REVIEW ARTICLE

Toxoplasma gondii Infections in Chickens (*Gallus domesticus*): Prevalence, Clinical Disease, Diagnosis and Public Health Significance

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Impacts

- Worldwide prevalence of *T. gondii* infection in chickens is reviewed and the role of infected chickens in the epidemiology of toxoplasmosis in humans is assessed. The prevalence of *T. gondii* in backyard chickens and chickens from organic farms reached up to 100%.
- Prevalence of viable *T. gondii* in chickens from commercial indoor farms was low and the ingestion of meat from these chickens was considered a low risk of transmission to humans. Although *T. gondii* is rarely excreted in chicken eggs, raw eggs should not be consumed by humans, for fear of being infected with *T. gondii* and *Salmonella* spp.
- Chickens are resistant to clinical toxoplasmosis and there are only a few reports of confirmed toxoplasmosis in chickens.

Keywords:
Toxoplasma gondii; toxoplasmosis; chickens; prevalence; public health

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Summary

Chickens are considered one of the most important hosts in the epidemiology of *Toxoplasma gondii* infection because they are an efficient source of infection for cats that excrete the environmentally resistant oocysts and because humans may become infected with this parasite after eating undercooked infected chicken meat. The objective of this study is to review worldwide prevalence of *T. gondii* infection in chickens and to assess the role of infected chickens in the epidemiology of toxoplasmosis in humans. A very high prevalence of the parasite was found in chickens raised in backyards (up to 100%) and free-range organic (30–50%) establishments.

Introduction

Toxoplasma gondii infections are widely prevalent in human beings and animals worldwide (Dubey and Beattie, 1988; Dubey, 2009). Humans become infected post-natally by ingesting tissue cysts from undercooked meat, consuming food or drink contaminated with oocysts or by accidentally ingesting oocysts from the environment. Felids are the most important host in the life cycle of *T. gondii* because they excrete environmentally resistant oocysts. Cats become infected with *T. gondii* by eating infected tissues from intermediate hosts.

Toxoplasma gondii infection in free-range chickens (FR) is considered important as FR chickens are one of the best indicators for soil contamination with *T. gondii* oocysts because they feed from the ground, and tissues of infected chickens are considered a good source of infection for cats. Additionally, ingestion of infected chicken meat can be a source of infection for *T. gondii* infection in humans and other animals. Rarely, toxoplasmosis can cause clinical disease in chickens.

Role of Infected Free-range Chickens in the Life Cycle and Epidemiology of *T. gondii*

Direct detection of *T. gondii* oocysts in soil is difficult. Therefore, chickens have been used as one of the indicators of soil contamination by oocysts. In 2002, we initiated a worldwide survey of *T. gondii* infection in FR chickens with the ultimate objective of studying the genetic diversity of *T. gondii* on a worldwide basis (Dubey et al., 2002; Lehmann et al., 2006; Dubey and Su, 2009). The samples were obtained from FR chickens from properties that were 1–2 km apart, so that the isolates of *T. gondii* obtained were independent and all chickens were tested for *T. gondii.* Most chickens were adults because the objective was to isolate viable *T. gondii* and not to estimate prevalence. Serum and tissues were obtained for *T. gondii* evaluation. Sera were tested for antibodies to *T. gondii* using the modified agglutination test (MAT) and tissues were bioassayed using a standardized protocol (Dubey et al., 2002, 2007a). Prevalence data from this study and others are summarized in Tables 1–3. The data were grouped into three tables because of the differences in techniques used for parasite isolation, serological testing and the sources of chickens.

Serological prevalence using MAT

Worldwide serological prevalences in free-range chickens using the MAT performed in one laboratory are given in Table 1. Prevalence varied from 2% to 100% depending on the source of chickens. The MAT detects only IgG antibodies because the mercaptoethanol used in the test destroys IgM-like substances that interfere with the specificity of the test. Because the data on the specificity of MAT have not been evaluated in chickens, all antibody titres are given in Table 1.

Serological prevalence in FR chickens by non-MAT tests

Serological prevalences using tests other than the MAT are presented in Table 2. The reason for splitting the data by test used is that none of these serological tests listed in Table 2 have been validated for *T. gondii* infection in chickens, and some serological tests do not detect *T. gondii* antibodies in chickens (see section on serological diagnosis). Data from the former USSR and the former eastern European block countries were summarized by Pak (1970) and are not included here; most were based on the complement fixation test (CFT) that is unreliable for serological diagnosis of *T. gondii* in chickens (see serological diagnosis section).

Prevalence of viable *T. gondii* in FR chickens

Viable *T. gondii* was isolated from up to 100% of chickens examined (Table 3). Reasons for this variability could be many, including the age of the chicken, number examined, method of bioassay and the tissues bioassayed. Of the 149 infected chickens whose individual tissues were bioassayed, 89.5% (129 of 144) of hearts, 49.2% (67 of 136) of brains, 44.1% (15 of 34) of leg muscle and 18.6% (16 of 86) of pectoral muscle were found to be infected (Table 4). These findings are biologically interesting because *T. gondii* was considered a neurotropic parasite based on studies in rodents. However, there are now ample data from chickens and other animals indicating that *T. gondii* encysts in muscle more efficiently than in the brain (Dubey and Beattie, 1988).

Data in Tables 3 and 4 were based on bioassay in mice. In addition to isolation data in Table 3, *T. gondii* was isolated from 11 of 11 chickens from a farm in Massachusetts (Dubey et al., 2003a; Lehmann et al., 2003), one of 15 chickens from Vietnam (Dubey et al., 2008a) and five of 20 cats fed seronegative chickens from India. Bioassay in cats, although expensive, is more sensitive than bioassay in mice because large volumes (500 g) can be fed to a cat, and cats fed even few bradyzoites shed oocysts. In one study, cats fed tissues of 235 chickens from Egypt shed oocysts (ElMassry et al., 1990).

Chickens as efficient intermediate hosts for *T. gondii*

In my opinion, FR chickens play an important role in the epidemiology of *T. gondii* in the rural environment, perhaps more than rodents, because they are clinically resistant to *T. gondii* (see later section) and live longer than rodents. Cats fed naturally infected chicken tissues can shed millions of oocysts (Dubey et al., 2002). Cats fed tissues of acutely infected chickens can shed oocysts (Ruiz and Frenkel, 1980). Chickens fed *T. gondii* oocysts were killed 1–2 weeks later and their tissues were fed to cats. Cats fed tissues of chickens that had been infected 10 days earlier shed oocysts (Ruiz and Frenkel, 1980). Chickens can harbour mouse-virulent *T. gondii* without showing any clinical signs (Dubey et al., 2002).

Clinical Toxoplasmosis in Naturally Exposed Chickens

Chickens are considered resistant to clinical toxoplasmosis. There are only a few reports of clinical toxoplasmosis in chickens worldwide. Historically, toxoplasmosis was first described by Hepding (1939) in a hen from Germany. Microscopically, sciatic nerve neuritis, chorioretinitis and

Table 1. Serological prevalence of *Toxoplasma gondii* in free-range chickens from different countries using the modified agglutination test modified agglutination test (MAT)

Country	No. chickens tested	No. positive (%)	No. chickens with MAT titres						Reference
			5	10	20	40	100	160	
Argentina									
La Plata	29	19 (65.5)	1	1	6	2	2	7	Dubey et al. (2003e)
Santiago	61	25 (40.9)	6	1	2	5	4	7	Dubey et al. (2005h)
Austria	830	302 (36.3)	ND	50	69	53	40	90	Dubey et al. (2005b)
Brazil									
Amazon	50	33 (66)	3	2	1	1	2	24	Dubey et al. (2006a)
Grande do Sul	50	19 (38)	ND	0	1	2	5	11	Dubey et al. (2007b)
Pará	34	20 (58.8)	ND	1	1	2	2	14	Dubey et al. (2007b)
Paraná	40	16 (40)	3	1	3	1	2	6	Dubey et al. (2003d)
Rio de Janeiro	198	129 (65.1)	ND	21			108		Silva et al. (2003)
São Paulo	82	33 (40.2)	ND	2	1	2	6	22	Dubey et al. (2002)
Pernambuco, Rio Grande do Norte, Maranhão, Bahia, Ceará, Sergipe and Alagoas	152	81 (53.3)	26	9	4	1	6	35	de Oliveira et al. (2009)
Chile	85	47 (55.3)	6	4	4	3	9	21	Dubey et al. (2006b)
Colombia	77	32 (44.4)	4	3	1	1	8	15	Dubey et al. (2005c)
Costa Rica	144	60 (40.1)	16	5	2	3	5	29	Dubey et al. (2006c)
Democratic Republic of Congo (DRC)	50	25 (50)	7	7	6	1	0	4	Dubey et al. (2005e)
Egypt	121	49 (40.4)	11	4	4	8	10	12	Dubey et al. (2003b)
	108	51 (47.2)	ND	ND	25	4	ND	22	El-Massry et al.(2000)
	150[a]	28 (18.7)	ND	ND	28	ND	ND	ND	Deyab and Hassanein (2005)
Ghana	64	41 (64)	5	15	1	4	3	9	Dubey et al. (2008a)
Grenada	102	53 (52)	6	4	4	4	15	20	Dubey et al. (2005a)
Guatemala	50	37 (74)	11	7	11	1	1	6	Dubey et al. (2005g)
Guyana	76	50 (65.8)	4	1	5	7	6	27	Dubey et al. (2007a)
India									
Chennai (Madras), Tamil Nadu	185	73 (39.5)	ND	ND	15	20	12	26	Devada et al. (1998)
Mumbai	741	133 (17.9)		22	18	2	3	0	Sreekumar et al. (2003)
Indonesia	90	24 (26.6)	11	5	3	1	2	2	Dubey et al. (2008a)
Israel	96	45 (46.8)	3	2	0	5	5	30	Dubey et al. (2004c)
Italy	80	11 (13.7)	4	3	1	0	2	1	Dubey et al. (2008a)
Kenya	30	4 (13.3)	0	0	0	0	1	3	Dubey et al. (2005e)
Mexico	208	13 (6.2)	ND	3	4	1	1	4	Dubey et al. (2004b)
Nicaragua	98	84 (85.7)	10	8	7	9	11	35	Dubey et al. (2006d)
Nigeria	79	5	ND	3	1	1	0	0	Velmurugan et al. (2008)
Peru	50	13 (26)	1	0	3	0	0	9	Dubey et al. (2004a)
Poland	20	6 (30)	2	3	0	0	0	1	Dubey et al. (2008a)
Portugal	225	61 (27.1)	8	6	3	23	5	16	Dubey et al. (2006e)
Sri Lanka	100	39 (39)	8	8	4	5	5	9	Dubey et al. (2005i)
Uganda	85	40 (47)	9	10	5	12	1	2	Lindström et al. (2008)
USA									
Ohio	118	20 (16.9)	5	5	1	1	4	4	Dubey et al. (2003a)
Illinois	11	11 (100)	0	0	0	1	0	10	Dubey et al. (2007c)
Venezuela	46	16 (32)	1	2	0	2	2	9	Dubey et al. (2005f)
Vietnam	330	80 (24.2)	34	22	13	1	1	9	Dubey et al. (2008a)

[a]Samples were from a slaughterhouse and the MAT was performed in the laboratory of Deyab and Hassanein (2005).
ND, no data.

encephalitis were the main lesions. *Toxoplasma gondii*-like organisms were found only in the eye and were confined to the retina. Individual and groups of protozoa were present in all layers of the retina. There was also mononuclear cell infiltration in the optic nerve, choroid and the ciliary body. Diffuse lymphocytic infiltrates were present in the sciatic nerve, and the brain had focal gliosis. Hepding (1939) proposed a new name, *Toxoplasma gallinarum*, for the

Table 2. Serological prevalence of *Toxoplasma gondii* in chickens from different countries using serological tests other than modified agglutination test

Country	Source of chickens	No. tested	No. positive (%)	Test	Cut-off titre	Reference
Brazil	SH	17	3 (24)	IHAT	1 : 64	Ferraroni et al. (1980)
	FR	28	15 (53.6)	IFAT	1 : 16	Brandão et al. (2006)
Costa Rica	FR	471	191 (40.5)	IFAT	1 : 16	Abrahams-Sandi and Vargas-Brenes (2005)
Czech Republic	C	1120	1 (0.01)	DT	1 : 4	Literák and Hejlíček (1993)
	C	205	11 (5.3)	DT	1 : 4	Zástěra et al. (1965)
	C	570	1 (0.16)	IFAT	1 : 40	Bártová et al. (2009)
Egypt	SH	30	15	DT	1 : 8	Rifaat et al. (1969)
		85	17 (20)	DT	1 : 8	Rifaat et al. (1977)
	C	600	200 (33.3)	IHAT	1 : 2	Hassanain et al. (1997)
			320 (53.3)	CFT	1 : 2	Hassanain et al. (1997)
						Hassanain et al. (1997)
India	SH	46	10 (21.70)	IFAT	1 : 8	Sreekumar et al. (2001)
Iran	FR	45	23 (51)	LAT	1 : 8	Zia-Ali et al. (2007)
	FR	109	33 (30.2)	IHAT	1 : 20	Ghorbani et al. (1990)
Italy	FR	56	30 (73.1)	IFAT	1 : 8	Zardi et al. (1967a,b))
	C	120	35 (29.0)	IFAT	1 : 8	Zardi et al. (1967a)
	FR	16	4 (25.0)	IFAT	1 : 8	Zardi et al. (1967b)
Japan						
Osaka	C	197	9 (4.6)	DT	1 : 16	Izutani (1958)
Hokkaido						
Tokyo	C	42	0	DT	1 : 16	Sato (1960)
Niigata	C	9	0	DT	1 : 16	Katsube et al. (1968)
						Hagiwara et al. (1977)
	C	110	0	DT	1 : 16	Maitani (1970)
Jordan	SH	165	60 (36)	IHAT	1 : 64	Morsy et al. (1978)
Mexico	SH	93	14 (5)	DT	1 : 16	Roch and Varela (1966)
Nigeria	SH	250	112 (44.8)	IHAT	1 : 64	Aganga and Belino (1984)
China	SH	503	15 (3)	IHAT	1 : 64	Zhang et al. (1989)
	FR	308	107 (34.7)	ELISA		Zhu et al. (2008)
	Caged	210	6 (2.8)	ELISA		
Poland	SH	1200	11	DT, CFT	1 : 5	Grzywiński (1967b)
	SH	84	3 (3.5)	CFT	1 : 8	Umiński et al. (1961)
Thailand	FR	303	194 (64)	IFAT	1 : 16	Chumpolbanchorn et al. (2009)
Turkey	FR	300	5 (1.6)	DT	1 : 16	Inci et al. (1998)
	C	328	2 (0.6)	DT	1 : 16	Cicek et al. (2004)
	C	287	1	DT	1 : 4	Altinöz et al. (2007)
USA	FR	60	1 (1.6)	DT	1 : 4	Eyles et al. (1959)

SH, slaughterhouse; FR, free-range; C, commercial farm; IFAT, indirect fluorescent antibody test; IHAT, indirect haemagglutination antibody test; DT, dye test; CFT, complement fixation test; LAT, latex agglutination test.

organism in this hen. This case was considered to be a primary infection with Marek's disease virus with secondary involvement of toxoplasmosis. Sparapani (1950) in Italy, and Fankhauser (1951) in Switzerland reported toxoplasmosis in two chickens. However, confirmed toxoplasmosis was first reported by Erichsen and Harboe (1953a) in a flock of 40 White Leghorn hens from Norway. Clinical signs observed were anorexia, emaciation, diarrhoea, blindness and sudden death. Three dead and six chickens that were killed were examined at necropsy. *Toxoplasma gondii* was demonstrated in six chickens either histologically or by bioassay in mice inoculated with tissues of chickens. Histologically, *T. gondii* was found in five chickens; in sections of brains and hearts of three, liver and lungs of one and small intestinal wall of one. *Toxoplasma gondii* isolated from chickens was avirulent to mice. Main microscopical lesions were encephalitis, myocarditis and pericarditis, and mononuclear cell infiltrates in the choroid of two, in the sciatic nerve of one chicken (Erichsen and Harboe, 1953a,b). Subsequently, clinical toxoplasmosis in chickens was reported from Brazil (Nóbrega et al., 1954, 1955), Germany (Schulte, 1954; Schellner and Vollbrechtshausen, 1955; Denmark (Biering-Sørensen, 1956), Argentina (Mayer, 1961), Canada (Beauregard et al., 1965), Spain (Jover

Table 3. Isolation of *Toxoplasma gondii* by bioassay in mice from free-range chickens from different countries

Country	No. chickens	No. chickens bioassay positive (%)	Tissues bioassayed	Strain designation	Molecular data	Reference
Argentina						
Laplata	19	9 (47.3)	H + B	Yes	Yes	Dubey et al. (2003e, 2005h)
Santiago	22	17 (77.2)	H + B + M	Yes	Yes	Dubey et al. (2005h)
Austria	209	56 (26.7)	H = B	Yes	Yes	Dubey et al. (2005b)
Brazil						
Amazon	33	24 (72.7)	B + H	Yes	Yes	Dubey et al. (2006a)
Rio de Janeiro	96	67 (69.7)	B + H	Yes	Yes	Silva et al. (2003)
						Dubey et al. (2003c)
Paraná	16	13 (81.2)	H + B	Yes	Yes	Dubey et al. (2003d)
São Paulo	29	22 (75.8)	H + B	Yes	Yes	Dubey et al. (2002)
Pará	20	15 (75)	H + B	Yes	Yes	Dubey et al. (2007b)
Rio Grande du Sul	19	18 (94.7)	H + B	Yes	Yes	Dubey et al. (2007b)
Rio de Janeiro	20	6	H + B	No	No	Peixoto and Lopes (1990)
Minas Gerais	28	11	H	Yes	Yes	Brandão et al. (2006)
7 Northeastern states	81	23 (28.3)	H + B	Yes	Yes	de Oliveira et al. (2009)
						Dubey et al. (2008b)
Burkina Faso	40	1 (2.5)	H + B	Yes	Yes	Dubey et al. (2005e)
Chile	47	9 (19.1)	H + B	Yes	Yes	Dubey et al. (2006b)
Colombia	31	23 (74.1)	H + B	Yes	Yes	Dubey et al. (2005c)
Costa Rica	68	32 (47)	H + B	Yes	Yes	Dubey et al. (2006c)
	50	27 (54)	B + M	No	No	Ruiz and Frenkel (1980)
Czech Republic	10	2 (20)	H + B + M	No	NO	Literák and Hejlíček (1993)
DRC	25	10 (40)	H + B + M	Yes	Yes	Dubey et al. (2005e)
Egypt	49	19 (38.7)	H + B	Yes-this study[a]	Yes	Dubey et al. (2003b)
Ghana	32	2 (6.2)	H + B	Yes	Yes	Dubey et al. (2008a)
Grenada	53	35 (66)	H + B + M	Yes	Yes	Dubey et al. (2005a)
Guatemala	19	8 (42.1)	H + B + M	Yes	Yes	Dubey et al., 2005g)
Guyana	50	35 (70)	H + B	Yes	Yes	Dubey et al. (2007a)
India	186	0	H + B			Sreekumar et al. (2003)
Indonesia	24	1 (4.1)	H + B	Yes	Yes	Dubey et al. (2008a)
Iran	23	6 (26)	H + B	Yes	Yes	Zia-Ali et al. (2007)
	109	6 (5.4)	B	No	No	Ghorbani et al. (1990)
Israel	45	19 (42.2)	H + B	No	Yes	Dubey et al. (2004c)
Italy	5	2 (40)	H + B	Yes	Yes	Dubey et al. (2008a)
	176	7 (3.9)	B	No	No	Zardi et al. (1967a)
Kenya	4	1 (25)	H + B	Yes	Yes	Dubey et al. (2005e)
Mali	48	5 (10.4)	H + B	Yes	Yes	Dubey et al. (2005e)
Mexico	13	6 (46.1)	H + B	Yes-this study[b]	Yes	Dubey et al. (2004b)
Nicaragua	66	47 (71.2)	H + B	Yes	Yes	Dubey et al. (2006d)
Nigeria	5	1	H	Yes	Yes	Velmurugan et al. (2008)
Peru	13	10 (76.9)	H + B + M	Yes	Yes	Dubey et al. (2004a)
Poland	5	2 (40)	H + B	Yes	Yes	Dubey et al. (2008a)
Portugal	61	16 (26.2)	H + B + M	Yes	Yes	Dubey et al. (2006e)
Sri Lanka	36	11 (30.5)	H + B	Yes	Yes	Dubey et al. (2005i)
Uganda	85	9 (10.6)	H + B	Yes-this study[c]	Yes	Lindström et al. (2008)
USA						
Montana	11	3 (27.2)	H + B	No	Yes	Dubey (1981)
Illinois	11	11 (100)	H + B + M	Yes	Yes	Dubey et al. (2007c)
Tennessee	7	3 (42.8)	B	No	No	Gibson and Eyles (1957)
	60	0	B			Eyles et al. (1959)
Texas	11	7 (71.4)	H + B	No	No	Foster et al. (1969)
Iowa	12	11 (91.6)	H + B + M	No	No	McCulloch (1968)
Ohio	20	11 (55)	H + B	Yes-this study[d]	Yes	Dubey et al. (2003a)
Massachusetts	11	11	Bioassay in cats	Yes-this study[e]	Yes	Dubey et al. (2003a)

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Table 3. Continued

Country	No. chickens	No. chickens bioassay positive (%)	Tissues bioassayed	Strain designation	Molecular data	Reference
Venezuela	13	12 (92.3)	H + B + M	Yes	Yes	Dubey et al. (2005f)

[a]These isolates are now designated as TgCk Eg 1–19 from chicken nos. 7, 9, 10, 22, 25, 28 and 30 from batch 1, chickens 8, 20, 28 and 40 from batch 2 and chicken 18, 25, 26, 29, 37, 45, 48 and 49 from batch 3 respectively; and the TgCkEg 20 from faeces of cat fed pooled chicken tissues (see Dubey et al., 2003b for the source of each chicken).
[b]These isolates are now designated as TgCk Mx 1–6 from chicken nos. 2, 20, 30, 93, 13 and 14 respectively (see Dubey et al., 2004b for the source of each chicken).
[c]These isolates are now designated as TgCk Ug 1–9 from chicken nos. 1, 2, 17, 52, 68, 70, 79, 81 and 82 respectively (see Table 1 in Lindström et al., 2008 for source of each chicken).
[d]These isolates are now designated as TgCk UsOh1-11 from chickens listed serially in Table 2 of Dubey et al. (2003a).
[e]These isolates are now designated as TgCkUsMa1-11 from chickens listed serially in Table 3 of Dubey et al. (2003a). These isolates were obtained as oocysts by feeding tissues of each chicken to a separate cat.
H, heart; B, Brain; M, muscle.

Table 4. Distribution of *Toxoplasma gondii* in tissues of naturally infected chickens

Country	No. chickens positive	Brain	Heart	Leg muscle	Breast muscle	Reference
Brazil	24	16	17	ND	ND	Dubey et al. (2006a)
	6	1	6	ND	ND	Peixoto and Lopes (1990)
DRC	9	3	9	ND	3	Dubey et al. (2005e)
Grenada	35	24	33	ND	2	Dubey et al. (2005a)
Guatemala	8	4	8	ND	3	Dubey et al. (2005g)
Peru	10	0	10	ND	0	Dubey et al. (2004a)
Portugal	7	2	6	5	ND	Dubey et al. (2006e)
USA						
Montana	3	1	3	ND	ND	Dubey (1981)
Illinois	11	5	11	8	ND	Dubey et al. (2007c)
Maryland	4	0	ND	1	ND	Jacobs and Melton (1966)
Iowa	12 (in pools of 2 chickens)	ND	11	1	5	McCulloch (1968)
Texas	7 (in pools of 2 chickens)	4	5	ND	ND	Foster et al. (1969)
Venezuela	12	7	10	ND	3	Dubey et al. (2005f)
Total	148	67 of 136 (49.2%)	129 of 144 (89.5%)	15 of 34 (44.1%)	16 of 86 (18.6%)	

ND, no data.

Moyano and Miranda Garcia, 1970), People's Republic of China (Liu et al., 1983; Li and Chen, 1990), the USA (Ostendorf and Henderson, 1962) and Czech Republic (Zástěra et al., 1965). Clinical signs reported were anorexia, jerking of neck and head, paralysis and loss of eye sight. Biering-Sørensen (1956) regarded necrosis of the optic chiasma as pathognomonic of toxoplasmosis in chickens. Out of three chickens (one dead, two severely ill) from a farm in Regensburg, Germany, Schellner and Vollbrechtshausen (1955) isolated viable *T. gondii* by bioassay of chicken brains into mice; one of these chickens also had Marek's disease. Additional details of early reports in chickens are given by Pak (1970) and Siim et al. (1963).

Clinical toxoplasmosis with these characteristics has not been reported in the literature in the last 40 years. Whether these reports were primary toxoplasmosis or were complicated by unrecognized viral infections is speculative because to my knowledge there is no archived material for a retrospective study. There are two recent reports of clinical toxoplasmosis in chickens from the USA (Goodwin et al., 1994; Dubey et al., 2007c). Goodwin et al. (1994) reported peripheral neuritis in three chickens from Georgia and the diagnosis was made immunohistochemically; however, herpes virus infection (Marek's disease) could not be ruled out. More recently, out of a group of 14 backyard chickens in Illinois, three birds died suddenly. Torticollis, an inability to stand, and lateral recumbency were the only clinical signs. One of these birds was necropsied. Marked lesions were limited to the brain which had multiple areas of necrosis, perivascular lymphocytic cuffs and gliosis. The protozoa in the brain reacted positively with *T. gondii* antibodies. An unusual finding was the presence of numerous tissue cysts and tachyzoites in the lesions. The remaining 11 chickens

remained asymptomatic and all contained viable *T. gondii* (see Table 3).

Pathogenesis of Clinical Disease

In general, chickens are resistant to clinical toxoplasmosis. Overall, attempts to reproduce the clinical syndrome described by Erichsen and Harboe (1953a) have been unsuccessful. Before the routine use of tissue culture to grow *T. gondii* in the laboratory, embryonated chicken eggs were used to propogate *T. gondii*. The parasite was inoculated on the chorioallointic membrane where it grew profusely producing pock-like lesions (MacFarlane and Ruchman, 1948; Jacobs and Jones, 1950; Jacobs and Melton, 1954). Chickens that hatched from intra-egg inoculation developed clinical toxoplasmosis (Wolfson, 1942; Čatár, 1961; Kinjo, 1961, 1972b; Abbas, 1967; Nockiewicz, 1972; Caballero-Servin, 1974; Que et al., 2004). Before the discovery of the natural (oral) route of transmission and the environmentally resistant stage of the parasite (oocyst), chickens of different ages were inoculated with *T. gondii* tachyzoites by parenteral routes (intramuscularly, intraperitoneally, subcutaneously or intracranially). These parenterally inoculated chickens generally remained asymptomatic (Geißler, 1952, 1955; Harboe and Erichsen, 1954, 1955a,b; Nóbrega et al., 1955; Jones et al., 1959; Kinjo, 1961, 1972a; Kulasiri and Prasad, 1961; Simitch et al., 1961b, 1966; Kulasiri, 1965; Bickford and Saunders, 1966; Boch et al., 1966; Jacobs and Melton, 1966; Grzywiński, 1967a,c; Maitani et al., 1968; Samir, 1969; Sokolov, 1970; Mahajan et al., 1982). Most severe disease was produced in chickens inoculated with *T. gondii* directly in the brain (Bickford and Saunders, 1966). One-day-old chickens inoculated intracerebrally developed incoordination, ataxia, torticollis and opisthotonos, and *T. gondii* was demonstrable in the brain histopathologically. Control chickens inoculated intramuscularly with a similar inoculum remained asymptomatic (Bickford and Saunders, 1966). Chickens fed *T. gondii* tissue cysts became infected but remained asymptomatic (Simitch et al., 1961a; Boch et al., 1966; Jacobs and Melton, 1966; Miller et al., 1972; Kaneto et al., 1997).

Chickens inoculated orally with *T. gondii* oocysts also remained asymptomatic or developed a mild illness (Miller et al., 1972; Biancifiori et al., 1986; Dubey et al., 1993; Kaneto et al., 1997). Of 13 hens each inoculated with 5000 oocysts directly into the crop, none became sick. The 13 hens given 50 000 oocysts also remained asymptomatic with the exception of a decrease in egg production and high mortality in embryonated eggs (Biancifiori et al., 1986). Dubey et al. (1993) examined the effects of infection induced by feeding oocysts of two (Me-49, GT1) strains of *T. gondii* in 1-month-old chickens. None of the 17 chickens fed 1000, 10 000 or 100 000 oocysts of the Me-49 strain developed clinical signs, whereas all five chickens fed 100 000 oocysts of the GT1 strain were anorectic 4–7 days p.i. and one bird died day 7 p.i; focal necrotic lesions were seen in the intestines, liver and spleen of this bird. Tissues of all chickens that were killed days 15–58 p.i. were examined histologically; a single tissue cyst was found in the brain of a chicken day 15 p.i. Of five chickens whose tissues were bioassayed day 68 p.i.; *T. gondii* was isolated from the brains of all five, heart of three and leg muscles of two, but not from the pectoral muscle and liver of any chicken (Dubey et al., 1993).

One-month-old broilers fed 500, 5000 or 50 000 oocysts of the P strain of *T. gondii* remained asymptomatic, except pyrexia in chickens fed 50 000 oocysts (Kaneto et al., 1997). In another related experiment by the same research group, *T. gondii* infection induced by feeding oocysts to broiler chickens was aggravated when co-infected with *Cryptosporodium baileyi* (Meireles et al., 1995).

Serological Diagnosis

Several serological tests, including the Sabin-Feldman dye test (DT), complement fixation test (CFT), complement inhibition test, indirect haemagglutination test (IHAT), indirect fluorescent antibody test (IFAT), latex agglutination test (LAT), enzyme-linked immunosorbent assay (ELISA) and the MAT, have been used to detect antibodies to *T. gondii* in sera of chickens. In the following section, I have attempted to evaluate efficiencies of these tests to detect antibodies in chicken sera.

Dye test

The DT is one of the most sensitive and specific tests for the diagnosis of toxoplasmosis in humans. However, it does not work with chicken sera or gives erratic results. *Toxoplasma gondii* has been isolated or seen histologically in tissues of naturally infected chickens that had no demonstrable antibodies in a 1 : 2 dilution of chicken sera (Erichsen and Harboe, 1953a; Nóbrega et al., 1955; Gibson and Eyles, 1957; Ruiz and Frenkel, 1980). The failure to detect antibodies by the DT is not related to antigenic differences between mammalian and avian strains of *T. gondii* and does not apply to all birds because pigeons develop high DT antibody titres (Frenkel, 1981). The DT is a complement-mediated test and erratic results with DT in chickens may be related to fixation of the first component of complement (Frenkel, 1981). Chickens experimentally infected with *T. gondii* either did not develop antibodies detectable by the DT or had only low titres (Harboe and Erichsen, 1955a,b; Schellner and

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Vollbrechtshausen, 1955; Jones et al., 1959; Bickford and Saunders, 1966; Boch et al., 1966; Miller et al., 1972; Dubey et al., 1993). These findings, therefore, should be considered in interpreting serological surveys based on the DT (see Table 2).

Complement fixation test and complement inhibition test

The CFT, like the DT, did not detect antibodies to *T. gondii* in chicken sera (Erichsen and Harboe, 1953a; Nóbrega et al., 1955; Biering-Sørensen, 1956; Robertson et al., 1963). Harboe and Reenaas (1957) developed a complement inhibition test to detect *T. gondii* antibodies in chicken sera. It is unfortunate that most of the surveys for *T. gondii* in chickens in USSR were based on CFT. In the light of the low sensitivity of CFT, one should be cautious while evaluating survey data based on CFT (Table 2). Robertson et al. (1963) reported success in detecting *T. gondii* antibodies in experimentally infected chickens using an indirect CFT utilizing chicken sera that were heated to 60°C.

Indirect haemagglutination test

The IHAT is easy to perform and kits are available commercially, hence it has been used in some surveys to detect antibodies to *T. gondii* in chicken sera (Table 2). However, it is an insensitive test. In 13 experimentally infected chickens, its sensitivity was 46% and specificity of a negative test was 25% (Frenkel, 1981). Ghorbani et al. (1990) isolated *T. gondii* from six of 109 (5.4%) chickens from Iran; 30% had IHAT titres of 1 : 20 or higher and the parasite was isolated from five seropositive and one seronegative chicken.

Indirect fluorescent antibody test

There is limited information on the accuracy of the IFAT test for the detection of *T. gondii* antibodies in chicken sera. Chickens fed 1000 or 100 000 *T. gondii* oocysts developed IFAT titres between 7 and 14 days p.i. and titres peaked by 28 days p.i. (Sedlák et al., 2000). Sreekumar et al. (2001) isolated viable *T. gondii* from naturally infected IFAT-positive chickens, but did not provide quantitative data. Brandão et al. (2006) isolated viable *T. gondii* from tissues of nine of 15 (60%) chickens with IFAT titres of 1 : 16 or higher and from two of 13 (15.3%) seronegative free-range chickens from Brazil.

Latex agglutination test

Limited information is available concerning the LAT. The LAT is simple to perform and kits are available commercially. In seven chickens fed *T. gondii* oocysts, sera were tested by the LAT at 15, 35, 49 and 68 days p.i. (Dubey et al., 1993). Antibodies were detected by LAT in four of seven chickens at day 15, 7 of 7 chickens at day 35, six of six chickens at day 49 and one of four at day 68. The highest titer detected was 1 : 128. In conclusion, only low titers were detected and in at least three chickens, antibody titers had declined to undetectable level between day 50 and day 68 p.i. (Dubey et al., 1993). Zia-Ali et al. (2007) isolated *T. gondii* from six of 23 seropositive chickens in Iran; one of these birds had a LAT titer of 1 : 8, considered negative in this test.

Enzyme-linked immunosorbent assay (ELISA)

The ELISA test has the advantage that it can be automated and is convenient for large-scale surveys. In chickens fed oocysts, Biancifiori et al. (1986) studied the kinetics of IgG-ELISA using the soluble fraction of tachyzoites. They reported IgG titres of 1 : 800 or higher starting at day 12 p.i. and titres peaked to 1 : 12 800 at day 41 when the experiment was terminated. Dubey et al. (1993) reported on the ELISA values in 10 chickens fed the Me-49 strain of *T. gondii.* Seroconversion was observed by day 14 p.i. Chicken sera were diluted in buffered 7% NaCl (instead of 0.85%) aqueous solution (saline) because most chicken immuoglobulins precipitate when diluted with phosphate-buffered saline.

Modified agglutination test

The sensitivity and specificity of the MAT in naturally exposed chickens is under investigation in my laboratory using isolation of the parasite as a reference standard. Preliminary data in Table 3 suggest that the MAT is efficient in detecting *T. gondii* antibodies in chickens. In chickens fed oocysts, MAT was positive in 1 : 100 dilution of serum by day 15 p.i. (Dubey et al., 1993).

Chickens as Source of *T. gondii* Infection for Humans

Raw poultry eggs

Although *T. gondii* has been isolated from ovaries and oviducts of naturally infected hens (Jacobs and Melton, 1966; McCulloch, 1968; Foster et al., 1969; Peixoto and Lopes, 1990), shelled eggs were not to be found infected with *T. gondii* (Jacobs and Melton, 1966). From experimentally infected hens only one of 323 eggs had viable *T. gondii*; the infected egg probably had a few organisms because only one of the five mice inoculated with egg homogenate became infected (Jacobs and Melton, 1966).

Table 5. Isolation of *Toxoplasma gondii* from indoor chickens or from slaughterhouse

Country	Source	No. bioassayed	Tissue	No. positive (%)	Reference remarks
Brazil	C	50	H	0	Brandão et al. (2006)
Croatia	SH	716	B	3 (0.4)	Kuticic and Wikerhauser (2000)
Czech Republic	C	1097	B + H + M	4	Literák and Hejliček (1993)
Egypt	SH	235	M	0	ElMassry et al. (1990)
Germany	Farms	1636	B and/or H	5 (0.3)	Boch et al. (1968)
Iran	SH	109	B	6	Ghorbani et al. (1990)
Italy	Farm	176	B	7	Zardi et al. (1967a,b))
Poland	SH	1200	?	0	Grzywiński (1967b)
USA					
Nationwide	Retail meat	2094	Pectoral muscle	0	Dubey et al. (2005d)
Maryland	SH	108	Ovary, B, leg muscle	4 (ovaries 3, leg muscle 1)	Jacobs and Melton (1966)
Yugoslavia	C	786	B	2	Simitch et al. (1961a)

SH, slaughterhouse; C, commercial farm, B, brain; M, muscle; H, heart.

In another study, none of the 2214 eggs laid by experimentally infected hens was positive for *T. gondii* (Boch et al., 1966). In both of these experiments, hens had been inoculated parenterally with large doses of *T. gondii* tachyzoites or tissue cysts. Pak (1969) found viable *T. gondii* in six of 408 eggs laid by 22 experimentally infected hens, whereas Sokolov (1970) did not find *T. gondii* in any of the 115 eggs laid by experimentally infected hens. Additionally, none of the 550 eggs laid by hens fed 5000 or 50 000 *T. gondii* oocysts contained viable organisms (Biancifiori et al., 1986).

A false report of the presence of *T. gondii* in eggs of poultry (Pande et al., 1961) led Kunert and Werner (1963) to investigate the survival of *T. gondii* in eggs experimentally inoculated with tachyzoites. *T. gondii* survived in egg yolk and albumin eggs boiled for 3 min and yolk of eggs fried for 3 min. In spite of these discrepant findings, raw eggs should not be consumed by humans, for fear of acquiring *T. gondii* infection, but more importantly salmonellosis. In conclusion, raw hen eggs are unlikely to be a source of infection for humans.

Free-range chickens

Data summarized in Table 3 provide ample evidence that chickens raised not only in backyard operations but also in large commercial free-range operations (Dubey et al., 2004c, 2005b) harbour viable *T. gondii*. In many instances, especially in developing countries, these chickens are killed at home or in unsupervised slaughter facilities and the viscera are left for scavengers or are improperly disposed off. *T. gondii* infection can be transmitted if care is not taken to wash hands thoroughly after cutting meat and during cooking of meat; however, risk assessment studies have not been undertaken.

Indoor or chickens raised in confinement

In the USA, the per capita yearly consumption of poultry is estimated as 37.2 kg and approximately 8.5 billion chickens are killed for human consumption. In a recent survey, *T. gondii* was not isolated from any of the 2094 chicken meat samples obtained from retail meat stores in the USA (Dubey et al., 2005d). Results of this study do not negate that infected chickens are not a source of infection for humans for several reasons. First, in this study, chicken breasts were selected for sampling because of the experimental design that called for testing 1 kg of boneless meat for each sample, although the authors were aware that the prevalence of *T. gondii* in chicken breast is lower than that in other tissues. *T. gondii* was isolated from breast meat of only 18.6% of infected chickens (Table 5). Second, many of the chicken breasts had been injected with enhancing solutions that have a deleterious effect on *T. gondii.* Third, antibodies to *T. gondii* were found in 1.3% of the juice extracted from the breast meat using an ELISA with values six times higher than in control chicken sera (Dubey et al., 2005d). Overall, prevalence of viable *T. gondii* in chickens raised indoors was low (Table 5).

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