 152°21′E) (Georges and Adams, 1996). *Emydura macquarii* prefers slow flowing water and is mostly omnivorous, though juveniles and breeding females are predominantly carnivorous (Cogger, 1996).

The previously recorded helminths of *E. macquarii* include 2 nematodes (Ferguson and Smales, 1998), 5 monogeneans (Rohde and Pearson, 1980; Rohde, 1984; Pichelin, 1995), 11 digeneans (Platt and Pichelin, 1994; Platt and Blair, 1996; Cribb and Pichelin, 1997; Jue Sue and Platt, 1998a, 1998b; 1999; Ferguson et al. 2001; Platt and Brooks, 2001; Platt, 2003), and 1 aspidogastrean (Ferguson et al. 1999).

In the present study, we examined the helminths occurring in *E. macquarii* collected from north and central Queensland. Three coastal waterways, Ross River (19°16′S, 146°49′E), Proserpine River (20°24′S, 148°35′E), and Fitzroy River (23°22′S, 150°32′E), were sampled for *E. macquarii* between 1994 and 1998. One turtle from the Gordonvale River (16°45′S, 145°47′E), and 1 from the Burnett River (24°52′S, 152°21′E) were also examined.

Turtles were collected using drum nets, crab pots, or hand lines baited with ox heart. Following capture, turtles were held in freshwater in fiberglass tanks for no more than 24 hr before killing by cervical injection of an overdose (>5 ml/kg bodyweight) of Nembutal® (pentobarbitone sodium). Carcasses were dissected and all internal organs, eyes, mouth, and body cavity examined for helminths. On removal, all worms were washed in 0.7% saline. Nematodes were fixed in hot formalin. Trematodes were relaxed in 0.1% ethanol and fixed by pipetting into near-boiling formalin. Cestodes were relaxed in tap water then fixed in formalin. All helminths were identified to species level.

The prevalence and mean abundance, as defined by Bush et al. (1997), of each species of helminth were determined for each locality. Specialist and generalist species were assigned according to Kennedy (1995). Species richness was calculated using Simpson's Reciprocal Index (1/D). Sorenson's Qualitative (presence/absence), and Quantitative (abundance) Indices (Magurran, 1988) were used to measure similarity between the helminth communities from each locality.

The SPSS 10 statistical package was used for calculations, and unless otherwise specified, significance levels are at 0.05 (5%). All work for this project was carried out under CQU Animal Ethics Clearance No. 95/7-105. All collections were performed under Environmental Protection Agency Permit Nos H0/000119/95/SAA, N0/001662/97/SAA, and C6/000077/98/SAA. All material was deposited in the South Australian Museum.

In total, 78 *E. macquarii*, 37 males and 41 females, were collected and 11 species of helminths were identified (Table I). The most common group was the Digenea, with 5 genera from 4 families. The Aspidogastrea, Monogenea, and Cestoda were each represented by a single species and the Nematoda by 2 species from 2 families. Nine species, *Choanocotyle elegans* Jue Sue and Platt, 1998, *C. nematoides* Jue Sue and Platt, 1998, *Aptorchis aequalis* Nicoll, 1918, *Notopronocephalus peekayi* Cribb and Pichelin, 1997, *Pretestis laticaecum* Ferguson, Cribb, and Smales, 2001, *Sigmapera cincta* Nicoll, 1918, *Sychnocotyle kholo* Ferguson, Cribb, and Smales, 1999, *Camallanus chelonius* Baker, 1983, and *Spiroxys chelodinae* Berry, 1985 were found in the intestine. Of these, *S. kholo*, *Si. Cincta*, and *C. chelonius* occupied the same habitat, the region immediately posterior to the stomach. *Polystomoides australiensis* Rohde and Pearson, 1980 was found in the accessory bladders, and *Gigantolina elongata* (Johnston 1931) was free in the body cavity.

The prevalences and mean abundances of the helminth species from each locality are summarized in Table I. *Polystomoides australiensis*, *S. kholo*, and *N. peekayi* were the most prevalent and *N. peekayi* was the most abundant in the host population (Table I). No helminth species was found in all turtles and 3 (i.e., *A. aequalis*, *P. australiensis*, and *S. kholo*) were found in all 3 localities. *Pretestis laticaecum* was the only specialist helminth (i.e., occurring only in *E. macquarii*.) Two species, *Ch. nematoides* and *Pr. laticaecum*, also occur in other species of *Emydura*. The remaining 8 species were generalist parasites of the Chelidae. No additional species were found in the Gordonvale and Burnett River hosts.

TABLE I. Prevalence % (P) and mean abundance (A) of 11 helminth species found in *Emydura macquarii* from 3 localities in Queensland.

	Ross River		Proserpine River		Fitzroy River	
Number of hosts	15		10		51	
	P	A	P	A	P	A
Digenea						
Choancotyle elegans	13	0.2	—	—	2.0	0.02
Choancotyle nematoides	13	0.3	—	—	2.0	0.02
Aptorchis aequalis	27	0.8	30	1.7	25	3.2
Sigmapera cincta	20	3.8	—	—	33	4.0
Pretestis laticaecum	—	—	—	—	6	0.6
Notopronocephalus peekayi	33	5.9	—	—	41	9.8
Aspidogastrea						
Sychnocotyle kholo	53	2.0	10	0.6	49	1.3
Monogenea						
Polystomoides australiensis	46	0.7	60	0.8	57	0.8
Amphilinidea						
Gigantolina elongata	—	—	10	0.1	—	—
Nematoda						
Camallanus chelonius	66	3.7	—	—	16	0.4
Spiroxys chelodinae	20	0.4	—	—	35	0.8

The Fitzroy River community, with 10 helminth species, had the highest species richness, followed by 9 from the Ross River community and 4 from the Proserpine River community. Simpson's Reciprocal Index (I/D) was highest for the Ross River community with an I/D of 4.68 (15 hosts), followed by the Fitzroy community with an I/D of 3.48 (51 hosts), and lowest for the Proserpine River community with an I/D of 2.77 (10 hosts). Comparison of the Ross River and Fitzroy River communities using Sorenson's Qualitative Index showed 95% similarity, and these 2 communities had 55% and 43% similarity with the Proserpine River community, respectively. Sorenson's Quantitative Index, however, indicated that similarity was low for all sites; only 6% similarity between the Fitzroy and Proserpine River communities, 17% similarity between the Proserpine and Ross River communities, and 35% similarity between the Ross and Fitzroy River communities. There was a significant difference in the number of helminth species in hosts from each area (ANOVA, $F_{2,70} = 5.440$, $P = 0.006$). An a posteriori Tukey pairwise comparison test showed that the Proserpine River community had significantly fewer species than the Ross River and Fitzroy River communities.

Data from each of the localities were pooled across time and must, therefore, be treated with caution; nevertheless some observations about the helminth communities at each location can be made. Of the 11 helminth species present, only 4 occurred in turtles from the Proserpine River. Although the sample size from both the Ross River and Proserpine River turtles was small (15 and 10 individuals) compared to the Fitzroy River sample (51), only a single additional helminth species was recovered from the Fitzroy River sample. The low number of helminths recovered from the Proserpine River may, therefore, be indicative of a small available pool of species, rather than the small sample size. Plagiorchiids dominated the community in terms of abundance (Table I), even though *S. kholo* (aspidogastrid) and *P. australiensis* (monogenean) were more prevalent. Only 1 species of pronocephalid was recorded. In contrast, Cribb and Pichelin (1997) reported 3 species occurring in *E. macquarii* from southern Queensland. No spirorchiids

were recovered from this community, though they were expected, as Platt and Pichelin (1994) reported 1 species from southern Queensland, and Platt and Blair (1996) reported 2 species from northern Queensland. The choanocotyliid, *Auriculotrema lechneri*, found in northern chelids by Platt (2003), was not seen in this study.

In *E. macquarii*, 9 of the 11 helminths reported (Table I) are transmitted via dietary items. Four species use tadpoles, snails, or the occasional crayfish as hosts of infective stages (Jue Sue and Platt, 1998a, 1998b, 1999). Two species employ copepods as intermediate hosts, together with a variety of aquatic species (snails, tadpoles, frogs, and fish) as potential paratenic hosts (Anderson, 2000). One species uses a parastacid crayfish (Rohde and Georgi, 1983), another has a snail (Ferguson et al. 2001) as an intermediate host, and 1 encysts on algae (Angel and Manter, 1970). The only active colonizer among the parasites is *P. australiensis* (Rohde and Pearson, 1980); in this case, the infective stage swims to the turtle host. There is no life cycle information available for *N. peekayi*. This suggests that the structure of the helminth community is influenced by host feeding patterns. Molluscs and algae form a large proportion of the diet of *E. macquarii*, although other food items are eaten if available (Cogger, 1996). Such opportunistic feeding habits are congruent with this notion. Consequently infection would be random, dependant on both the availability of particular foods and their selection by the turtle host.

The communities of helminths in the Fitzroy and Ross Rivers were the most similar, with 9 species in common, whereas that in the Proserpine River had significantly fewer species. Random factors, i.e., the availability of infected food items and the food choices of the host, as indicated above, appear to be the determinants of the poor species richness found in the Proserpine River helminth community.

Potential species richness was never realized in any of the individual hosts or host populations. Of the 11 species found in this study, the greatest number of species in any 1 host was 6. The total number of species recorded for *E. macquarii* in Queensland is 19, and approximately 60% of these species were recovered in the present study. The findings in the present study are consistent with the conclusion of Bush et al. (1990) that the habitat of the host is an important determinant of species richness, and that the helminth infracommunities of *E. macquarii* are most likely the product of random or stochastic events (Esch and Fernandez, 1993; Poulin, 1996).

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