Parasitology

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Epidemiologic significance of *Toxoplasma gondii* infections in chickens (*Gallus domesticus*): the past decade

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Review

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Abstract

Toxoplasma gondii infections are common in humans and animals worldwide. Domestic free-range chickens (*Gallus domesticus*) are excellent sentinels of environmental contamination with *T. gondii* oocysts because they feed on the ground. Chickens can be easily infected with *T. gondii*; however, clinical toxoplasmosis is rare in these hosts. Chickens are comparatively inexpensive and thus are good sentinel animals for *T. gondii* infections on the farms. Here, the authors reviewed prevalence, the persistence of infection, clinical disease, epidemiology and genetic diversity of *T. gondii* strains isolated from chickens worldwide for the past decade. Data on phenotypic and molecular characteristics of 794 viable *T. gondii* strains from chickens are discussed, including new data on *T. gondii* isolates from chickens in Brazil. This paper will be of interest to biologists, epidemiologists, veterinarians and parasitologists.

Introduction

Toxoplasma gondii infections are prevalent in humans and animals worldwide. The ingestion of undercooked infected meat or the consumption of food and water contaminated with oocysts excreted in cat feces are the main sources of infection. Cats are everywhere and a single cat can excrete millions of oocysts that can remain viable in the environment for months under natural conditions. Estimation of oocyst contamination of the environment is difficult because of low numbers present in soil or water and because there are no molecular markers to distinguish live *vs* dead oocysts.

Domestic free-range (FR) chickens (*Gallus domesticus*) are excellent sentinels of environmental contamination with *T. gondii* oocysts because they feed on the ground, they are comparatively inexpensive, can be easily infected with *T. gondii* and seldom develop clinical toxoplasmosis (Ruiz and Frenkel, 1980; Dubey, 2010*a*, 2010*b*).

Until 2000, *T. gondii* was generally considered to have low genetic diversity and strains were considered clonal. Interest in genetic diversity of *T. gondii* was spurred because some isolates were found to be more virulent (as assessed in mice) than others and certain genotypes were associated with clinical toxoplasmosis in humans (Dubey, 2010*a*).

Beginning in 2000, a collaborative research project was initiated at the United States Department of Agriculture (USDA) facility in Beltsville, Maryland, and the project terminated in 2019. The main objective was to study the genetic diversity of *T. gondii* using DNA derived from live parasites. Our initial focus was South America because, until then, little was known of the genetic diversity of *T. gondii* in this part of the world. The plan was to obtain tissues from chickens and bioassay them in outbred Swiss Webster mice and in cats at Beltsville. Thus, the biology of isolates could be compared using identical conditions. The ease of availability and the cost of purchasing chicken was also a factor in selecting this host species. Secondly, there was no restriction on importing chicken tissues into the USA at that time compared with no imports of tissues from other livestock (pigs, sheep, goats, cattle). A decade later, restrictions on the import of chickens were imposed because of H5N1 virus infection. The greatest success was obtained through collaboration with scientists in several institutions in Brazil. It was possible to isolate viable *T. gondii* from most regions of Brazil (discussed later). This was very labour-intensive and costly research. Initially, a door to door survey of houses with backyard chickens in Rio de Janeiro was conducted. The chicken sampled were from properties that were about 1 km apart and no more than 10 chickens were sampled from each property (da Silva *et al.*, 2003; Dubey *et al.*, 2003*a*). It meant purchasing chickens from individual houses, holding them live at a local facility, euthanizing them a day before departure from Brazil, and bringing them personally or by overnight courier service to Beltsville for bioassay. The project was extended to 19 other countries (see Dubey *et al.*,

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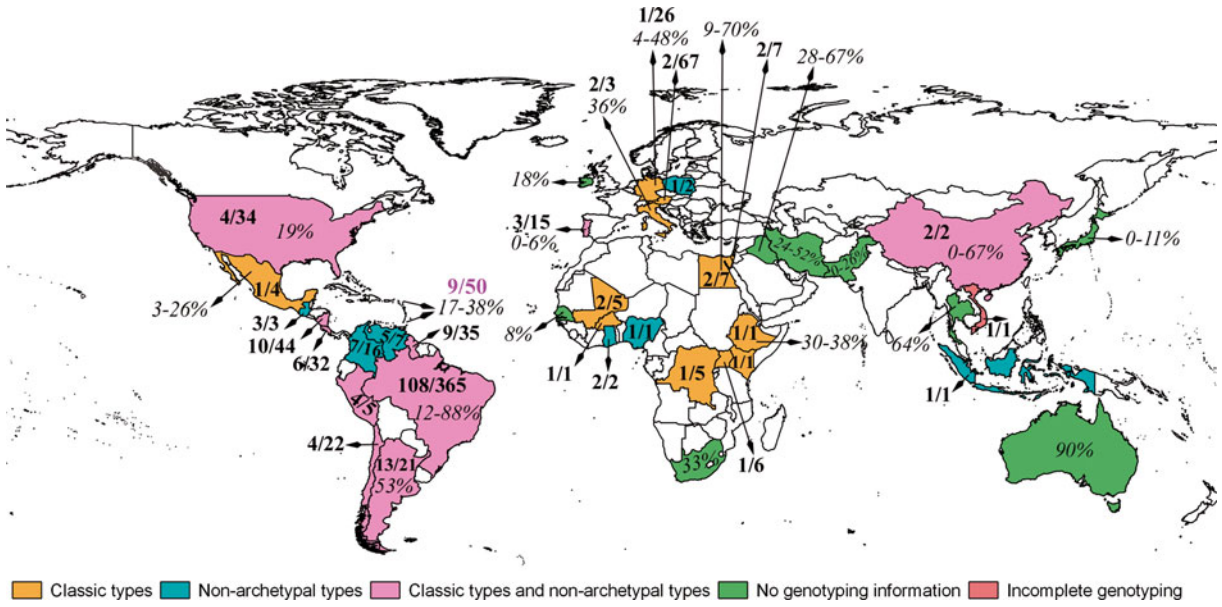

Fig. 1. Worldwide distribution of *T. gondii* infections in chickens. Numbers in bold are the number of *T. gondii* genotypes/number of viable isolates. Seroprevalences are given as %.

2016). At Beltsville, tissues were bioassayed in outbred Swiss Webster mice (five for each tissue) so that mortality data could be compared. A system was proposed to designate the *T. gondii* isolates-Tg (for *T. gondii*) Ck (for chicken) and the country (e.g. Br for Brazil). Information on all viable *T. gondii* isolates is scattered in many publications. Genotyping was performed using PCR-RFLP, and the results were published piecemeal as more markers were developed in the last two decades (Su and Dubey, 2020). We now summarize all data using 10 PCR-RFLP markers and correct errors in reporting.

Here, we review toxoplasmosis in chickens for the past decade, add new genotyping data, and correct mistakes in the literature. The review is divided into natural and experimental infections.

Natural infections

Prevalence

Serologic investigations

Worldwide serologic prevalences are summarized in Table 1 and Fig 1. Results varied with management, serological techniques and the cut-off values used. Virtually all chickens become infected after hatching because vertical transmission is extremely rare in chickens (Dubey, 2010*b*). Several studies documented an increasing prevalence with age (Table 1). Infections were higher in poorly managed farms. Infections were low (3.7% of 384) even in adult laying hens in large farms (>1000 per unit) compared with 11.7% of 470 backyard chickens in Germany; all chickens tested had outdoor access (Schares *et al.*, 2017*a*). Infections in caged chickens were lower than in FR chickens (Yan *et al.*, 2009; Cui *et al.*, 2010; Tian and Cui, 2010; Xu *et al.*, 2012; Mahmood *et al.*, 2014; Matsuo *et al.*, 2014; Rodrigues *et al.*, 2019; Duong *et al.*, 2020).

Results of different reports in Table 1 are difficult to compare because several serological tests with different cut-off values were used. Among those serological tests, the MAT was most commonly used (discussed later).

Several enzyme-linked immunosorbent assay (ELISA), indirect fluorescent antibody (IFA), and indirect haemagglutination (IHA) tests were used to detect *T. gondii* antibodies in chicken sera. Of these, the IHA is generally considered insensitive (Dubey, 2010*a*), but the results vary with the cut-off used, and the stability of reagents. In a study from Brazil, of 510 sera tested by MAT and IHA, the seropositivity was 40.4% by IHA (cut-off 1:16), compared with 38.8% by MAT (1:25) but different cut-off values were compared (Beltrame *et al.*, 2012). In another study from Brazil, four serological tests were compared: of 135 sera from chickens tested, *T. gondii* antibodies were detected in 67 (49.6%) by MAT (1:16), in 82 (60.7%) by IFA (1:16), in 49 (36.2%) by IHA (1:16) and in 81 (60.0%) by ELISA (Casartelli-Alves *et al.*, 2014) indicating variation among different tests.

The IHA test was used almost exclusively in surveys in China (Table 1); data on validation of this test in naturally infected chickens are not available. In the surveys that used IHA for *T. gondii* antibodies in chickens in China, the results of different studies are comparable because the same test kit and the same cut-off (1:64) were employed (Dong *et al.*, 2018).

Different antigens, including recombinant antigens and total lysate antigens (TLAs), and total soluble antigens (TSAs), have been employed in ELISA (Sun *et al.*, 2015). Similar results were obtained by using GRA1, GRA7, TSA and western blotting (Sun *et al.*, 2015). Comparable results were obtained by MAT (51.8%, 1:16) and an ELISA (48.1%) on 106 chicken sera in Iran (Hamidinejat *et al.*, 2014), and between IFA (17.2%, 1:16) and ELISA (21.2%) in Brazil (Millar *et al.*, 2012). Several immunoreactive proteins were identified in sera of experimentally infected chickens; these may be useful for use in western blots for confirmation of results obtained with other tests (Wen *et al.*, 2019).

Parasitologic investigation

Isolation of viable T. gondii: Results of isolation of viable *T. gondii* and genotyping of each isolate are summarized in Tables 2 and 3. Most isolations were made from FR chickens in Brazil.

Detection of T. gondii DNA: Results are summarized in Table 4. Results varied among different studies depending on the source of chickens, PCR method used, type and the amount of tissue tested. A study from Iran reported *T. gondii* DNA in tissues of 27 of 29 seropositive chickens (Asgari *et al.*, 2009). Usually, it is difficult to get good quality DNA from naturally infected tissues for genotyping; however, Zou *et al.* (2017) successfully genotyped four isolates as ToxoDB genotype #9. Given that PCR is known to be sensitive to contamination, it is often difficult to assess the PCR results without obtaining live parasites. A

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Table 1. Seroprevalence of *T. gondii* in chickens (2009–2020).

Country	Area	Source	No. tested	No. positive	% positive	Test[a]	Cut-off	Remarks[b]	Reference
Argentina	INTA-Balcarce	FR	32	17	53.0	IFA	1:100	Tg	Moré *et al.* (2012)
Australia	Western	Abattoir	20	18	90.0	IFA	1:64	Td	Chumpolbanchorn *et al.* (2013)
Brazil	Alagoas	FR-7 farms	200	72	36.0	IFA	1:16		dos Santos Silva *et al.* (2020)
Brazil	Espírito Santo	FR-33 small farms	510	198 206	38.8 40.4	MAT IHA[2]	1:25 1:16	Tg	Beltrame *et al.* (2012)
Brazil	Espírito Santo	NS	58	13	22.4	IHA[2]	1:32	Tg	Ferreira *et al.* (2018)
Brazil	Fernando de Noronha	FR	50	42	84.0	MAT	1:5	Tg	Dubey *et al.* (2010)
Brazil	Fernando de Noronha	FR	100	80	80.0	MAT	1:5		Costa *et al.* (2012)
Brazil	Fernando de Noronha	FR	430	380	88.4	IFA	1:16	RF	Magalhães *et al.* (2016)
Brazil	Mato Grosso do Sul	FR	201	46	22.8	MAT	1:25		Marques *et al.* (2009)
Brazil	Mato Grosso do Sul	FR-8 farms	40	27	67.5	MAT	1:5	Tg	Holsback *et al.* (2012)
Brazil	Minas Gerais	FR	108	77	71.3	MAT	1:16	Tg	Lopes *et al.* (2016)
Brazil	Northeastern	FR-22 municipalities	152	81	53.3	MAT	1:5	Tg	de Oliveira *et al.* (2009)
Brazil	Paraíba	FR-5 municipalities	483	152	31.5	IFA	1:16	Tg	Feitosa *et al.* (2016)
Brazil	Paraná	FR-24 farms	386	64 102	16.6 26.4	MAT IFA	1:16 1:16	Tg	Vieira *et al.* (2018)
Brazil	Pernambuco	FR-16 properties	212	86	40.5	IFA	1:16	Td	Fernandes *et al.* (2016)
Brazil	Pernambuco	FR-29 farms	629	176	27.9	IFA	1:16	RF	de Sá *et al.* (2017)
Brazil	Rio de Janeiro	FR-22 farms	220	64	29.1	IFA	1:16		Casartelli-Alves *et al.* (2012)
Brazil	Rio de Janeiro	FR-48 farms	135	82 81 49 67	60.7 60.0 36.2 49.6	IFA ELISA IHA[2] MAT	1:16 1:16 1:16		Casartelli-Alves *et al.* (2015)
Brazil	Rio de Janeiro	FR Caged	350 460	116 56	33.1 12.2	IFA	1:16	RF	Millar *et al.* (2012)
Brazil	Rio Grande do Sul	9 rural districts	597	294	49.2	IFA	1:16	RF	Camillo *et al.* (2018)
Brazil	Santa Catarina	FR-small farms	21	11	52.4	MAT	1:5	Tg	Pena *et al.* (2018)

(*Continued*)

Table 1. (*Continued.*)

Country	Area	Source	No. tested	No. positive	% positive	Test[a]	Cut-off	Remarks[b]	Reference
Caribbean islands	Antigua and Barbuda	FR	45	9	20.5	MAT[1]	1:6	Td	Hamilton *et al.* (2019*a*)
	Dominica		76	29	38.2				
	Trinidad and Tobago		41	7	17.1				
Caribbean islands	Grenada	FR	145	39	26.9	MAT	1:25		Chikweto *et al.* (2017)
China	13 provinces	FR	1173	226	19.3	ELISA[1]	1:5	Circulating *T. gondii* antigens detected in 119 (16.9%) of sera.RF	Zhao *et al.* (2012*b*)
China	AnHui	FR	60	0	0	ELISA[1]		Season	Shen (2010)
	FuJian	FR	64	10	15.6				
	JiangSu	FR	165	58	35.2				
	JiangXi	FR	111	25	22.5				
	ShangHai	FR	234	32	13.7				
	HeNan	FR	135	17	12.6				
	HeNan	Caged	93	2	2.2				
	GuangDong	FR	72	14	19.4				
	GuangXi	FR	140	39	27.9				
China	FuNing	FR	100	53	53.0	ELISA[1]	21 samples positive by TCA		Zhao *et al.* (2012*a*)
China	GanSu	Caged	605	10	1.7	IHA[1]	1:64		He *et al.* (2016)
China	GanSu	FR	92	9	9.8	IHA[1]	1:64		Wang *et al.* (2016)
		Caged	187	6	3.2				
China	GuangDong	FR	83	31	37.3	IHA[1]	1:64		Liu *et al.* (2013)
		Caged	380	63	16.6	IHA[1]	1:64		
China	GuangZhou	FR	361	41	11.4	MAT	1:5		Yan *et al.* (2009)
		Caged	244	10	4.1				
China	HeBei	FR	364	24	6.6	IHA[1]	1:64		Cui *et al.* (2010)
		Caged	120	0	0				
China	HeBei	FR	345	38	11.0	IHA[1]	1:64		Tian and Cui (2010)
		Caged	235	5	2.1				

China	HuBei	Caged	400	77	19.3	IHA[1]	1:64		Long (2013)
		FR	296	81	27.4				
China	HeNan	Caged	551	31	5.6	IHA[1]	1:64		Li (2015)
China	HeNan	FR	700	132	18.9	MAT	1:25		Feng *et al.* (2016)
China	HuBei	-Wild	571	72	12.6	IHA[1]	1:64		Luo *et al.* (2017)
China	JiangSu	FR	309	53	17.2	ELISA[1]			Ding *et al.* (2012)
		Caged	150	4	2.7				
China	JiLin	FR	110	17	15.5	ELISA Western blot			Sun *et al.* (2015)
				16	14.5	Western blot			
China	JiLin	FR	96	10	10.4	ELISA			Wang (2018)
China	JiLin	Farms	339	66	19.5	ELISA[2]			Wu *et al.* (2018)
China	JiLin	Farms	337	30	9.0	IHA[1]	1:16		Yin (2019)
China	JinZhou	FR	160	30	18.8	MAT	1:25	1.3% of 160 boilers, 7.9% of 190 layers, and 8.0% of 100 breeders-seropositive	Xu *et al.* (2012)
		Caged	450	25	5.6				
China	LanZhou	FR	108	11	10.2	MAT	1:5		Cong *et al.* (2012)
		Caged	305	19	6.2				
China	LiaoNing	FR	110	11	10.0	MAT			Yang *et al.* (2012*a*)
		Caged	392	13	3.3				
China	LiaoNing	FR	206	23	11.2	MAT	1:25		Wang *et al.* (2014*a*)
		Caged	296	14	4.7				
China	LiaoNing	FR	160	30	18.8	MAT	1:20		Xu *et al.* (2014)
		Caged	450	25	5.6				
China	NanJing	FR	350	235	67.1	ELISA	1:10	41 of 100 soil samples positive for *T. gondii* DNA	Liu *et al.* (2017)
China	Northeastern		96	13	13.5	Oocyst-specific protein ELISA		9 positive with sporozoite-specific protein ELISA	Liu *et al.* (2019)
China	ShangHai	FR	234	32	13.7	IHA[1]	1:64		Zhu *et al.* (2015)
		Caged	95	1	1.1				

(*Continued*)

Table 1. (*Continued.*)

Country	Area	Source	No. tested	No. positive	% positive	Test[a]	Cut-off	Remarks[b]	Reference
China	ShenYang	FR	206	23	11.2	MAT	1:25		Yang *et al.* (2012*b*)
		Caged	296	14	4.7				
China	XinJiang	FR	100	12	12.0	IHA[1]	1:64		Lei *et al.* (2015)
Czech Republic	Abattoir	Caged	480	2	0.4	IFA	1:40		Bartova *et al.* (2009)
Egypt	Benni Suef	FR Abattoir	90 125	18 12	20.0 9.6	IHA[1]			Aboelhadid *et al.* (2013)
Egypt	Cairo, Giza, Kalubiya	FR	88	33	37.5	ELISA			ElFadaly *et al.* (2017)
Egypt	Delta	FR Abattoir	97 207	16 18	16.4 8.6	ELISA			Ibrahim *et al.* (2016)
Egypt	Kafr sheikh	FR	84	32	38.1	IHA[3]	1:80		Harfoush and Tahoon (2010)
Egypt	Several	FR Abattoir	108 331	75 227	69.5 68.5	ELISA			Barakat *et al.* (2012)
Ethiopia	Addis Ababa	FR	125	48	38.4	MAT[1]	1:5	Tg	Tilahun *et al.* (2013)
Ethiopia	Central	FR	601	183	30.5	MAT[1]	1:60	RF	Gebremedhin *et al.* (2015)
Germany	Eastern	Large farms Backyards	384 86	14 41	3.7 47.7	ELISA		Tg, RF	Schares *et al.* (2017*a*)
Iran	Ahvaz	FR	106	55 51	51.8 48.1	MAT ELISA	1:10	Td	Hamidinejat *et al.* (2014)
Iran	Fars	FR	231	58	24.5	IFA	1:16	Td	Asgari *et al.* (2009)
Iraq	Al-Najaf, Al-Qadisyia, Babylon	FR Industrial	200 200	134 62	67.0 31.0	LAT[2]			Alkhaled *et al.* (2012)
Iraq	Sulaimani	FR	65	18	27.6	LAT[2]	1:64	21 chickens had titers of 1:2 -1:32	Mohammed and Abdullah (2013)
Ireland	Abattoirs	FR	364	65	18.0	LAT[3]	1:64		Halová *et al.* (2013)
Italy	Piacenza	FR	66	24	36.4	ELISA		Meat juice used for testing. PCR-negative.	Vismarra *et al.* (2016)
Japan	Gifu	Caged FR	103 103	0 0		LAT[1]	1:64		Matsuo *et al.* (2014)
Japan	Miyazaki	Boilers FR	100 267	0 29	0 10.9	ELISA			Duong *et al.* (2020)

Mexico	Durango	Backyards (49 homes) Farms-Sinola Farms-Nayarit	51 289 179	13 18 5	25.5 6.2 2.8	MAT	1:25	RF	Alvarado-Esquivel *et al.* (2012)
Nigeria	Oyo	FR	50	50	100.0	MAT	1:5	MAT titers 1;5 in 8, 1:25 in 9, 1:100 in 19, and 1:500 in 14	Ayinmode and Dubey (2012)
Nigeria	Oyo	FR	225	91	40.4	MAT	1:20		Ayinmode and Olaosebikan (2014)
Nigeria	Oyo	FR	241	26	10.8	IFA	1:25	Titers of 1:25 in 26, 1:50 in 5, and 1:100 in none	Ayinmode and Jones-Akinbobola (2015)
Pakistan	Bannu Khyber Pakhtunkhwa	Shaver chicken	85	0	0	ELISA[3]			Khan *et al.* (2018)
Pakistan	Khyber Pakhtunkwa	Caged FR	68 468	4 97	5.9 20.7	IHA	1:80		Mahmood *et al.* (2014)
Pakistan	Khyber Pakhtunkwa	Domestic Boilers	168 230	44, IgM 25, IgG 27, IgM 10, IgG	26.2 14.8 11.7 4.3	ELISA[3]			Khan *et al.* (2020)
Portugal	Central	Abattoirs	Boilers-170 FR-178	0 10	0 5.6	MAT	1:10		Rodrigues *et al.* (2019)
Senegal	Saint-Louis, Sahelian	FR	665	51	7.6	MAT	1:20	RF	Sarr *et al.* (2020)
South Africa	NS		137	46	33.3	LAT	1:64		Tagwireyi *et al.* (2019)
Thailand	Bangkok	Backyards	303	194	64.0	IFA	1:16		Chumpolbanchorn *et al.* (2009)
Thailand	Khon Kaen	FR	257	26	10.1	MAT	1:40		Saichua *et al.* (2017)
USA	Maryland	Grocery stores	1185	230	19.4	MAT	1:5	*T. gondii* not isolated from 230 seropositive hearts.	Ying *et al.* (2017)

[a]ELISA = enzyme-linked immunosorbent assay. Unless stated otherwise, ELISA = ELISA in-house. [1]ELISA (R&B Scientific, USA); [2]ELISA (Military Veterinary Institute, Chinese Academy of Military Medical Sciences, Changchun, Jilin Province, China); [3](Bio-ELISA toxo-IgM and IgG kits (Biokit, S.S., Barcelona, Spain).
IFA = Indirect fluorescent antibody test.
IHA = Indirect hemagglutination antibody test. [1]IHA (Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Science, Lanzhou, Gansu Province, China); [2]Immuno-HAI Toxo, Wama Diagnostics, São Paulo, Brazil); [3]IHA (SERFIB, France)
LAT = Latex agglutination test. [1]Toxocheck-MT, Eiken Chemical, Tokyo, Japan; (1) Toxo-HAI Fumouze Diagnostics, Le Malesherbes, Levallois Perret, France; [2]Toxoplasmosis Latex Test (Plasmatec, United Kingdom); [3]Toxoreagent RST701, Mast Group, United Kingdom.
MAT = Modified agglutination test (Dubey and Desmonts, 1987). [1]MAT (Toxo-Screen DA®, Biomerieux, Marcy l'Etiole, France). This is the same test as MAT.
[b]RF = risk factors, Td = *T. gondii* DNA detected. Tg = viable *T. gondii* isolated. NS = not stated.

Table 2. Isolation and genetic characterization of viable *T. gondii* from feral chickens by bioassay in mice.

Country	Location	No. tested	Tissues	No. isolated	Strain designation	PCR-RFLP genotype (ToxoDB)	Notes	Reference
Argentina				10 isolated before 2009		6 genotypes: #2, Type III (3, TgCkAr2,6,24), #7 (2, TgCkAr16, 18), #11 (1, TgCkAr1), #15 (1, TgCkAr25), #17 (2, TgCkAr27,28), #48 (1, TgCkAr7)		Bernstein *et al.* (2018); Dubey *et al.* (2003*e*); Rajendran *et al.* (2012)
Argentina	Buenos Aires	17 seropositive	B, H	6	TgCkN21Arg, TgCkP22Arg, TgCkP24Arg, TgCkC24Arg, TgCkC25Arg, TgCkC26Arg	3 genotypes based on 9 of the 10 RFLP markers: #1, clonal Type II (2, TgCkP22Arg, TgCkP24Arg), #8 (1, TgCkN21Arg), #123 (3,TgCk24Arg,TgCk25Arg,TgCk26Arg)	3 strains mouse virulent	Bernstein *et al.* (2018); Moré *et al.* (2012)
Argentina	Misiones	18	B	5	TgCk11-9Arg TgCk13-5Arg TgCk14-5Arg TgCk14-6Arg TgCk14-7Arg	4 genotypes #19 (1, TgCk11-9Arg) #116 (1, TgCk13-5Arg) #14 (1, TgCk14-5Arg) #283 (2, TgCk14-6Arg TgCk14-7Arg)		Pardini *et al.* (2016); Bernstein *et al.* (2018)
Austria				67 isolated before 2009		2 genotypes: #1, clonal Type II (1, TgCkAt46), #1 or 3, Type II (38, TgCkAt1,8,9,10,11,12,13,14,15,16,25,26,28,34,36,40,41,44, 45,47,48,51,52,53,54,55,56,57,58,59,60,61,62,63,64,65,66,67), #3 (28, TgCkAt2,3,4,5,6,7,17,18,19,20,21,22,23,24,27,29,30, 31,32,33,35,37,38,39,42,43,49,50)		Dubey *et al.* (2005*b*); Verma *et al.* (2015)
Brazil	Alagoas	8 seropositive	B, H	4	TgCkBr184-187	2 genotypes: #13 (2, TgCkBr184,185), #88 (1, TgCkBr186), Mixed (1, TgCkBr187)		de Oliveira *et al.* (2009); Dubey *et al.* (2008*b*); Shwab *et al.* (2014)
Brazil	Alagoas		B	2	TgCkAL01,02	2 genotypes: #146 (1, TgCkAL01), #277 (1, TgCkAL02)		dos Santos Silva *et al.* (2020)
Brazil	Alagoas	2	B, H	2	TgCkBrAL01,02	2 genotypes: #114 (1, TgCkBrAL02), #277 (1, TgCkBrAL01)		Ribeiro-Andrade *et al.* (2019)
Brazil	Amazonas	10 pools of 5 chickens each	B, H	5	TgCkBr282,283	2 genotypes: #257 (1, TgCkBr282), #258 (1, TgCkBr283), Mixed (3, not named)		Vitaliano *et al.* (2015)
Brazil	Bahia			25	TgCkBr284-308	8 genotypes: #8 (2, TgCkBr285,286), #13 (6, TgCkBr288,289,290,291,292,293), #36 (1, TgCkBr307), #122 (4, TgCkBr296,301,305,308), #235 (2, TgCkBr294,295),		Costa *et al.* (2008); Gonçalves *et al.* (2012); Rocha *et al.* (2018)

						#302, new1 (1, TgCkBr302), #303, new2 (1, TgCkBr303), #304, new3 (1, TgCkBr304), Mixed (5, TgCkBr284,287,297,298,299), Incomplete (2, TgCkBr300,306)		
Brazil	Bahia	10	B, H	4	TgCkBr173-176	2 genotypes: #13 (2, TgCkBr174,176), #81 (1, TgCkBr173), Mixed (1, TgCkBr175)		de Oliveira *et al.* (2009); Dubey *et al.* (2008*b*); Shwab *et al.* (2014)
Brazil	Ceará	17 seropositive	B, H	6	TgCkBr177-182	5 genotypes: #7 (1, TgCkBr182), #13 (2, TgCkBr179,180), #48 (1, TgCkBr181), #109 (1, TgCkBr177), #134 (1, TgCkBr178)		de Oliveira *et al.* (2009); Dubey *et al.* (2008*b*); Shwab *et al.* (2014)
Brazil	Espírito Santo	64 seropositive	B, H, Sk-64	44	TgCkBr234-281	11 genotypes: #6 (4, TgCkBr265,273,277,281), #14 (3, TgCkBr236,237,241), #65 (1, TgCkBr280), #75 (1, TgCkBr272), #108 (17, TgCkBr234,235,238,239,240,242,243,247, 248,253,254,255,256,261,262,263,264), #109 (3, TgCkBr249,250,252), #162 (5, TgCkBr267,268,269,270,271), #206 (5, TgCkBr244,245,246,278,279), #213 (3, TgCkBr258,259,260), #214 (1, TgCkBr257), #215 (1, TgCkBr274)	44 of 44 strains mouse virulent	Beltrame *et al.* (2012); Pena *et al.* (2013)
Brazil	Espírito Santo	13 seropositive	B, H	5	TgCkBrEs1-5	3 genotypes: #6 (2, TgCkBrEs4,5), #36 (2, TgCkBrEs2,3), #206 (1, TgCkBrEs1)	All strains mouse virulent	Ferreira *et al.* (2018)
Brazil	Fernando de Noronha	40-seropositive	B, H, Sk	24	TgCkBr210-233	6 genotypes: #2, Type III (1, TgCkBr231), #3, Type II variant (5, TgCkBr221, 225,226,228,230), #142 (1, TgCkBr222), #146 (15, TgCkBr210,211,212,213,214,215, 216,217,218,219,223,224,227,229, 233), #153 (1, TgCkBr232), #163 (1, TgCkBr220)		Dubey *et al.* (2010); Shwab *et al.* (2014)
Brazil	Maranhão	14 seropositive	B, H	2	TgCkBr171,172	1 genotype: #57 (2, TgCkBr171,172)		de Oliveira *et al.* (2009); Dubey *et al.* (2008*b*); Shwab *et al.* (2014)
Brazil	Maranhão	15 seropositive	B, H	5	TgCkBrMA1-5	4 genotypes: #6 (1, TgCkBrMA4), #7 (2, TgCkBrMA2,3), #109 (1, TgCkBrMA1), #269 (1, TgCkBrMA5)		Sousa *et al.* (2016)

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Table 2. (*Continued.*)

Country	Location	No. tested	Tissues	No. isolated	Strain designation	PCR-RFLP genotype (ToxoDB)	Notes	Reference
Brazil	Mato Grosso do Sul	27 seropositive	B, H	11		Isolate designation and genotyping included in Soares *et al.* (2011)		Holsback *et al.* (2012)
Brazil	Mato Grosso do Sul-Pantanal	90	B, H	22	TgCkBr188-209	11 genotypes: #6 (3, TgCkBr201,203,207), #7 (1, TgCkBr196), #8 (2, TgCkBr194,195), #19 (2, TgCkBr205,209), #157 (2, TgCkBr202,204), #158 (1, TgCkBr206), #159 (1, TgCkBr200), #164 (1, TgCkBr208), #161 (1, TgCkBr199), #172 (6, TgCkBr188-193), #174 (1, TgCkBr197), Mixed (1, TgCkBr198)	5 strains mouse virulent	Soares *et al.* (2011); Shwab *et al.* (2014)
Brazil	Minas Gerais		H	12	CH1-12	7 genotypes: #2, Type III (1, CH6), #6 (2, CH4,5), #8 (1, CH12), #11 (4, CH7,9,10,11), #19 (2, CH2,3), #163 (1, CH1), #206 (1, CH8)		Brandão *et al.* (2006); Silva *et al.* 2014)
Brazil	Minas Gerais	77 seropositive	B, H	2	TgChBrUD1,2	2 genotypes: #11 (1, TgChBrUD1), #6 (1, TgChBrUD2)		Lopes *et al.* (2016)
Brazil	Pará			15 isolated before 2009		10 genotypes: #6 (1, TgCkBr144), #7 (2, TgCkBr111,112), #25 (1, TgCkBr110), #28 (3, TgCkBr115,142,145), #29 (1, TgCkBr114), #30 (1, TgCkBr113), #70 (2, TgCkBr107,108), #77 (1, TgCkBr141), #96 (1, TgCkBr109), #105 (1, TgCkBr143), Incomplete (1, TgCkBr116)		Dubey *et al.* (2007*b*); Shwab *et al.* (2014)
Brazil	Paraíba	71	B, H	33	TgCkBrPB1-33	9 genotypes from 29 of 33 isolates: #8 (1, TgCkBrPB30), #11 (1, TgCkBrPB9), #13 (14, TgCkBrPB3,10,13,15,16, 17,18,19,20,22,23,24,25,27), #48 (3, TgCkBrPB4,5,6), #88 (2, TgCkBrPB28,29), #116 (2, TgCkBrPB1,2), #273 (3, TgCkBrPB11,12,14), #274 (1, TgCkBrPB26), #277 (2, TgCkBrPB7,8)	16 isolates mouse virulent	Feitosa *et al.* (2016, 2017)

Brazil	Paraná			11 isolated before 2009		6 genotypes: #6 (4, TgCkBr98,101,102,104), #11 (1, TgCkBr97), #21 (1, TgCkBr95), #47 (2, TgCkBr99,100), #53 (1, TgCkBr96), #69 (2, TgCkBr93,94)		Dubey *et al.* (2003*d*, 2006*a*, 2008*b*); Shwab *et al.* (2014)
Brazil	Paraná	119 seropositive	B, H, Li, Lu	38	TgCkBrPr1-18	10 genotypes: #6 (2, TgCkBrPr2,3), #19 (1, TgCkBrPr5), #21 (2, TgCkBrPr7,13), #111 (2, TgCkBrPr4,15), #152 (1, TgCkBrPr10), #175 (1, TgCkBrPr8), #248 (1, TgCkBrPr16), #251(1, TgCkBrPr11), #252 (1, TgCkBrPr14), #253 (1, TgCkBrPr17) 5 samples no data		Vieira *et al.* (2018)
Brazil	Pernambuco	10 seropositive	B, H	2	TgCkBr165,166	2 genotypes: #13 (1, TgCkBr165), #114 (1, TgCkBr166)		de Oliveira *et al.* (2009); Dubey *et al.* (2008*b*); Shwab *et al.* (2014)
Brazil	Rio de Janeiro	153 (123 + 30)	B, H, Sk	45 (tissue cysts or tachyzoites)		ND	RF. Note: 123 chickens were common in both papers-personal communication with authors, JPD -13 April 2020)	Casartelli-Alves *et al.* (2014, 2015)
Brazil	Rio de Janeiro			56 isolated before 2009		23 genotypes: #2, Type III (2, TgCkBr31,56), #6 (4, TgCkBr55,79,86,87), #11 (2, TgCkBr57,64), #14 (2, TgCkBr82,90), #17 (1, TgCkBr81), #19 (5, TgCkBr28,33,50,52,58), #22 (8, TgCkBr27,38,44,51,65,66, 78,80), #33 (5, TgCkBr41,42,49,60,62), #36 (4, T gCkBr30,34,59,67), #37 (4, TgCkBr32,36,84,85) #40 (3, TgCkBr75,76,92), #51 (1, TgCkBr46), #59 (2, TgCkBr40,47), #65 (1, TgCkBr89), #71 (2, TgCkBr26,69), #75 (2, TgCkBr48,88), #82 (1, TgCkBr54), #93 (1, TgCkBr61), #107 (1, TgCkBr37), #135 (1, TgCkBr45), #138 (1, TgCkBr74)		Dubey, *et al.* (2003*a*, 2006*a*); Shwab, *et al.* (2014)

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Table 2. (*Continued.*)

Country	Location	No. tested	Tissues	No. isolated	Strain designation	PCR-RFLP genotype (ToxoDB)	Notes	Reference
						#71 (1, TgCkBr71), #242, new (1, TgCkBr83), #243, new (1, TgCkBr63)		THIS STUDY (genotyping)
Brazil	Rio Grande do Norte	30 seropositive	B	13	TgCkBrRN1-13	1 genotype: #163 (7, TgCkBrRN1,2,3,4,10,12,13), Incomplete likely #163 (6, TgCkBrRN5,6,7,8,9,11)		Clementino Andrade *et al.* (2013)
Brazil	Rio Grande do Norte	17 seropositive	B, H	4	TgCkBr167-170	3 genotypes: #13 (2, TgCkBr167,170), #78 (1, TgCkBr169), #129 (1, TgCkBr168)		de Oliveira *et al.* (2009); Dubey *et al.* (2008*b*); Shwab *et al.* (2014)
Brazil	Rio Grande do Sul	12 seropositive	B, H	9	Pains #1,2 (P1, P2). Santa Flora (SF1, 306, SF439), BM, AS, AG	7 genotypes: #11 (2, P1,2), #55 (1, SF306), #64 (1, SF1), #140 (1, SF439), #163 (1, BM), #271 (1, AG), #308 (1, AS), Incomplete (1, SA)	Strains mouse virulent	Camillo (2015); Cadore *et al.* (2018)
Brazil	Rio Grande do Sul			19 isolated before 2009		7 genotypes: #2, Type III (3, TgCkBr158,161,164), #10, Type I (1, TgCkBr146), #14 (1, TgCkBr153), #17 (7, TgCkBr147,148,151,154,160,162,163), #26 (4, TgCkBr149,150,152,157), #76 (2, TgCkBr155,159), #87 (1, TgCkBr156)	First Type I genotype in this host	Dubey *et al.* (2007*b*, 2008*b*); Shwab *et al.* (2014)
Brazil	Rio Grande do Sul	2 clinical -(see text)	Lu	2	TgCkBrRS20,21	1 genotype: #280 (2, TgCkBrRS20,21)	Strain mouse virulent	Vielmo *et al.* (2019)
Brazil	Rondônia			20 isolated before 2009		6 genotypes: #6 (2, TgCkBr123,124), #8 (4, TgCkBr131,132,133,134), #15 (7, TgCkBr119,120,122,129,135, 137,140), #41 (3, TgCkBr136,138,139), #45 (3, TgCkBr117,126,127), #116 (1, TgCkBr130)		Dubey *et al.* (2006*a*, 2008*b*); Shwab *et al.* (2014)
Brazil	Santa Catarina	11 seropositive	B, H	4	TgCkBrSC1-4	4 genotypes: #10, Type I (1, TgCkBrSC1), #26 (1, TgCkBrSC2), #53 (1, TgCkBrSC3), #278 (1, TgCkBrSC4)	Type I typing confirmed by microsatellite typing	Pena *et al.* (2018)
Brazil	Santa Catarina	30-seropositive	B, H	8		5 genotypes: #26 (2, Ck32,35), #53 (1, Ck103), #120 (2, Ck89,102), #305, new NEO1 (1, Ck56),		Trevisani *et al.* (2017)

						#306, new NEO2 (1, Ck127), Mixed (1, Ck128)		
Brazil	São Paulo		B, H	17 isolated before 2009		11 genotypes: #6 (1, TgCkBr10), #8 (3, TgCkBr7,11,17), #63 (2, TgCkBr13,23), #64 (2, TgCkBr19,24), #94 (1, TgCkBr16), #125 (1, TgCkBr8)		Dubey, *et al.* (2002, 2006*a*, 2008*b*); Shwab, *et al.* (2014)
						#6 (1, TgCkBr4), #63 (1, TgCkBr6), #69 (1, TgCkBr21), #227 (1, TgCkBr3), #244 (1, TgCkBr18), #245 (1, TgCkBr5), #246 (1, TgCkBr14)		THIS STUDY (genotyping)
Brazil	Sergipe	5 seropositive	B, H	1	TgCkBr183	1 genotype: #13 (1, TgCkBr183)		de Oliveira *et al.* (2009); Dubey *et al.* (2008*b*); Shwab *et al.* (2014)
Burkina Faso				1 isolated before 2009		1 genotype: #2, Type III (1, TgCkBF-1)		Dubey *et al.* (2005*d*); Velmurugan *et al.* (2008); Shwab *et al.* (2014)
Caribbean islands	St. Kitts	81	B, H	21	TgCkStK1-21	6 genotypes: #1, clonal Type II (6, TgCkStk5,6,8,12,20,21), #2, Type III (1, TgCkStk19), #13 (3, TgCkStk7,9,11), #141 (7, TgCkStk3,4,10,13,14,15,16), #265 (3, TgCkStk2,17,18), #264 (1, TgCkStk1)	Genotypes #13 and #141-mouse virulent	Hamilton *et al.* (2017, 2019*a*, 2019*b*)
Chile				22 isolated before 2009		4 genotypes: #1, clonal Type II (4, TgCkCh6,10,11,16), #2, Type III (4, TgCkCh13,15,18,20), #3, Type II variant (13, TgCkCh2,4,5,7,8,9,12, 13,14,17,19,21,22), #14 (1, TgCkCh1)		Dubey *et al.* (2006*a*); Rajendran *et al.* (2012)
China			B, H, K, Li, Lu, Sp	21	1	1 genotype: #10, Type I	Isolation made after 3 passages. I (JPD) suspects laboratory contamination)	Zhao *et al.* (2012*a*)
China	Anhui		B	24	1, TgChsz1	1 genotype: #225 (1, TgCksz1)		Wang *et al.* (2013)
Colombia				16 isolated before 2009		7 genotypes: #14 (1, TgCkCo1), #28 (1, TgCkCo5), #29 (1, TgCkCo20),		Dubey *et al.* (2005*c*); Rajendran *et al.* (2012)

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Table 2. (*Continued.*)

Country	Location	No. tested	Tissues	No. isolated	Strain designation	PCR-RFLP genotype (ToxoDB)	Notes	Reference
						#38 (9, TgCkCo6,8,10,12,13,15,21,23,24), #178 (1, TgCkCo4), #179 (2, TgCkCo17,22), #188 (1, TgCkCo9)		
Congo				5 isolated before 2009		1 genotype: #2, Type III (5, TgCkDROC-3,6,8,9,10)		Dubey *et al.* (2005*d*); Velmurugan *et al.* (2008); Shwab *et al.* (2014)
Costa Rica				32 isolated before 2009		6 genotypes: #2, Type III (1, TgCkCr11), #7 (17, TgCkCr8,12,13,14,15,16,17, 18,19,20, 21,22,23,24,25,26,27), #24 (6, TgCkCr2,28,29,30,31,32), #35 (4, TgCkCr3,4,5,6), #43 (3, TgCkCr7,8,9), #91 (1, TgCkCr1)		Dubey *et al.* (2006*c*); Rajendran *et al.* (2012)
Egypt				7 isolated before 2009		2 genotypes: #1, clonal Type II (5, TgCkEg12,13,14,16,17), #2, Type III (2, TgCkEg15,19),		Dubey *et al.* (2003*b*); Dubey (2010*b*); Shwab *et al.* (2014); Velmurugan *et al.* (2008)
Ethiopia	Addis Ababa	115 (72-seronegative 43 seropositive)	H	1		1 genotype: #1, clonal Type II		Tilahun *et al.* (2013)
Ethiopia	Central	41 seropositive	B, H	29 (tissue cysts in 24, 5 positive by serology only)		ND		Gebremedhin *et al.* (2014)
Germany		61–41 seropositive 20 seronegative	H, Sk	26 from hearts (3 from legs also)	1 isolate from seronegative chicken	All Type II (8 RFLP markers used)		Schares *et al.* (2017*a*)
Ghana				2 isolated before 2009		2 genotypes: #132 (1, TgCkGh2), #137 (1, TgCkGh1)		Dubey *et al.* (2008*a*); Shwab *et al.* (2014); Velmurugan *et al.* (2008)
Grenada, West Indies		39 seropositive	H	20	TgCkGr37-57	4 genotypes: #2, Type III (15, TgCkGr39,40,41,42,43, 47,48,49,50,51,52,53,54,55,57), #7 (1, TgCkGr37), #13 (3, TgCkGr45,46,56), #259 (1, TgCkGr38)		Chikweto *et al.* (2017)

Grenada, West Indies	9 isolated before 2009		3 genotypes: #2, Type III (5, TgCkGr12,16,23,24,29), #13 (2, TgCkGr25,26), #187 (2, TgCkGr17,18)	Dubey *et al.* (2005*a*); Rajendran *et al.* (2012)
Guatemala	3 isolated before 2009		3 genotypes: #7 (1, TgCkGa6), #190 (1, TgCkGa1), #191 (1, TgCkGa4)	Dubey *et al.* (2005*f*); Rajendran *et al.* (2012)
Guyana	35 isolated before 2009		9 genotypes: #2, Type III (2, TgCkGy26,27), #7 (2, TgCkGy23,24), #12 (12, TgCkGy2,3,5,6,9,12,16,19, 20,25,28,32), #25 (5, TgCkGy8,10,14,15,35), #30 (4, TgCkGy7,11,13,31), #31 (5, TgCKGh1,4,29,30,33), #48 (2, TgCkGy21,22), #68 (2, TgCkGy17,18), #123 (1, TgCkGy34),	Dubey *et al.* (2007*a*); Shwab *et al.* (2014)
Indonesia	1 isolated before 2009		1 genotype: #89 (1, TgCkId1)	Dubey *et al.* (2008*a*)
Israel	7 isolated before 2009		2 genotypes: #1 or 3, Type II (6, chicken 1,4,7,8,91,98), #3 (1, chicken 40)	Dubey *et al.* (2004*c*); Verma *et al.* (2015)
Italy	3 isolated before 2009		2 genotypes: #1(1, TgCkIt3) #3 (2, TgCkIt1,2)	Dubey et al. (2008*a*)
Kenya	1 isolated before 2009		1 genotype: #3, Type II variant (1, TgCKKen-1)	Dubey *et al.* (2005*d*); Velmurugan *et al.* (2008); Shwab *et al.* (2014)
Mali	5 isolated before 2009	TgCkMal-2-5	2 genotypes: #2, Type III (4, TgCkMal-1,2,3,4), #3, Type II variant (1, TgCKMal5)	Dubey *et al.* (2005*d*); Velmurugan *et al.* (2008); Shwab *et al.* (2014)
Mexico	4 isolated before 2009		1 genotype: #2, Type III (4, TgCkMx1,2,3,4)	Dubey *et al.* (2004*b*); Dubey (2010*b*); Shwab *et al.* (2014)
Nicaragua	44 isolated before 2009		10 genotypes: #2, Type III (6, TgCkNi3,8,13,44,48,2x), #4 (3, TgCkNi18,39,42), #7 (5, TgCkNi14,15,20,25,30), #16 (11, TgCkNi1,11,22,23,26,29,33, 36,38,46,47y), #23 (7, TgCkNi4,6,10,17,21,34,37), #27 (5, TgCkNi7,9,40,41,43), #50 (3, TgCkNi16,45,7x), #52 (2, TgCkNi12,32),	Dubey *et al.* (2006*d*); Rajendran *et al.* (2012)

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Table 2. (*Continued.*)

Country	Location	No. tested	Tissues	No. isolated	Strain designation	PCR-RFLP genotype (ToxoDB)	Notes	Reference
						#102 (1, TgCkNi35), #140 (1, TgCkNi27)		
Nigeria		5 seropositive	B, H	1	TgCkNg1	1 genotype: #15 (1, TgCkNg1)		Shwab *et al.* (2014); Velmurugan *et al.* (2008)
Peru				5 isolated before 2009		4 genotypes: #2, Type III (1, TgCkPe2), #17 (2, TgCkPe4,6), #116 (1, TgCkPe3), #189 (1, TgCkPe189)		Dubey *et al.* (2004*a*); Rajendran *et al.* (2012)
Poland				2 isolated before 2009		1 genotype: #15 (2, TgCkPo1,2)		Dubey *et al.* (2008*a*);
Portugal				15 isolated before 2009		3 genotypes: #1 or #3, Type II (7, TgCkPr3,6,7,8,10, 11,12), #2, Type III (4, TgCkPr1,2,4,5), #254 (4, TgCkPr13,14,15,16)		Dubey *et al.* (2006*e*); Verma *et al.* (2015)
Uganda		20 seropositive	B, H	9	TgCkUg 1-9	6 were clonal Type II based on 6 RFLP markers.		Dubey (2010*b*); Lindström *et al.* (2008)
Venezuela				7 isolated before 2009		5 genotypes: #8 (1, TgCkVe1), #14 (1, TgCkVe3), #48 (3, TgCkVe4,5,10), #116 (1, TgCkVe11), #185 (1, TgCkVe6)		Dubey *et al.* (2005*e*); Rajendran *et al.* (2012)
Vietnam				1 isolated before 2009		Partial data (SAG1-u-1, SAG2-II, SAG3-III, c22-8-II)		Dubey *et al.* (2008*a*);
USA	Illinois			11 isolated before 2009	TgCkUsIl 1-11	1 genotype: #1, clonal Type II (11, TgCkUsI1-11)		Dubey *et al.* (2007*c*); Shwab *et al.* (2014)
USA	Massachusetts, Ohio			15 isolated before 2009 8 isolated before 2009		2 genotypes: #1, clonal Type II (7, TgCkUsMa1,7,9, 10,11,12,13), #2, Type III (7, TgCkUsMa3,5,6,14,15,16,17) Mixed (1, TgCkUsMa8) 3 genotypes: #2, Type III (4, TgCkUsOh2,3,4,5), #3, Type II variant (1, TgCkUsOh11), #170 (3, TgCkUsOh8,9,10)		Dubey *et al.* (2003*c*); Dubey (2010*b*); Ying *et al.* (2017)
USA	Massachusetts, Rhode Island, Connecticut	31 -sentinel chickens	B, H, Sk	27		ND		Dubey *et al.* (2015)

ND, no data; B, brain; H, heart; Li, liver; Lu, lung; Sk, skeletal muscle

udy from Argentina (Pardini *et al.*, 2016; Bernstein *et al.*, 2018) also had success in isolating good quality DNA from the brains of two naturally infected chickens from Argentina; one sample was ToxoDB genotype #19, the other was #286 based on 10 PCR-RFLP markers (Table 4).

Histopathology and immunohistochemistry: The *T. gondii* burden in tissues of asymptomatic chickens is low (Dubey, 2010*a*). Thus, the chances of detection of the parasite by histopathology and immunohistochemistry are low (Casartelli-Alves *et al.*, 2014; Ibrahim *et al.*, 2016). In a study from Brazil, serologic results, bioassay and histopathology results were compared. Histological sections of the brain, heart and thigh muscle were examined microscopically and after immunohistochemical staining (IHC) reactivity with *T. gondii* antibodies. *Toxoplasma gondii* was detected in tissues of eight (5.9%) of naturally infected chickens, in hearts of five and in brains of three. Only tissue cysts were found, and they were not associated with lesions (Casartelli-Alves *et al.*, 2014). In a study from Egypt, blood and brains of 304 chickens were tested for *T. gondii* infection; 34 (11.8%) of blood sera were seropositive by ELISA and *T. gondii* was detected histologically in sections of formalin preserved brains of 21 (6.9%) (Ibrahim *et al.*, 2016). The finding of *T. gondii* in Giemsa stained smears of livers (38.4%), kidney (20.5%) and spleen (12.8%) of 39 seropositive but asymptomatic chickens in another study from Egypt is an overestimation of infection (Mohammed and Abudullah, 2013); the illustrations provided indicate that artefacts were diagnosed as *T. gondii* (J.P.D. own opinion).

Validation of MAT serologic results with the isolation of viable T. gondii

Among the serological tests, the MAT was most commonly used in the studies in the last two decades. As stated earlier, a unique opportunity became available to evaluate the efficiency of detection of *T. gondii* in naturally exposed chickens (Dubey *et al.*, 2016). In that study, 2066 FR chickens from Argentina (Dubey *et al.*, 2003*e*; Dubey *et al.*, 2005*g*), Austria (Dubey *et al.*, 2005*b*), Brazil (Dubey *et al.*, 2002, 2003*a*; da Silva *et al.*, 2003; Dubey *et al.*, 2003*d*; Dubey *et al.*, 2006*a*; Dubey *et al.*, 2010), Chile (Dubey *et al.*, 2006*b*), Colombia (Dubey *et al.*, 2005*c*), Congo (Dubey *et al.*, 2005*d*), Costa Rica (Dubey *et al.*, 2006*c*), Egypt (Dubey *et al.*, 2003*b*), Grenada (Dubey *et al.*, 2005*a*), Israel (Dubey *et al.*, 2004*c*), Italy (Dubey *et al.*, 2008*a*), Mexico (Dubey *et al.*, 2004*b*), Nicaragua (Dubey *et al.*, 2006*d*), Peru (Dubey *et al.*, 2004*a*), Poland (Dubey *et al.*, 2008*a*), Portugal (Dubey *et al.*, 2006*e*), Sri Lanka (Dubey *et al.*, 2005*h*), USA (Dubey *et al.*, 2003*c*; Dubey *et al.*, 2007*c*), Venezuela (Dubey *et al.*, 2005*e*) were serologically tested by MAT and chicken hearts were bioassayed for the isolation of viable *T. gondii* (Dubey *et al.*, 2016). These chickens would have been exposed to many pathogens, including protozoans *Eimeria* species, *Cryptosporidium* species, *Sarcocystis* species, *Neospora caninum*, various helminthic and bacterial infections that may react with *T. gondii*. Thus, there was a chance to study cross-reactivity against other pathogens. Needless to say that these studies were very expensive to conduct with respect to money, time and resources. All chickens were bioassayed, irrespective of serological status. In many instances, seronegative chicken hearts were pooled and fed to cats and the feces of cats were tested for excretion of *T. gondii* oocysts; cats excrete oocysts even after ingesting few *T. gondii* (Dubey, 2010*a*). All serological results were done by one operator, minimizing procedure variability.

Viable *T. gondii* was isolated from 528 of 2066 chickens by bioassay in mice (Dubey *et al.*, 2016). The isolation rate of *T. gondii* generally increased with the MAT titer. It is noteworthy that viable *T. gondii* was isolated from six of 1025 chickens with MAT titer of <1:5 (considered seronegative). Likely, these chickens had not yet seroconverted or there was a prozone (the lower dilutions are negative, but higher dilutions are positive). The isolation rates with different titers in increasing order were 15.2% of 105 at a titer of 1:5, 11.4% of 79 at a titer of 1:10, 42.9% of 98 at a titer of 1:20 and 59.9% of 759 chickens at titers of 1:40 or higher (Dubey *et al.*, 2016). This result suggests that the higher the titer, the higher the parasite tissue load in chickens.

Additionally, hearts pooled from 1028 chickens were bioassayed in 29 cats. It was noteworthy that the 23 cats fed hearts pooled from 802 seronegative (MAT < 1:5) chickens did not excrete *T. gondii* oocysts, thus supporting the specificity of the test (Dubey *et al.*, 2016). Cats are highly sensitive to *T. gondii* infection after ingestion of *T. gondii* stages. Experimentally, cats orally inoculated with single bradyzoites (freed from tissue cysts) excreted millions of oocysts (Dubey, 2001). Cats can consume more than 200–500 g of tissues in a matter of 3–4 days and excrete oocysts in feces that can be easily detected by microscopic examination of feces. Thus, it was assumed that a cat would have excreted oocysts if any of the 802 hearts fed to cats were infected with *T. gondii* (Dubey *et al.*, 2016).

Comparison of serology, PCR techniques, and bioassay for the detection of T. gondii

An extensive study was conducted to determine the efficacy of 3 serological tests (MAT, IFAT, ELISA), magnetic-capture (MC) real-time PCR (RT PCR), and *T. gondii* burden in brain, heart, drumstick in chickens (Schares et al. 2018). Two PCR methods (conventional RT PCR, and on acidic pepsin digests [PD-RT PCR]) were used to detect DNA. Antibodies to *T. gondii* were determined using blood serum and meat juice. The following conclusions were drawn: (i) substantial agreement was found between the mouse bioassay and MC-RT PCR or the mouse bioassay and conventional PD-RT PCR. (ii) The PD-RT PCR was more sensitive than MC-RT PCR. (iii) The organ tested affected the diagnostic sensitivity of MC-RT PCR;100 times higher parasite burdens were found in brain and heart tissues than pectoral muscles, thigh or drumstick muscles. (iv) using sera of naturally exposed chickens, diagnostic sensitivities of ELISA, IFAT and MAT were: 87.5%, 87.5% and 65.2%, respectively, and diagnostic specificities of 86.2%, 82.8% and 100%, respectively. Testing of meat juice by three serological tests revealed that the MAT with meat juice from pectoral muscles was less consistent than those of ELISA and IFAT and the MAT performed similar to ELISA and IFAT when applied to test meat juice samples collected from heart, thigh or drumstick musculature (Schares *et al.*, 2018).

Genetic diversity of viable *T. gondii* isolates

Genotypes of *T. gondii* from chickens in each publication are summarized in Table 2, and by continent in Table 3 and Fig 1. Viable *T. gondii* parasites were isolated from most geographical regions, including Africa, Europe, Caribbean, Central America and South America. Data from Asia are very limited (Tables 2 and 3). Overall, genotype distribution follows the global patterns recognized previously (Shwab *et al.*, 2014), with ToxoDB genotypes #1 and #3 (collectively known as Type II), and genotype #2 (known as Type III) being dominant in Africa and Europe (Table 3). In the Caribbean region, genotypes #2 and #13 were frequently identified, and diverse genotypes were also present. In Central America, genotype #7 is common in chickens, as well as the presence of many unique genotypes. *Toxoplasma gondii* isolates are highly diverse and there is no clear dominance of any genotypes in South America. Of the 471, *T. gondii* samples analysed in South America, 365 were from Brazil, from which

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Table 3. Distribution of PCR-RFLP (ToxoDB) *T. gondii* genotypes from chickens from different continents/countries; data were based on genotyping from viable *T. gondii* isolates.

		Classic types			ToxoDB-RFLP genotype								
Continent/country	Total typed	I (ToxoDB #10)	II (ToxoDB #1 or #3	III (ToxoDB #2)	#4	#6	#7	#9	#11	#13	#15	Others	
AFRICA													
Burkina Faco	1	0	0	1	0	0	0	0	0	0	0		Velmurugan *et al.* (2008); Shwab *et al.* (2014)
Congo	5	0	0	5	0	0	0	0	0	0	0		Velmurugan *et al.* (2008); Shwab *et al.* (2014)
Egypt	7	0	5	2	0	0	0	0	0	0	0		Velmurugan *et al.* (2008); Shwab *et al.* (2014)
Ethiopia	1	0	1	0	0	0	0	0	0	0	0	0	Tilahun *et al.* (2013)
Ghana	2	0	0	0	0	0	0	0	0	0	0	2 (#132-1, #137-1)	Velmurugan *et al.* (2008); Shwab *et al.* (2014)
Kenya	1	0	1	0	0	0	0	0	0	0	0	0	Velmurugan *et al.* (2008); Shwab *et al.* (2014)
Mali	5	0	1	4	0	0	0	0	0	0	0		Velmurugan *et al.* (2008); Shwab *et al.* (2014)
Nigeria	1	0	0	0	0	0	0	0	0	0	1	0	Velmurugan *et al.* (2008); Shwab *et al.* (2014)
Uganda	6	0	6	0	0	0	0	0	0	0	0	0	Lindstrom *et al.* (2008); Dubey (2010*b*)
Total Africa	29	0	14	12	0	0	0	0	0	0	1	2	
ASIA													
China	6	1	0	0	0	0	0	4	0	0	0	1 (#225-1)	Zhao *et al.* (2012*a*); Wang *et al.* (2013)
Israel	7	0	7	0	0	0	0	0	0	0	0	0	Verma *et al.* (2015)
Indonesia	1	0	0	0	0	0	0	0	0	0	0	1 (#89-1)	Dubey *et al.* (2008*a*)
Total Asia	14	1	7	0	0	0	0	4	0	0	0	2	
EUROPE													
Austria	67	0	67	0	0	0	0	0	0	0	0	0	Verma *et al.* (2015)
Germany	26	0	26	0	0	0	0	0	0	0	0	0	Schares *et al.* (2017*a*, 2017*b*)
Italy	3	0	3	0	0	0	0	0	0	0	0	0	Dubey *et al.* (2008*a*)
Poland	2	0	0	0	0	0	0	0	0	0	2	0	Dubey *et al.* (2008*a*)
Portugal	15	0	7	4	0	0	0	0	0	0	0	4 (#254-4)	Verma *et al.* (2015)
Total Europe	113	0	103	4	0	0	0	0	0	0	2	4	

CARIBBEAN													
Grenada	29	0	0	20	0	0	1	0	0	5	0	3 (#187-2, #259-1)	Rajendran *et al.* (2012); Chikweto *et al.* (2017)
St. Kitts	21	0	6	1	0	0	0	0	0	3	0	11 (#141-7, #265-3, #264-1)	Hamilton *et al.* (2017, 2019*a*, 2019*b*)
Total Caribbean	50	0	6	21	0	0	1	0	0	8	0	14	
CENTRAL AMERICA													
Costa Rica	32	0	0	1	0	0	17	0	0	0	0	14 (#24-6, #35-4, #43-3, #91-1)	Rajendran *et al.* (2012)
Guatemala	3	0	0	0	0	0	1	0	0	0	0	2 (#190-1, #191-1)	Rajendran *et al.* (2012)
Nicaragua	44	0	0	6	3	0	5	0	0	0	0	30 (#16-11, #23-7, #27-5, #50-3, #52-2, #102-1, #140-1)	Rajendran *et al.* (2012)
Total Central America	79	0	0	7	3	0	23	0	0	0	0	46	
SOUTH AMERICA													
Argentina	21	0	2	3	0	0	2	0	1	0	1	12 (#8-1, #14-1, #17-2, #19-1, #48-1, #116-1, #123-3, #283-2)	Rajendran *et al.* (2012); Pardini *et al.* (2016); Bernstein *et al.* (2018)
Brazil	365	2	5	7	0	28	6	0	11	30	7	269 (100 different genotypes)	See Supplementary Tables 1, 2
Chile	22	0	17	4	0	0	0	0	0	0	0	1 (#14-1)	Rajendran *et al.* (2012)
Colombia	16	0	0	0	0	0	0	0	0	0	0	16 (#14-1, #28-1, #29-1, #38-9, #178-1, #179-2, #188-1)	Rajendran *et al.* (2012)
Guyana	35	0	0	2	0	0	2	0	0	0	0	31 (#12-12, #25-5, #30-4, #31-5, #48-2, #68-2, #123-1)	Dubey *et al.* (2007*a*); Shwab *et al.* (2014)
Peru	5	0	0	1	0	0	0	0	0	0	0	4 (#17-2, #116-1, #189-1)	Rajendran *et al.* (2012)
Venezuela	7	0	0	0	0	0	0	0	0	0	0	7 (#8-1, #14-1, #48-3, #116-1, #185-1)	Rajendran *et al.* (2012)
Total South America	471	2	24	17	0	28	10	0	12	30	8	340	
NORTH AMERICA													
Mexico	4	0	0	4	0	0	0	0	0	0	0	0	Dubey (2010*b*); Shwab *et al.* (2014)
USA	34	0	19	11	0	0	0	0	0	0	0	4 (#170-3, mixed-1)	Dubey *et al.* (2003*c*); Dubey *et al.* (2007*c*); Dubey (2010*b*); Shwab *et al.* (2014)
Total North America	38	0	19	15	0	0	0	0	0	0	0	4	
Grand total	794	3	173	76	3	28	34	4	12	38	11	412	

108 genotypes were identified (Supplementary Tables S1, S2; Supplementary Fig.1).

Clinical infections

Chickens are considered resistant to *T. gondii* and hence there are only rare reports of clinical toxoplasmosis in chickens (Dubey, 2010*a*). An outbreak of clinical toxoplasmosis was reported on an avian farm from Brazil that had 47 FR chickens (Vielmo *et al.*, 2019). Of these, 13 adult chickens were sick and nine died. The birds had apathy and diarrhea. Four of these nine chickens were examined at necropsy. The affected chickens were in poor body condition. Microscopically, necrosis and inflammation were noted in several tissues, including air sacs, myocardium, brain, kidney, lungs, liver, small intestine and spleen, tissue cysts or tachyzoites were identified in lesions. Viable *T. gondii* was isolated from tissues of two chickens by bioassay in mice. PCR-RFLP genotyping revealed a unique ToxoDB genotype, designated #280 and the results were confirmed by microsatellite typing. Antibodies to *T. gondii* were detected in the serum of one dead chicken and sera of four other chickens; the MAT titers were 10, 320 and 2560 (three chickens).

Epidemiology and use of sentinel chickens

In a study in China, *T. gondii* DNA was found in 41 of 100 soil samples on chicken farms, indicating the presence of oocysts (Liu *et al.*, 2017). Serological results using oocyst-based protein ELISA indicated that chickens acquired infection by ingesting oocysts (Liu *et al.*, 2019). Follow up of *T. gondii* infection in sentinel chickens can provide valuable information concerning the epidemiology of toxoplasmosis on farms. The results of two studies in Argentina and the USA are summarized here.

Moré *et al.* (2012) studied *T. gondii* infection in 202, one-week-old sentinel chickens placed on 10 chicken farms in Argentina. The chickens were bled 68 or 74 days later; 13 chickens developed *T. gondii* antibodies in the IFA test (1:100 in eight and 1:200 in five); however, attempts to isolate viable *T. gondii* were not successful by bioassay in mice inoculated with tissues of any of the 13 seropositive chickens.

An experiment in the USA was initiated to study the epidemiology of *T. gondii* transmission on three pig farms in three New England states that had a high prevalence of *T. gondii* infection (Dubey, 2010*a*). *Toxoplasma gondii* seronegative, sentinel chickens were placed on three (30 each) swine farms in November 2003. Chickens were bled monthly and their sera were tested for *T. gondii* antibodies by MAT (cut-off 1:25). Chickens that seroconverted were euthanized on the farm and their tissues were bioassayed in mice, cats or both. Over the course of the experiment (7 months), 31 of 71 chickens seroconverted (MAT 1:100 or higher); three chickens seroconverted after 1 month, eight chickens after 2 months, five chickens after 3 months, two chickens after 4 months, one chicken after 5 months, and seven chickens after 6 months. Tissues of 26 seropositive chickens were bioassayed in both cats and mice; viable *T. gondii* was isolated, by bioassay in mice, from hearts (whole) of all 26 chickens, brains (whole) of three chickens and leg muscles (25 g) of 11 chickens; 21 of 26 cats fed 250 g of leg muscle from seropositive chickens excreted *T. gondii* oocysts. Results confirmed earlier findings that indicated low *T. gondii* burden in poultry skeletal muscle and heart being the tissue of choice for isolation of viable parasites (Dubey, 2010*b*).

The number of mice that became infected with *T. gondii* was higher when inoculated with heart tissue *vs* the brain and leg muscles; of 130 mice used for bioassay, 5 (3.8%), 28 (21.5%) and 115 (88.4%) mice became infected with *T. gondii* after inoculation with brain, leg muscle and heart, respectively. Of the 27 cats fed leg muscles from 27 seropositive chickens, 23 excreted *T. gondii* oocysts. The two cats fed tissues of 40 seronegative chickens did not excrete oocysts. As stated earlier, in another investigation, viable *T. gondii* was isolated from 26 chickens, hearts of all 26 and legs of only three (Schares *et al.*, 2017*a*). Thus, the heart is confirmed once more as the organ of choice for isolating viable *T. gondii* in chickens.

Little is known of the dynamics of *T. gondii* in chickens under natural conditions. While feeding from the ground provides exposure to *T. gondii* oocysts, the USA study was performed during the winter months. It is not clear how chickens became infected with *T. gondii* during the winter months. Winters in New England states are harsh, and the ground is frozen; thus, chickens are unlikely to ingest oocysts on the ground from the previous year. It is more likely that the grain fed to these chickens was contaminated with oocysts excreted by cats on the farm. It is interesting to note, that among the three farms studied, no chickens were infected on one farm, a few were infected on the second farm, and all chickens were infected on the third farm, indicating that risk factors differed among these three farms (Dubey, 2010*a*).

Experimental infections

Clinical and diagnosis

Chickens inoculated intravenously with *T. gondii* tachyzoites generally remained asymptomatic, irrespective of the dose (Chumpolbanchorn *et al.*, 2009; Geuthner *et al.*, 2014; Schares *et al.*, 2017*a*,*b*). Chickens orally inoculated with oocysts can develop diarrhoea, and the effect may be neurogenic rather than the destruction of enterocytes (Bonapaz *et al.*, 2010; Braga *et al.*, 2011).

Chickens inoculated with *T. gondii* seroconverted as early as 4 days post-inoculation (p.i.), but more commonly between 10 and 21 days p.i. (Geuthner *et al.*, 2014; Wang *et al.*, 2014*b*; Hiob *et al.*, 2017; Maksimov *et al.*, 2018). Antibody titers (IFA) persisted until euthanasia at 10 weeks p.i. (Geuthner *et al.*, 2014). In some chickens, antibodies declined to undetectable levels by 4 weeks p.i. (Geuthner *et al.*, 2014). Based on DNA detection, *T. gondii* burden was sparse and detectable in heart, retina, pancreas and drumstick of four of 12 chickens euthanized at 10 weeks p.i. (Geuthner *et al.*, 2014); clinical acute toxoplasmosis developed in 7–10 days old chickens inoculated intraperitoneally with large numbers (1–50 million) of five strains of *T. gondii* in China (Wang *et al.*, 2014*b*; Wang *et al.*, 2015). Age was a factor in the pathogenesis of acute toxoplasmosis. Of the chickens infected at 7, 14, 21 and 28 days of age, only the chickens inoculated at 7-day old, died of acute toxoplasmosis, chickens inoculated at 14 days had mild signs, but no mortality and those inoculated on 21 and 28 days old chickens remained asymptomatic (Wang *et al.*, 2014*a*).

In an experiment from China, 30 chickens (35-day old) were inoculated intravenously with 4.3–10 million tachyzoites (Yan *et al.*, 2010). The chickens were euthanized on 7, 14, 21, 28 and 35 days p.i. and their tissues were tested for parasite DNA and sera were evaluated by the MAT and IHA. This study provided valuable information concerning a commercial IHA kit marketed by Lanzhou Veterinary Research Institute, China; this IHA kit has been used extensively for *T. gondii* serological surveys in animals in China, including chickens (Table 1). The inoculated chickens remained asymptomatic. By MAT, antibodies (titer 1:160 or 1:640) peaked around 21 days p.i. and were present in low titers (1:10, 1:40, 1:40, 1:160) in four chickens killed on day 35 p.i. By IHA, peak titers (1:10, 1:10, 1:160, 1:160) were detected in four chickens euthanized on day 21 p.i. and the titers had dropped

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Table 4. *Toxoplasma gondii* DNA in tissues of chickens.

Country	Location	No. tested	Tissues	PCR target	No. positive	% positive	Reference
Argentina	Misiones	33	B	Tox5-Tox8	10 (2 DNA samples typed based on 10 PCR-RFLP markers, genotype #19 for 1, #286 for 1)	30.3	Bernstein *et al.* (2018); Pardini *et al.* (2016)
Australia	Western	50	B, S	B1	3 of 27 brains, 3 of 23 spleens	12.0	Chumpolbanchorn *et al.* (2013)
Brazil	Mato Grosso do Sul	40	B, H	B1	16	40.0	Holsback *et al.* (2012)
Brazil	Pernambuco	12	B, H, Li, Lu	REP-529	2	16.7	Fernandes *et al.* (2016)
Canada	Quebec, Ontario, British Columbia	94	breast	B1 and REP-529	7	7.5	Iqbal *et al.* (2018)
Caribbean islands	Antigua and Barbuda Dominica Trinidad and Tobago	45 76 41	B, H	ITS1	11 13 7	24.4 17.1 17.1	Hamilton *et al.* (2019*b*)
Caribbean islands	St. Kitts	81	B, H	ITS1	23	28.0	Hamilton *et al.* (2017)
China	Henan	25	H	450bp	4	16.0	Feng *et al.* (2016)
China	Tai'an	360-super market	H	ITS1	8	2.2	Wang *et al.* (2020)
		360- farmers market	H	ITS1	69	19.2	
China	Shandong	257	Sk	B1 (3 DNA samples typed as ToxoDB genotype #9 (TgCk1-3)	21	8.2	Zou *et al.* (2017)
China	Shandong	1653-supermarkets	H	Nested PCR	204	12.3	Sun (2018)
Colombia	Sincelejo-Sucre	40	NS	B1	14	35.0	Campo-Portacio *et al.* (2014)
Colombia	Bogota	60	Sk	B1	33	55.0	Franco-Hernandez *et al.* (2016)
Iran	Ahvaj	106	B, H, Li	ITS1	49	46.2	Hamidinejat *et al.* (2014)
Iran	Khuzestan	103	B, H	B1	16 (B of 6, H of 16)	15.5	Khademvatan *et al.* (2013)
Iran	Fars	29 seropositive	B, H, Li	B1	*T. gondii* DNA in 27 of 29: livers 25 brains and 16 hearts	93.1	Asgari *et al.* (2009)
Iran	Bandar, Haji	200	Eggs	529 bp	22	11.0	Khademi *et al.* (2018)
Iran	Northwestern	50	NS	B1	4	8.0	Mahami-Oskouei *et al.* (2017)
Kenya	Thika	105	B1	529 bp (1 isolate by mouse bioassay, details missing)	83	79.0	Mose *et al.* (2016); Mose *et al.* (2017)
Pakistan	Khyber Pakhtunkwa	Domestic-65 Boilers-230	H, Li, Sk	B1	13 32	20.0 10.8	Khan *et al.* (2020)
Taiwan		100 grocery store	H, Li, Sk	B1	4	4.0	Fuh *et al.* (2013)

B, brain; H, heart; Li, liver; Lu, lung; Sk, skeletal muscle, NS = Not stated

to 1:5 or <1:5 on days 28 or 35 p.i. Thus, the results obtained by IHA were inconsistent and mostly below the cut-off of 1:64 used in various surveys (Table 1). *Toxoplasma gondii* DNA was extracted from several tissues of these chickens; the heart and lungs were more consistently infected (Yan *et al*., 2010).

Valuable serological diagnostic information was obtained from chickens orally inoculated with different strains of *T. gondii* oocysts (Hotop *et al*., 2014; Geuthner *et al*., 2019). Unusual and inconsistent results were obtained by ELISA using recombinant proteins: by rGRA1- and rGRA9-based ELISA, high levels of antibodies were detected only between days 7 and 10 p.i., dropped to undetectable levels and mildly increased between 42 and 63 days p.i. By the rGRA6-ELISA, the initial peak was between days 14 and 21 p.i. and antibodies persisted until day 63 p.i. By rSAG1-ELISA, antibodies peaked between days 14 and 21 and then were not detectable (Hotop *et al*., 2014). By contrast, chickens developed MAT antibodies between 4 and 7 days p.i., and antibodies persisted until the termination of the experiment on day 84 p.i. (Hotop *et al*., 2014). Seroconversion and the rate of parasitization varied among chickens inoculated with different strains (Geuthner *et al*., 2019). Antibody titers as high as 1: 512 000 were detected in chickens by IFA. Parasite DNA was detectable in many tissues, but the heart was the most persistently infected tissue (Geuthner *et al*., 2019).

An extensive investigation was undertaken by a Japanese study concerning the use of recombinant and nascent proteins for the serodiagnosis of toxoplasmosis in chickens (Appiah-Kwarteng *et al*., 2019). Chickens ($n = 21$) were inoculated with 10 or 100 million tachyzoites intravenously (three strains, RH, CTG, PLK) or intravenously and intraperitoneally (ME49) and were tested for antibodies and parasites. The chickens remained asymptomatic and were bled on days 7, 14, 21 and 28 p.i. Antibodies were assessed by the commercial latex agglutination test (LAT, Eiken Kagaku, Japan), western blot and ELISA using nascent and recombinant proteins (SAG1, GRA7). By LAT, antibodies were detected only on day 7 p.i., but not afterwards; this is a noteworthy observation because LAT has been used to detect *T. gondii* antibodies in many species of animals, including chickens. By ELISA, antibodies peaked day 7 or 14 p.i. and were detectable until the termination of the experiment on day 28 p.i.; the antibody response was stronger and more consistent by using nascent proteins then recombinant *E. coli*-derived recombinant proteins (Appiah-Kwarteng *et al*., 2019). The results were confirmed by western blotting using crude *T. gondii* lysate. To locate the *T. gondii* in tissues, 7-day old chickens were inoculated with a fluorescent-tagged protein *T. gondii* strain, TgCatJpGi1/TaJ/GRA Red. Fluorescent-tagged parasite images were visible in the hearts, lungs, livers and brains of the three of seven chickens that died 7 days p.i., but not in tissues of chickens that survived the acute phase; results were confirmed by bioassay in mice. These observations are in marked contrast to the findings that viable parasites are easily isolated from the hearts of chronically infected mice (see isolation Table 2). A luciferase-linked GRA8-ELISA was developed in Japan for the detection of *T. gondii* antibodies in sera of experimentally infected chickens (Duong *et al*., 2020).

Serotyping

Toxoplasma gondii strains are genetically diverse but strains from Europe, North America and Africa fall into two main lineages (Types II, III). The information is based on the characterization of parasite DNA extracted from live *T. gondii* isolated from infected hosts. Only limited information is available based on the serotyping of samples from humans (Maksimov *et al*., 2018). Information on a large panel of 101 synthetic peptides was obtained on sera from chickens intravenously inoculated with tachyzoites of three strains of *T. gondii* (RH-Type I, Me49-Type II and NED-Type III). The authors concluded that by using selected peptides, it was possible to serotype strains up to 9 weeks p.i. (Maksimov *et al*., 2018).

Effect of breed/strain of chickens, T. gondii genotype on toxoplasmosis in chickens

Breed or strain of chicken can influence the course of *T. gondii* infection (Schares *et al*., 2017*b*). One-day-old chickens of two lines (white layer, line A, brown layer, line B) inoculated intravenously with tachyzoites of a cross line of Type II/Type III *T. gondii* strain; higher mortality was observed in line A chickens (Schares *et al*., 2017*b*). Serum antibody levels assessed by SAG1-ELISA at 31 days p.i. were higher in chickens of line B. By using RT-PCR and 25 mg aliquots of brain and lungs, *T. gondii* burden was higher in the brain than in lungs.

Concurrent infections

Coccidial infections are common in chickens and *Eimeria tenella* is the most pathogenic among the seven or more species of *Eimeria* that infect chickens. Chickens are also commonly infected with *T. gondii*. Therefore, the effect of concurrent infections of these two coccidians was investigated. Results indicated that *E. tenella* and *T. gondii* could interact *in vivo* and *in vitro* (Zou *et al*., 2011; Tang *et al*., 2016; Hiob *et al*., 2017; Zhang *et al*., 2018). By using moderate doses of *E. tenella* and *T. gondii*, an adverse or synergistic effect was not demonstrated in dually infected chickens (Hiob *et al*., 2017).

Conclusions

Here, we summarized seroprevalence, clinical disease, epidemiology and genetic diversity of *T. gondii* strains isolated from chickens worldwide for the past decade. It is obvious that *T. gondii* infection in FR chickens is common and chickens are excellent sentinels to monitor *T. gondii* contamination in the environment. Chickens, in general, are resistant to *T. gondii* infection. Genetic studies revealed low genetic diversity in Europe, Asia, Africa and the USA, intermediate diversity in Caribbean Islands, but higher diversity of *T. gondii* from FR chickens in South America. Controlled experiments using chickens on farms in Argentina and the USA revealed the dynamic of infection and distribution of the parasites in these animals. It will be good to have similar studies from other parts of the world and to conduct genetic analyses of *T. gondii* isolates from sentinel chickens over a period time, which can shed light on the dynamics of *T. gondii* infections on farms, to reveal single or multiple exposures *T. gondii* strains.

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