

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

Review

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Author for correspondence:

J. P. Dubey,
E-mail: jitender.dubey@ars.usda.gov

J. P. Dubey¹ , H. F. J. Pena², C. K. Cerqueira-Cézar¹, F. H. A. Murata¹, O. C. H. Kwok¹, Y. R. Yang³, S. M. Gennari^{2,4} and C. Su⁵

¹United States Department of Agriculture, Agricultural Research Service, Animal Parasitic Diseases Laboratory, Beltsville Agricultural Research Center, Building 1001, Beltsville, MD 20705-2350, USA; ²Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Av. Prof. Orlando Marques de Paiva, 87, Cidade Universitária, São Paulo, SP, CEP 05508-000, Brazil; ³Laboratory of Veterinary Pathology, College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou 450002, PR China; ⁴Programa de Pós-Graduação em Medicina Veterinária, Universidade Santo Amaro, Rua Prof. Enéas Siqueira Neto, 340, São Paulo, SP, CEP 04829-900, Brazil and ⁵Department of Microbiology, University of Tennessee, Knoxville, TN 37996-0845, USA

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, 2010a, 2010b).

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, 2010a).

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.*, 2003a). 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.*,

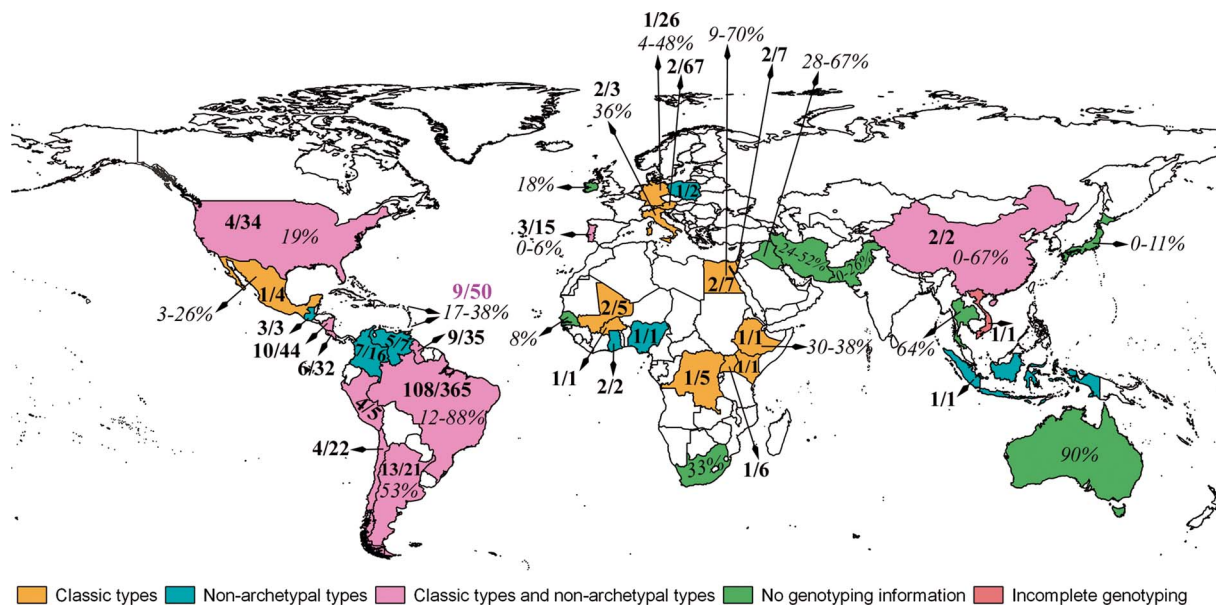


Fig. 1. Worldwide distribution of *T. gondii* infections in chickens. Numbers in bold are the number of *T. gondii* genotypes/number of viable isolates. Seroprevalences are given as %.

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, 2010b). 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.*, 2017a). 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, 2010a), 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

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	More 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 <i>et al.</i> (2019a)
	Dominica		76	29	38.2				
	Trinidad and Tobago		41	7	17.1				
Caribbean islands	Grenada	FR	145	39	26.9	MAT	1:25		Chikweto <i>et al.</i> (2017)
China	13 provinces	FR	1173	226	19.3	ELISA ¹	1:5	Circulating <i>T. gondii</i> antigens detected in 119 (16.9%) of sera.RF	Zhao <i>et al.</i> (2012b)
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				
	FuNing	FR	100	53	53.0	ELISA ¹	21 samples positive by TCA		Zhao <i>et al.</i> (2012a)
China	GanSu	Caged	605	10	1.7	IHA ¹	1:64		He <i>et al.</i> (2016)
China	GanSu	FR	92	9	9.8	IHA ¹	1:64		Wang <i>et al.</i> (2016)
		Caged	187	6	3.2				
China	GuangDong	FR	83	31	37.3	IHA ¹	1:64		Liu <i>et al.</i> (2013)
		Caged	380	63	16.6	IHA ¹	1:64		
China	GuangZhou	FR	361	41	11.4	MAT	1:5		Yan <i>et al.</i> (2009)
China		Caged	244	10	4.1				
	HeBei	FR	364	24	6.6	IHA ¹	1:64		Cui <i>et al.</i> (2010)
		Caged	120	0	0				
China	HeBei	FR	345	38	11.0	IHA ¹	1:64		Tian and Cui (2010)
China		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	Xu et al. (2012)
							1.3% of 160 boilers, 7.9% of 190 layers, and 8.0% of 100 breeders-seropositive	
		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. (2012a)
		Caged	392	13	3.3			
China	LiaoNing	FR	206	23	11.2	MAT	1:25	Wang et al. (2014a)
		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	Liu et al. (2017)
							41 of 100 soil samples positive for <i>T. gondii</i> DNA	
China	Northeastern		96	13	13.5	Oocyst-specific protein ELISA		Liu et al. (2019)
							9 positive with sporozoite-specific protein ELISA	
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 <i>et al.</i> (2012b)
		Caged	296	14	4.7				
China	XinJiang	FR	100	12	12.0	IHA ¹	1:64		Lei <i>et al.</i> (2015)
Czech Republic	Abattoir	Caged	480	2	0.4	IFA	1:40		Bartova <i>et al.</i> (2009)
Egypt	Benni Suef	FR	90	18	20.0	IHA ¹			Aboelhadid <i>et al.</i> (2013)
		Abattoir	125	12	9.6				
Egypt	Cairo, Giza, Kalubiya	FR	88	33	37.5	ELISA			ElFadaly <i>et al.</i> (2017)
Egypt	Delta	FR	97	16	16.4	ELISA			Ibrahim <i>et al.</i> (2016)
		Abattoir	207	18	8.6				
Egypt	Kafr sheikh	FR	84	32	38.1	IHA ³	1:80		Harfoush and Tahoon (2010)
Egypt	Several	FR	108	75	69.5	ELISA			Barakat <i>et al.</i> (2012)
		Abattoir	331	227	68.5				
Ethiopia	Addis Ababa	FR	125	48	38.4	MAT ¹	1:5	Tg	Tilahun <i>et al.</i> (2013)
Ethiopia	Central	FR	601	183	30.5	MAT ¹	1:60	RF	Gebrenehin <i>et al.</i> (2015)
Germany	Eastern	Large farms	384	14	3.7	ELISA		Tg, RF	Schares <i>et al.</i> (2017a)
		Backyards	86	41	47.7				
Iran	Ahvaz	FR	106	55	51.8	MAT	1:10	Td	Hamidinejat <i>et al.</i> (2014)
				51	48.1	ELISA			
Iran	Fars	FR	231	58	24.5	IFA	1:16	Td	Asgari <i>et al.</i> (2009)
Iraq	Al-Najaf, Al-Qadisiya, Babylon	FR	200	134	67.0	LAT ²			Alkhaled <i>et al.</i> (2012)
		Industrial	200	62	31.0				
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 <i>et al.</i> (2013)
Italy	Piacenza	FR	66	24	36.4	ELISA		Meat juice used for testing. PCR-negative.	Vismarra <i>et al.</i> (2016)
Japan	Gifu	Caged	103	0		LAT ¹	1:64		Matsuo <i>et al.</i> (2014)
		FR	103	0					
Japan	Miyazaki	Boilers	100	0	0	ELISA			Duong <i>et al.</i> (2020)
		FR	267	29	10.9				

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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 <i>et al.</i> (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 <i>et al.</i> (2018)
Pakistan	Khyber Pakhtunkhwa	Caged FR	68 468	4 97	5.9 20.7	IHA	1:80		Mahmood <i>et al.</i> (2014)
Pakistan	Khyber Pakhtunkhwa	Domestic Boilers	168 230	44, IgM 25, IgG 27, IgM 10, IgG	26.2 14.8 11.7 4.3	ELISA ³			Khan <i>et al.</i> (2020)
Portugal	Central	Abattoirs	Boilers-170 FR-178	0 10	0 5.6	MAT	1:10		Rodrigues <i>et al.</i> (2019)
Senegal	Saint-Louis, Sahelian	FR	665	51	7.6	MAT	1:20	RF	Sarr <i>et al.</i> (2020)
South Africa	NS		137	46	33.3	LAT	1:64		Tagwireyi <i>et al.</i> (2019)
Thailand	Bangkok	Backyards	303	194	64.0	IFA	1:16		Chumpolbanchorn <i>et al.</i> (2009)
Thailand	Khon Kaen	FR	257	26	10.1	MAT	1:40		Saichua <i>et al.</i> (2017)
USA	Maryland	Grocery stores	1185	230	19.4	MAT	1:5	<i>T. gondii</i> not isolated from 230 seropositive hearts.	Ying <i>et al.</i> (2017)

^aELISA = enzyme-linked immunosorbent assay. Unless stated otherwise, ELISA = ELISA in-house. ¹ELISA (R&B Scientific, USA); ²ELISA (Military Veterinary Institute, Chinese Academy of Military Medical Sciences, Changchun, Jilin Province, China); ³Bio-ELISA toxo-IgM and IgG kits (Biokit, S.S., Barcelona, Spain).

IFA = Indirect fluorescent antibody test.

IHA = Indirect hemagglutination antibody test. ¹IHA (Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Science, Lanzhou, Gansu Province, China); ²Immuno-HAI Toxo, Wama Diagnostics, São Paulo, Brazil); ³IHA (SERFIB, France)

LAT = Latex agglutination test. ¹Toxochek-MT, Eiken Chemical, Tokyo, Japan; (1) Toxo-HAI Fumouze Diagnostics, Le Malesherbes, Levallois Perret, France; ²Toxoplasmosis Latex Test (Plasmatec, United Kingdom); ³Toxoreagent RST701, Mast Group, United Kingdom.

MAT = Modified agglutination test (Dubey and Desmonts, 1987). ¹MAT (Toxo-Screen DA[®], Biomerieux, Marcy l'Etoile, France). This is the same test as MAT.

^bRF = 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	Buenos Aires	17 seropositive	B, H	6	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)	3 strains mouse virulent	Bernstein <i>et al.</i> (2018); Dubey <i>et al.</i> (2003e); Rajendran <i>et al.</i> (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 <i>et al.</i> (2018); Moré <i>et al.</i> (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 <i>et al.</i> (2016); Bernstein <i>et al.</i> (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 <i>et al.</i> (2005b); Verma <i>et al.</i> (2015)
Brazil	Alagoas	8 seropositive	B, H	4	TgCkBr184-187	2 genotypes: #13 (2, TgCkBr184,185), #88 (1, TgCkBr186), Mixed (1, TgCkBr187)		de Oliveira <i>et al.</i> (2009); Dubey <i>et al.</i> (2008b); Shwab <i>et al.</i> (2014)
Brazil	Alagoas		B	2	TgCKAL01,02	2 genotypes: #146 (1, TgCKAL01), #277 (1, TgCKAL02)		dos Santos Silva <i>et al.</i> (2020)
Brazil	Alagoas	2	B, H	2	TgCkBrAL01,02	2 genotypes: #114 (1, TgCkBrAL02), #277 (1, TgCkBrAL01)		Ribeiro-Andrade <i>et al.</i> (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 <i>et al.</i> (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 <i>et al.</i> (2008); Gonçalves <i>et al.</i> (2012); Rocha <i>et al.</i> (2018)

Brazil	Bahia	10	B, H	4	TgCkBr173-176	#302, new1 (1, TgCkBr302), #303, new2 (1, TgCkBr303), #304, new3 (1, TgCkBr304), Mixed (5, TgCkBr284,287,297,298,299), Incomplete (2, TgCkBr300,306)	de Oliveira <i>et al.</i> (2009); Dubey <i>et al.</i> (2008b); Shwab <i>et al.</i> (2014)
Brazil	Ceará	17 seropositive	B, H	6	TgCkBr177-182	2 genotypes: #13 (2, TgCkBr174,176), #81 (1, TgCkBr173), Mixed (1, TgCkBr175)	de Oliveira <i>et al.</i> (2009); Dubey <i>et al.</i> (2008b); Shwab <i>et al.</i> (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)	Beltrame <i>et al.</i> (2012); Pena <i>et al.</i> (2013)
Brazil	Espírito Santo	13 seropositive	B, H	5	TgCkBrEs1-5	3 genotypes: #6 (2, TgCkBrEs4,5), #36 (2, TgCkBrEs2,3), #206 (1, TgCkBrEs1)	Ferreira <i>et al.</i> (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 <i>et al.</i> (2010); Shwab <i>et al.</i> (2014)
Brazil	Maranhão	14 seropositive	B, H	2	TgCkBr171,172	1 genotype: #57 (2, TgCkBr171,172)	de Oliveira <i>et al.</i> (2009); Dubey <i>et al.</i> (2008b); Shwab <i>et al.</i> (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 <i>et al.</i> (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 <i>et al.</i> (2011)		Holsback <i>et al.</i> (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 <i>et al.</i> (2011); Shwab <i>et al.</i> (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 <i>et al.</i> (2006); Silva <i>et al.</i> (2014)
Brazil	Minas Gerais	77 seropositive	B, H	2	TgChBrUD1,2	2 genotypes: #11 (1, TgChBrUD1), #6 (1, TgChBrUD2)		Lopes <i>et al.</i> (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 <i>et al.</i> (2007b); Shwab <i>et al.</i> (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, TgCkBrPB1,12,14), #274 (1, TgCkBrPB26), #277 (2, TgCkBrPB7,8)	16 isolates mouse virulent	Feitosa <i>et al.</i> (2016, 2017)

Brazil	Paraná	119 seropositive	B, H, Li, Lu	38	TgCkBrPr1-18	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. (2003a, 2006a, 2008b); 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. (2008b); 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	153 (123 + 30)	B, H, Sk	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, TgCkBr30,34,59,67), #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. (2003a, 2006a); 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
Brazil	Rio Grande do Norte	30 seropositive	B	13	TgCkBrRN1-13	#71 (1, TgCkBr71), #242, new (1, TgCkBr83), #243, new (1, TgCkBr63)		THIS STUDY (genotyping)
Brazil	Rio Grande do Norte	17 seropositive	B, H	4	TgCkBr167-170	1 genotype: #163 (7, TgCkBrRN1,2,3,4,10,12,13), Incomplete likely #163 (6, TgCkBrRN5,6,7,8,9,11) 3 genotypes: #13 (2, TgCkBr167,170), #78 (1, TgCkBr169), #129 (1, TgCkBr168)		Clementino Andrade <i>et al.</i> (2013) de Oliveira <i>et al.</i> (2009); Dubey <i>et al.</i> (2008b); Shwab <i>et al.</i> (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 <i>et al.</i> (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 <i>et al.</i> (2007b, 2008b); Shwab <i>et al.</i> (2014)
Brazil	Rio Grande do Sul	2 clinical (see text)	Lu	2	TgCkBrRS20,21	1 genotype: #280 (2, TgCkBrRS20,21)	Strain mouse virulent	Vielmo <i>et al.</i> (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 <i>et al.</i> (2006a, 2008b); Shwab <i>et al.</i> (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 <i>et al.</i> (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),		Travisani <i>et al.</i> (2017)

Brazil	São Paulo	B, H	17 isolated before 2009		#306, new NEO2 (1, Ck127), Mixed (1, Ck128)	11 genotypes: #6 (1, TgCkBr10), #8 (3, TgCkBr7,11,17), #63 (2, TgCkBr13,23), #64 (2, TgCkBr19,24), #94 (1, TgCkBr16), #125 (1, TgCkBr8) #6 (1, TgCkBr4), #63 (1, TgCkBr6), #69 (1, TgCkBr21), #227 (1, TgCkBr3), #244 (1, TgCkBr18), #245 (1, TgCkBr5), #246 (1, TgCkBr14)	Dubey, <i>et al.</i> (2002, 2006a, 2008b); Shwab, <i>et al.</i> (2014)
Brazil	Sergipe	B, H	1	TgCkBr183	1 genotype: #13 (1, TgCkBr183)	THIS STUDY (genotyping)	de Oliveira <i>et al.</i> (2009); Dubey <i>et al.</i> (2008b); Shwab <i>et al.</i> (2014)
Burkina Faso			1 isolated before 2009		1 genotype: #2, Type III (1, TgCkBF-1)		Dubey <i>et al.</i> (2005d); Velmurugan <i>et al.</i> (2008); Shwab <i>et al.</i> (2014)
Caribbean islands	St. Kitts	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 <i>et al.</i> (2017, 2019a, 2019b)
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 <i>et al.</i> (2006a); Rajendran <i>et al.</i> (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 <i>et al.</i> (2012a)
China	Anhui	B	24	1, TgChsz1	1 genotype: #225 (1, TgCkszl)		Wang <i>et al.</i> (2013)
Colombia			16 isolated before 2009		7 genotypes: #14 (1, TgCkCo1), #28 (1, TgCkCo5), #29 (1, TgCkCo20),		Dubey <i>et al.</i> (2005c); Rajendran <i>et al.</i> (2012)

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Table 2. (Continued.)

Country	Location	No. tested	Tissues	No. isolated	Strain designation	PCR-RFLP genotype (ToxoDB)	Notes	Reference
Congo				5 isolated before 2009		#38 (9, TgCkCo6,8,10,12,13,15,21,23,24), #178 (1, TgCkCo4), #179 (2, TgCkCo17,22), #188 (1, TgCkCo9)		Dubey <i>et al.</i> (2005d); Velmurugan <i>et al.</i> (2008); Shwab <i>et al.</i> (2014)
Costa Rica				32 isolated before 2009		1 genotype: #2, Type III (5, TgCkDROC-3,6,8,9,10) 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 <i>et al.</i> (2006c); Rajendran <i>et al.</i> (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 <i>et al.</i> (2003b); Dubey (2010b); Shwab <i>et al.</i> (2014); Velmurugan <i>et al.</i> (2008)
Ethiopia	Addis Ababa	115 (72-seronegative 43 seropositive)	H	1		1 genotype: #1, clonal Type II		Tilahun <i>et al.</i> (2013)
Ethiopia	Central	41 seropositive	B, H	29 (tissue cysts in 24, 5 positive by serology only)		ND		Gebremedhin <i>et al.</i> (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 <i>et al.</i> (2017a)
Ghana				2 isolated before 2009		2 genotypes: #132 (1, TgCkGh2), #137 (1, TgCkGh1)		Dubey <i>et al.</i> (2008a); Shwab <i>et al.</i> (2014); Velmurugan <i>et al.</i> (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 <i>et al.</i> (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 <i>et al.</i> (2005a); Rajendran <i>et al.</i> (2012)
Guatemala	3 isolated before 2009	3 genotypes: #7 (1, TgCkGa6), #190 (1, TgCkGa1), #191 (1, TgCkGa4)	Dubey <i>et al.</i> (2005f); Rajendran <i>et al.</i> (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 <i>et al.</i> (2007a); Shwab <i>et al.</i> (2014)
Indonesia	1 isolated before 2009	1 genotype: #89 (1, TgCkId1)	Dubey <i>et al.</i> (2008a)
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 <i>et al.</i> (2004c); Verma <i>et al.</i> (2015)
Italy	3 isolated before 2009	2 genotypes: #1(1, TgCkIt3) #3 (2, TgCkIt1,2)	Dubey <i>et al.</i> (2008a)
Kenya	1 isolated before 2009	1 genotype: #3, Type II variant (1, TgCKKen-1)	Dubey <i>et al.</i> (2005d); Velmurugan <i>et al.</i> (2008); Shwab <i>et al.</i> (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 <i>et al.</i> (2005d); Velmurugan <i>et al.</i> (2008); Shwab <i>et al.</i> (2014)
Mexico	4 isolated before 2009	1 genotype: #2, Type III (4, TgCKMx1,2,3,4)	Dubey <i>et al.</i> (2004b); Dubey (2010b); Shwab <i>et al.</i> (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 <i>et al.</i> (2006d); Rajendran <i>et al.</i> (2012)

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Table 2. (Continued.)

Country	Location	No. tested	Tissues	No. isolated	Strain designation	PCR-RFLP genotype (ToxoDB)	Notes	Reference
Nigeria		5 seropositive	B, H	1	TgCKNg1	#102 (1, TgCKNi35), #140 (1, TgCKNi27) 1 genotype: #15 (1, TgCKNg1)		Shwab <i>et al.</i> (2014); Velmurugan <i>et al.</i> (2008)
Peru				5 isolated before 2009		4 genotypes: #2, Type III (1, TgCKPe2), #17 (2, TgCKPe4,6), #116 (1, TgCKPe3), #189 (1, TgCKPe189)		Dubey <i>et al.</i> (2004a); Rajendran <i>et al.</i> (2012)
Poland				2 isolated before 2009		1 genotype: #15 (2, TgCKPo1,2)		Dubey <i>et al.</i> (2008a);
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 <i>et al.</i> (2006e); Verma <i>et al.</i> (2015)
Uganda		20 seropositive	B, H	9	TgCKUg 1-9	6 were clonal Type II based on 6 RFLP markers.		Dubey (2010b); Lindström <i>et al.</i> (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 <i>et al.</i> (2005e); Rajendran <i>et al.</i> (2012)
Vietnam				1 isolated before 2009		Partial data (SAG1-u-1, SAG2-II, SAG3-III, c22-8-II)		Dubey <i>et al.</i> (2008a);
USA	Illinois			11 isolated before 2009	TgCKUsil 1-11	1 genotype: #1, clonal Type II (11, TgCKUsil1-11)		Dubey <i>et al.</i> (2007c); Shwab <i>et al.</i> (2014)
USA	Massachusetts, Ohio			15 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)		Dubey <i>et al.</i> (2003c); Dubey (2010b); Ying <i>et al.</i> (2017)
				8 isolated before 2009		3 genotypes: #2, Type III (4, TgCKUsOh2,3,4,5), #3, Type II variant (1, TgCKUsOh11), #170 (3, TgCKUsOh8,9,10)		
USA	Massachusetts, Rhode Island, Connecticut	31 -sentinel chickens	B, H, Sk	27	ND			Dubey <i>et al.</i> (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, 2010a). 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.*, 2003e; Dubey *et al.*, 2005g), Austria (Dubey *et al.*, 2005b), Brazil (Dubey *et al.*, 2002, 2003a; da Silva *et al.*, 2003; Dubey *et al.*, 2003d; Dubey *et al.*, 2006a; Dubey *et al.*, 2010), Chile (Dubey *et al.*, 2006b), Colombia (Dubey *et al.*, 2005c), Congo (Dubey *et al.*, 2005d), Costa Rica (Dubey *et al.*, 2006c), Egypt (Dubey *et al.*, 2003b), Grenada (Dubey *et al.*, 2005a), Israel (Dubey *et al.*, 2004c), Italy (Dubey *et al.*, 2008a), Mexico (Dubey *et al.*, 2004b), Nicaragua (Dubey *et al.*, 2006d), Peru (Dubey *et al.*, 2004a), Poland (Dubey *et al.*, 2008a), Portugal (Dubey *et al.*, 2006e), Sri Lanka (Dubey *et al.*, 2005h), USA (Dubey *et al.*, 2003c; Dubey *et al.*, 2007c), Venezuela (Dubey *et al.*, 2005e) 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, 2010a). 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 (Schaes *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 (Schaes *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

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.

Continent/country	Total typed	Classic types			ToxoDB-RFLP genotype									
		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	0	Velmurugan et al. (2008); Shwab et al. (2014)	
Congo	5	0	0	5	0	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	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	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 (2010b)	
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. (2012a); 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. (2008a)	
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. (2017a, 2017b)	
Italy	3	0	3	0	0	0	0	0	0	0	0	0	Dubey et al. (2008a)	
Poland	2	0	0	0	0	0	0	0	0	0	2	0	Dubey et al. (2008a)	
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	0	0	0	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	0	0	3	0	11 (#141-7, #265-3, #264-1)	Hamilton et al. (2017, 2019a, 2019b)
Total Caribbean	50	0	6	21	0	0	0	1	0	0	0	8	0	14	
CENTRAL AMERICA															
Costa Rica	32	0	0	0	1	0	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	0	1	0	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	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	0	0	46	
SOUTH AMERICA															
Argentina	21	0	2	3	0	0	2	0	1	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	0	1 (#14-1)	Rajendran et al. (2012)	
Colombia	16	0	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	0	31 (#12-12, #25-5, #30-4, #31-5, #48-2, #68-2, #123-1)	Dubey et al. (2007a); Shwab et al. (2014)	
Peru	5	0	0	1	0	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	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	0	Dubey (2010b); Shwab et al. (2014)	
USA	34	0	19	11	0	0	0	0	0	0	0	0	4 (#170-3, mixed-1)	Dubey et al. (2003c); Dubey et al. (2007c); Dubey (2010b); Shwab et al. (2014)	
Total North America	38	0	19	15	0	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, 2010a). 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.

More *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, 2010a). *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, 2010b).

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 (Schaes *et al.*, 2017a). 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, 2010a).

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; Schaes *et al.*, 2017a,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.*, 2014b; 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.*, 2014b; 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.*, 2014a).

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

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 <i>et al.</i> (2018); Pardini <i>et al.</i> (2016)
Australia	Western	50	B, S	B1	3 of 27 brains, 3 of 23 spleens	12.0	Chumpolbanchorn <i>et al.</i> (2013)
Brazil	Mato Grosso do Sul	40	B, H	B1	16	40.0	Holsback <i>et al.</i> (2012)
Brazil	Pernambuco	12	B, H, Li, Lu	REP-529	2	16.7	Fernandes <i>et al.</i> (2016)
Canada	Quebec, Ontario, British Columbia	94	breast	B1 and REP-529	7	7.5	Iqbal <i>et al.</i> (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 <i>et al.</i> (2019b)
Caribbean islands	St. Kitts	81	B, H	ITS1	23	28.0	Hamilton <i>et al.</i> (2017)
China	Henan	25	H	450bp	4	16.0	Feng <i>et al.</i> (2016)
China	Tai'an	360-super market	H	ITS1	8	2.2	Wang <i>et al.</i> (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 <i>et al.</i> (2017)
China	Shandong	1653-supermarkets	H	Nested PCR	204	12.3	Sun (2018)
Colombia	Sincelejo-Sucre	40	NS	B1	14	35.0	Campo-Portacio <i>et al.</i> (2014)
Colombia	Bogota	60	Sk	B1	33	55.0	Franco-Hernandez <i>et al.</i> (2016)
Iran	Ahvaj	106	B, H, Li	ITS1	49	46.2	Hamidinejat <i>et al.</i> (2014)
Iran	Khuzestan	103	B, H	B1	16 (B of 6, H of 16)	15.5	Khademvatan <i>et al.</i> (2013)
Iran	Fars	29 seropositive	B, H, Li	B1	<i>T. gondii</i> DNA in 27 of 29: livers 25 brains and 16 hearts	93.1	Asgari <i>et al.</i> (2009)
Iran	Bandar, Haji	200	Eggs	529 bp	22	11.0	Khademi <i>et al.</i> (2018)
Iran	Northwestern	50	NS	B1	4	8.0	Mahami-Oskouei <i>et al.</i> (2017)
Kenya	Thika	105	B1	529 bp (1 isolate by mouse bioassay, details missing)	83	79.0	Mose <i>et al.</i> (2016); Mose <i>et al.</i> (2017)
Pakistan	Khyber Pakhtunkwa	Domestic-65 Boilers-230	H, Li, Sk	B1	13 32	20.0 10.8	Khan <i>et al.</i> (2020)
Taiwan		100 grocery store	H, Li, Sk	B1	4	4.0	Fuh <i>et al.</i> (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 than 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, TgCatpGi1/Ta//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 (Schaes *et al.*, 2017b). 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 (Schaes *et al.*, 2017b). 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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References

- Aboelhadid SM, Abdel-Ghany AE, Ibrahim MA and Mahran HA (2013) Seroprevalence of *Toxoplasma gondii* infection in chickens and humans in Beni Suef, Egypt. *Global Veterinaria* **11**, 139–144.
- Alkhaled MJA, Yakoob AY and Al-Hamadani AHU (2012) An investigation of toxoplasmosis in free range chickens, industrial chickens and duck in mid Euphrates area of Iraq. *Al-Qadisiya Journal of Veterinary Medicine Sciences* **11**, 17–24.
- Alvarado-Esquivel C, González-Salazar AM, Alvarado-Esquivel D, Ontiveros-Vázquez F, Vitela-Corralles J, Villena I and Dubey JP (2012) Seroprevalence of *Toxoplasma gondii* infection in chickens in Durango State, Mexico. *Journal of Parasitology* **98**, 431–432.
- Appiah-Kwarteng C, Saito T, Toda N, Kitoh K, Nishikawa Y, Adenyo C, Kayang B, Owusu EO, Ohya K, Inoue-Murayama M, Kawahara F, Nagamune K and Takashima Y (2019) Native SAG1 in *Toxoplasma gondii* lysates is superior to recombinant SAG1 for serodiagnosis of *T. gondii* infections in chickens. *Parasitology International* **69**, 114–120.
- Asgari, Q., Motazedian, M. H., Esmaelzadeh, B., Kalantari, M. and Hatam, GhR (2009). The prevalence of *Toxoplasma* infection among free-ranging chickens in southern Iran using IFA and nested-PCR. *Iranian Journal of Parasitology* **4**, 29–36.
- Ayinmode AB and Dubey JP (2012) *Toxoplasma gondii* infection in free-range chicken: mini-review and seroprevalence study in Oyo State, Nigeria. *African Journal Biomedical Research* **15**, 145–148.
- Ayinmode AB and Jones-Akinbobola R (2015) Detection of *Toxoplasma gondii* IgG antibodies in Nigerian free-range chickens using indirect fluorescent antibody test (IFAT). *Alexandria Journal of Veterinary Sciences* **47**, 187–190.
- Ayinmode AB and Olaosebikan RIA (2014) Seroprevalence of *Toxoplasma gondii* infection in free ranged chicken from rural and urban settlements in Oyo State, Nigeria. *African Journal of Medicine and Medical Sciences* **43**, 51–57.
- Barakat AM, Salem LM, El-Newishy AM, Shaapan RM and El-Mahllawy EK (2012) Zoonotic chicken toxoplasmosis in some Egyptians governorates. *Pakistan Journal of Biological Sciences* **15**, 821–826.
- Bártová E, Sedláč K and Literák I (2009) Serologic survey for toxoplasmosis in domestic birds from the Czech Republic. *Avian Pathology* **38**, 317–320.
- Beltrame MAV, Pena HFJ, Ton NC, Lino AJB, Gennari SM, Dubey JP and Pereira FEL (2012) Seroprevalence and isolation of *Toxoplasma gondii* from free-range chickens from Espírito Santo state, southeastern Brazil. *Veterinary Parasitology* **188**, 225–230.
- Bernstein M, Pardini L, Moré G, Unzaga JM, Su C and Venturini MC (2018) Population structure of *Toxoplasma gondii* in Argentina. *Infection Genetics and Evolution* **65**, 72–79.
- Bonapaz RS, Hermes-Uliana C, Santos FN, da Silva AV, Araújo EJA and Sant'Ana DMG (2010) Effects of infection with *Toxoplasma gondii* oocysts on the intestinal wall and the myenteric plexus of chicken (*Gallus gallus*). *Pesquisa Veterinária Brasileira* **30**, 787–792.
- Braga CF, Silva AV, Sant'Ana DMG and Araújo EJA (2011) Infecção toxoplásmica causa hipertrofia da parede do cólon de frangos. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* **63**, 340–347.
- Brandão GP, Ferreira AM, Melo MN and Vitor RWA (2006) Characterization of *Toxoplasma gondii* from domestic animals from Minas Gerais, Brazil. *Parasite* **13**, 143–149.
- Cadore GC, Camillo G, Sangioni LA and Vogel FSF (2018) Virulência e multiplicação de isolados de *Toxoplasma gondii* da região central do Rio Grande do Sul. *Pesquisa Veterinária Brasileira* **38**, 1026–1029.
- Camillo G (2015) *Toxoplasma gondii* em galinhas domésticas: epidemiologia, isolamento e caracterização molecular. Universidade Federal de Santa Maria, RS. *Brasil tese de doutorado*, p 1–87.
- Camillo G, Machado MEA, Weber A, Cadore GC, Menezes FR, Pardini L, Sangioni LA and Vogel FSF (2018) Prevalência de anticorpos e fatores de risco associados à infecção por *Toxoplasma gondii* em galinhas domésticas da zona rural de Santa Maria, Rio Grande do Sul. *Pesquisa Veterinária Brasileira* **38**, 1351–1357.
- Campo-Portacio DM, Discuviche-Rebolledo MA, Blanco-Tuirán PJ, Montero-Pérez YM, Orozco-Méndez KE and Assia-Mercado YM (2014) Detección de *Toxoplasma gondii* por amplificación del gen B1 en carnes de consumo humano. *Infectio* **18**, 93–99.
- Casartelli-Alves L, Ferreira LC, Vicente RT, Millar PR, Oliveira RVC, Amendoeira MRR, Schubach TMP and Menezes RC (2012) Prevalência da infecção por *Toxoplasma gondii* em galinhas criadas extensivamente em Rio Bonito, Rio de Janeiro. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* **64**, 1398–1401.
- Casartelli-Alves L, Boechat VC, Macedo-Couto R, Ferreira LC, Nicolau JL, Neves LB, Millar PR, Vicente RT, Oliveira RVC, Muniz AG, Bonna ICF, Amendoeira MRR, Silva RC, Langoni H, Schubach TMP and Menezes RC (2014) Sensitivity and specificity of serological tests, histopathology and immunohistochemistry for detection of *Toxoplasma gondii* infection in domestic chickens. *Veterinary Parasitology* **204**, 346–351.
- Casartelli-Alves L, Amendoeira MRR, Boechat VC, Ferreira LC, Carreira JCA, Nicolau JL, de Freitas Trindade EP, de Barros Peixoto JN, Magalhães MAFM, de Oliveira RVC, Schubach TMP and Menezes RC (2015) Mapping of the environmental contamination of *Toxoplasma gondii* by georeferencing isolates from chickens in an endemic area in Southeast Rio de Janeiro State, Brazil. *Geospatial Health* **10**, 311.
- Chikweto A, Sharma RN, Tiwari KP, Verma SK, Calero-Bernal R, Jiang T, Su C, Kwok OC and Dubey JP (2017) Isolation and RFLP genotyping of *Toxoplasma gondii* in free-range chickens (*Gallus domesticus*) in Grenada, West Indies revealed widespread and dominance of clonal type III parasites. *Journal of Parasitology* **103**, 52–55.
- Chumpolbanchorn K, Anankeitikul P, Ratanasak W, Wiengcharoen J, Thompson RCA and Sukthana Y (2009) Prevalence of *Toxoplasma gondii* indirect fluorescent antibodies in naturally- and experimentally-infected chickens (*Gallus domesticus*) in Thailand. *Acta Parasitologica* **54**, 194–196.
- Chumpolbanchorn K, Lymbery AJ, Pallant LJ, Pan S, Sukthana Y and Thompson RCA (2013) A high prevalence of *Toxoplasma* in Australian chickens. *Veterinary Parasitology* **196**, 209–211.
- Clementino Andrade MM, Pinheiro BV, Cunha MM, Carneiro ACAV, Andrade Neto VF and Vitor RWA (2013) New genotypes of *Toxoplasma gondii* obtained from farm animals in Northeast Brazil. *Research in Veterinary Science* **94**, 587–589.
- Cong W, Huang SY, Zhou DH, Xu MJ, Wu SM, Yan C, Zhao Q, Song HQ and Zhu XQ (2012) First report of *Toxoplasma gondii* infection in market-sold adult chickens, ducks and pigeons in northwest China. *Parasites and Vectors* **5**, 110.
- Costa KS, Santos SL, Uzêda RS, Pinheiro AM, Almeida MAO, Araújo FR, McAllister MM and Gondim LFP (2008) Chickens (*Gallus domesticus*) are natural intermediate hosts of *Neospora caninum*. *International Journal for Parasitology* **38**, 157–159.
- Costa DGC, Marvulo MFV, Silva JSA, Santana SC, Magalhães FJR, Lima Filho CDF, Ribeiro VO, Alves LC, Mota RA, Dubey JP and Silva JCR (2012) Seroprevalence of *Toxoplasma gondii* in domestic and wild animals from the Fernando de Noronha, Brazil. *Journal of Parasitology* **98**, 679–680.
- Cui P, Fang SF, Gu XL, Guo B and Sun XM (2010) A survey of toxoplasmosis in chickens and rabbits in Bashang district. *Shijiazhuang city, China Animal Health Inspection* **27**, 46–47 (in Chinese).
- da Silva DS, Bahia-Oliveira LMG, Shen SK, Kwok OCH, Lehman T and Dubey JP (2003) Prevalence of *Toxoplasma gondii* in chickens from an area in southern Brazil highly endemic to humans. *Journal of Parasitology* **89**, 394–396.
- de Oliveira LN, Costa Junior LM, de Melo CF, Ramos Silva JC, Bevilacqua CML, Azevedo SS, Muradian V, Araújo DAFV, Dubey JP and Gennari SM (2009) *Toxoplasma gondii* isolates from free-range chickens from the northeast region of Brazil. *Journal of Parasitology* **95**, 235–237.
- de Sá SG, Ribeiro-Andrade M, Silva LTR, Neto OLS, Lima DCV, Pedrosa CM, Bezerra MJG and Mota RA (2017) Risk factors associated with *Toxoplasma gondii* infection in free-range chickens in the semiarid region of Brazil. *Brazilian Journal of Veterinary Parasitology* **26**, 221–225.
- Ding GE, Xu MB, Zhou YH, Fang F and Cui HP (2012) Seroepidemiological survey of chickens infected with *Toxoplasma gondii* in Wuxi city. *Chinese Journal of Schistosomiasis Control* **24**, 243–245 (in Chinese).
- Dong H, Su R, Lu Y, Wang M, Liu J, Jian F and Yang Y (2018) Prevalence, risk factors, and genotypes of *Toxoplasma gondii* in food animals and humans (2000–2017) from China. *Frontiers in Microbiology* **9**, 2108.

- dos Santos Silva AC, de Barros LD, Barros VMC, de Alcântara AM, Andrade MR, Garcia JL, Mota RA and Porto WJN (2020). Occurrence of atypical and new genotypes of *Toxoplasma gondii* in free-range chickens intended for human consumption in Brazil. *Acta Parasitologica*, 1–5. doi: 10.2478/s11686-020-00194-2. [Epub ahead of print]
- Dubey JP (2001) Oocyst shedding by cats fed isolated bradyzoites and comparison of infectivity of bradyzoites of the VEG strain *Toxoplasma gondii* to cats and mice. *Journal of Parasitology* **87**, 215–219.
- Dubey, J. P. (2010a). *Toxoplasmosis of Animals and Humans*, 2nd Edn. Boca Raton, Florida: CRC Press, pp 1–313.
- Dubey JP (2010b) *Toxoplasma gondii* infections in chickens (*Gallus domesticus*): prevalence, clinical disease, diagnosis, and public health significance. *Zoonoses and Public Health* **57**, 60–73.
- Dubey JP and Desmonts G (1987) Serological responses of equids fed *Toxoplasma gondii* oocysts. *Equine Veterinary Journal* **19**, 337–339.
- Dubey JP, Graham DH, Blackston CR, Lehmann T, Gennari SM, Ragozo AMA, Nishi SM, Shen SK, Kwok OCH, Hill DE and Thulliez P (2002) Biological and genetic characterisation of *Toxoplasma gondii* isolates from chickens (*Gallus domesticus*) from São Paulo, Brazil: unexpected findings. *International Journal for Parasitology* **32**, 99–105.
- Dubey JP, Graham DH, da Silva DS, Lehmann T and Bahia-Oliveira LMG (2003a) *Toxoplasma gondii* isolates of free-ranging chickens from Rio de Janeiro, Brazil: mouse mortality, genotype, and oocyst shedding by cats. *Journal of Parasitology* **89**, 851–853.
- Dubey JP, Graham DH, Dahl E, Hilali M, El-Ghaysh A, Sreekumar C, Kwok OCH, Shen SK and Lehmann T (2003b) Isolation and molecular characterization of *Toxoplasma gondii* from chickens and ducks from Egypt. *Veterinary Parasitology* **114**, 89–95.
- Dubey JP, Graham DH, Dahl E, Sreekumar C, Lehmann T, Davis MF and Morishita TY (2003c) *Toxoplasma gondii* isolates from free-ranging chickens from the United States. *Journal of Parasitology* **89**, 1060–1062.
- Dubey JP, Navarro IT, Graham DH, Dahl E, Freire RL, Prudencio LB, Sreekumar C, Vianna MC and Lehmann T (2003d) Characterization of *Toxoplasma gondii* isolates from free range chickens from Paraná, Brazil. *Veterinary Parasitology* **117**, 229–234.
- Dubey JP, Venturini MC, Venturini L, Piscopo M, Graham DH, Dahl E, Sreekumar C, Vianna MC and Lehmann T (2003e) Isolation and genotyping of *Toxoplasma gondii* from free-ranging chickens from Argentina. *Journal of Parasitology* **89**, 1063–1064.
- Dubey JP, Levy MZ, Sreekumar C, Kwok OCH, Shen SK, Dahl E, Thulliez P and Lehmann T (2004a) Tissue distribution and molecular characterization of chicken isolates of *Toxoplasma gondii* from Peru. *Journal of Parasitology* **90**, 1015–1018.
- Dubey JP, Morales ES and Lehmann T (2004b) Isolation and genotyping of *Toxoplasma gondii* from free-ranging chickens from Mexico. *Journal of Parasitology* **90**, 411–413.
- Dubey JP, Salant H, Sreekumar C, Dahl E, Vianna MCB, Shen SK, Kwok OCH, Spira D, Hamburger J and Lehmann TV (2004c) High prevalence of *Toxoplasma gondii* in a commercial flock of chickens in Israel, and public health implications of free-range farming. *Veterinary Parasitology* **121**, 317–322.
- Dubey JP, Bhaiyat MI, de Allie C, Macpherson CNL, Sharma RN, Sreekumar C, Vianna MCB, Shen SK, Kwok OCH, Miska KB, Hill DE and Lehmann T (2005a) Isolation, tissue distribution, and molecular characterization of *Toxoplasma gondii* from chickens in Grenada, West Indies. *Journal of Parasitology* **91**, 557–560.
- Dubey JP, Edelhofer R, Marcet P, Vianna MCB, Kwok OCH and Lehmann T (2005b) Genetic and biologic characteristics of *Toxoplasma gondii* infections in free-range chickens from Austria. *Veterinary Parasitology* **133**, 299–306.
- Dubey JP, Gomez-Marin JE, Bedoya A, Lora F, Vianna MCB, Hill D, Kwok OCH, Shen SK, Marcet PL and Lehmann T (2005c) Genetic and biologic characteristics of *Toxoplasma gondii* isolates in free-range chickens from Colombia, South America. *Veterinary Parasitology* **134**, 67–72.
- Dubey JP, Karhemere S, Dahl E, Sreekumar C, Diabaté A, Dabiré KR, Vianna MCB, Kwok OCH and Lehmann T (2005d) First biologic and genetic characterization of *Toxoplasma gondii* isolates from chickens from Africa (Democratic Republic of Congo, Mali, Burkina Faso, and Kenya). *Journal of Parasitology* **91**, 69–72.
- Dubey JP, Lenhart A, Castillo CE, Alvarez L, Marcet P, Sreekumar C and Lehmann T (2005e) *Toxoplasma gondii* infections in chickens from Venezuela: isolation, tissue distribution, and molecular characterization. *Journal of Parasitology* **91**, 1332–1334.
- Dubey JP, Lopez B, Alvarez M, Mendoza C and Lehmann T (2005f) Isolation, tissue distribution, and molecular characterization of *Toxoplasma gondii* from free-range chickens from Guatemala. *Journal of Parasitology* **91**, 955–957.
- Dubey JP, Marcet PL and Lehmann T (2005g) Characterization of *Toxoplasma gondii* isolates in free-range chickens from Argentina. *Journal of Parasitology* **91**, 1335–1339.
- Dubey JP, Rajapakse RPVJ, Ekanayake DK, Sreekumar C and Lehmann T (2005h) Isolation and molecular characterization of *Toxoplasma gondii* from chickens from Sri Lanka. *Journal of Parasitology* **91**, 1480–1482.
- Dubey JP, Gennari SM, Labruna MB, Camargo LMA, Vianna MCB, Marcet PL and Lehmann T (2006a) Characterization of *Toxoplasma gondii* isolates in free-range chickens from Amazon, Brazil. *Journal of Parasitology* **92**, 36–40.
- Dubey JP, Patitucci AN, Su C, Sundar N, Kwok OCH and Shen SK (2006b) Characterization of *Toxoplasma gondii* isolates in free-range chickens from Chile, South America. *Veterinary Parasitology* **140**, 76–82.
- Dubey JP, Su C, Oliveira J, Morales JA, Bolaños RV, Sundar N, Kwok OCH and Shen SK (2006c) Biologic and genetic characteristics of *Toxoplasma gondii* isolates in free-range chickens from Costa Rica, Central America. *Veterinary Parasitology* **139**, 29–36.
- Dubey JP, Sundar N, Pineda N, Kyvsgaard NC, Luna LA, Rimbaud E, Oliveira JB, Kwok OCH, Qi Y and Su C (2006d) Biologic and genetic characteristics of *Toxoplasma gondii* isolates in free-range chickens from Nicaragua, Central America. *Veterinary Parasitology* **142**, 47–53.
- Dubey JP, Vianna MCB, Sousa S, Canada N, Meireles S, da Costa C, Marcet JM, Lehmann PL, Dardé T and Thulliez ML (2006e) Characterization of *Toxoplasma gondii* isolates in free-range chickens from Portugal. *Journal of Parasitology* **92**, 184–186.
- Dubey JP, Applewhaite L, Sundar N, Velmurugan GV, Bandini LA, Kwok OCH, Hill R and Su C (2007a) Molecular and biological characterization of *Toxoplasma gondii* isolates from free-range chickens from Guyana, South America, identified several unique and common parasite genotypes. *Parasitology* **134**, 1559–1565.
- Dubey JP, Sundar N, Gennari SM, Minervino AHH, Farias NAR, Ruas JL, dos Santos TRB, Cavalcante GT, Kwok OCH and Su C (2007b) Biologic and genetic comparison of *Toxoplasma gondii* isolates in free-range chickens from the northern Pará state and the southern state Rio Grande do Sul, Brazil revealed highly diverse and distinct parasite populations. *Veterinary Parasitology* **143**, 182–188.
- Dubey JP, Webb DM, Sundar N, Velmurugan GV, Bandini LA, Kwok OCH and Su C (2007c) Endemic avian toxoplasmosis on a farm in Illinois: clinical disease, diagnosis, biologic and genetic characteristics of *Toxoplasma gondii* isolates from chickens (*Gallus domesticus*), and a goose (*Anser anser*). *Veterinary Parasitology* **148**, 207–212.
- Dubey JP, Huong LTT, Lawson BWL, Subekti DT, Tassi P, Cabaj W, Sundar N, Velmurugan GV, Kwok OCH and Su C (2008a) Seroprevalence and isolation of *Toxoplasma gondii* from free-range chickens in Ghana, Indonesia, Italy, Poland, and Vietnam. *Journal of Parasitology* **94**, 68–71.
- Dubey JP, Velmurugan GV, Chockalingam A, Pena HFJ, Nunes de Oliveira L, Leifer CA, Gennari SM, Bahia Oliveira LMG and Su C (2008b) Genetic diversity of *Toxoplasma gondii* isolates from chickens from Brazil. *Veterinary Parasitology* **157**, 299–305.
- Dubey JP, Rajendran C, Costa DGC, Ferreira LR, Kwok OCH, Qu D, Su C, Marvulo MFV, Alves LC, Mota RA and Silva JCR (2010) New *Toxoplasma gondii* genotypes isolated from free-range chickens from the Fernando de Noronha, Brazil: unexpected findings. *Journal of Parasitology* **96**, 709–712.
- Dubey JP, Lehmann T, Lautner F, Kwok OCH and Gamble HR (2015) Toxoplasmosis in sentinel chickens (*Gallus domesticus*) in New England farms: seroconversion, distribution of tissue cysts in brain, heart, and skeletal muscle by bioassay in mice and cats. *Veterinary Parasitology* **214**, 55–58.
- Dubey JP, Laurin E and Kwok OCH (2016) Validation of the modified agglutination test for detection of *Toxoplasma gondii* in free-range chickens by using cat and mouse bioassay. *Parasitology* **143**, 314–319.
- Duong HD, Appiah-Kwarteng C, Takashima Y, Aye KM, Nagayasu E and Yoshida A (2020) A novel luciferase-linked antibody capture assay (LACA) for the diagnosis of *Toxoplasma gondii* infection in chickens. *Parasitology International* **77**, 102125.
- Elfadaly HA, Hassanain NA, Shaapan RM, Hassanain MA, Barakat AM and Abdelrahman KA (2017) Molecular detection and genotyping of

- Toxoplasma gondii* from Egyptian isolates. *Asian Journal of Epidemiology* **10**, 37–44.
- Feitosa TF, Vilela VLR, de Almeida-Neto JL, dos Santos A, de Moraes DF, Athayde ACR, de Azevedo SS and Pena HFJ (2016) First study on seroepidemiology and isolation of *Toxoplasma gondii* in free-range chickens in the semi-arid region of Paraíba state, Brazil. *Parasitology Research* **115**, 3983–3990.
- Feitosa TF, Vilela VLR, de Almeida-Neto JL, de Melo LRB, de Moraes DF, Alves BF, Nakashima F, Gennari SM, Athayde ACR and Pena HFJ (2017) First report of typical Brazilian *Toxoplasma gondii* genotypes from isolates of free-range chickens (*Gallus gallus domesticus*) circulating in the state of Paraíba, Northeast Brazil. *Parasitology Research* **116**, 2265–2270.
- Feng Y, Lu Y, Wang Y, Liu J, Zhang L and Yang Y (2016) *Toxoplasma gondii* and *Neospora caninum* in free-range chickens in Henan Province of China. *BioMed Research International* **2016**, 8290536.
- Fernandes MFTS, Cavalcanti EFTSF, da Silva JG, Mota AR, de Souza Neto OL, Santos AS, de Albuquerque PPF, de Lima DCV and Mota RA (2016) Occurrence of anti-*Toxoplasma gondii* antibodies and parasite DNA in backyard chicken breeding in Northeast Brazil. *Brazilian Journal of Veterinary Parasitology* **25**, 105–108.
- Ferreira TCR, Buery JC, Moreira NIB, Santos CB, Costa JGL, Pinto LV, Baraviera RCA, Vitor RWA and Fux B (2018) *Toxoplasma gondii*: isolation, biological and molecular characterisation of samples from free-range *Gallus gallus domesticus* from countryside Southeast Brazil. *Brazilian Journal of Veterinary Parasitology* **27**, 384–389.
- Franco-Hernandez EN, Acosta A, Cortés-Vecino J and Gómez-Marín JE (2016) Survey for *Toxoplasma gondii* by PCR detection in meat for human consumption in Colombia. *Parasitology Research* **115**, 691–695.
- Fuh YB, Lin CS, Liao AT, Pong YM, Tung MC, Fei Cy and Lin DS (2013) Survey of *Toxoplasma gondii* in Taipei: Livestock meats, internal organs, cat and dog sera. *Thai Journal of Veterinary Medicine* **43**, 15–21.
- Gebremedhin EZ, Tesfamaryam G, Duguma R, Tilahun G, Di Marco V and Vitale M (2014) Majority of *T. gondii* Seropositive chickens (*Gallus domesticus*) in Central Ethiopia carries the infective parasite. *Acta Veterinaria Scandinavica* **56**, 60.
- Gebremedhin EZ, Tesfamaryam G, Yunus HA, Duguma R, Tilahun G, Di Marco V and Vitale M (2015) Seroepidemiology of *Toxoplasma gondii* infection in free-range chickens (*Gallus domesticus*) of Central Ethiopia. *Epidemiology and Infection* **143**, 608–617.
- Geuthner AC, Koethe M, Ludewig M, Pott S, Schares G, Dauschies A and Bangoura B (2014) Persistence of *Toxoplasma gondii* tissue stages in poultry over a conventional fattening cycle. *Parasitology* **141**, 1359–1364.
- Geuthner AC, Koethe M, Ludewig M, Pott S, Schares G, Maksimov P, Dauschies A and Bangoura B (2019) Development of an *in vivo* model for *Toxoplasma gondii* infections in chickens and turkeys simulating natural routes of infection. *Veterinary Parasitology* **276**, 108956.
- Gonçalves IN, Uzêda RS, Lacerda GA, Moreira RRN, Araújo FR, Oliveira RHM, Corbellini LG and Gondim LFP (2012) Molecular frequency and isolation of cyst-forming coccidia from free ranging chickens in Bahia state, Brazil. *Veterinary Parasitology* **190**, 74–79.
- Halová D, Mulcahy D, Rafter P, Turčáková L, Grant T and de Waal T (2013) *Toxoplasma gondii* in Ireland: seroprevalence and novel molecular detection method in sheep, pigs, deer and chickens. *Zoonoses and Public Health* **60**, 168–173.
- Hamidinejat H, Nabavi L, Mayahi M, Ghourbanpoor M, Pourmehdi Borojeni M, Norollahi Fard S and Shokrollahi M (2014) Comparison of three diagnostic methods for the detection of *Toxoplasma gondii* in free range chickens. *Tropical Biomedicine* **31**, 507–513.
- Hamilton CM, Kelly PJ, Boey K, Corey TM, Huynh H, Metzler D, Villena I, Su C, Innes EA and Katzer F (2017) Predominance of atypical genotypes of *Toxoplasma gondii* in free-roaming chickens in St. Kitts, West Indies. *Parasites & Vectors* **10**, 104.
- Hamilton CM, Black L, Oliveira S, Burrells A, Bartley PM, Melo RPB, Chianini F, Palarea-Albaladejo J, Innes EA, Kelly PJ and Katzer F (2019a) Comparative virulence of Caribbean, Brazilian and European isolates of *Toxoplasma gondii*. *Parasites & Vectors* **12**, 104.
- Hamilton CM, Robins R, Thomas R, Oura C, Oliveira S, Villena I, Innes EA, Katzer F and Kelly PJ (2019b) Prevalence and genetic diversity of *Toxoplasma gondii* in free-ranging chickens from the Caribbean. *Acta Parasitologica* **64**, 738–744.
- Harfoush M and Tahoon AE (2010) Seroprevalence of *Toxoplasma gondii* antibodies in domestic ducks, free-range chickens, turkeys and rabbits in Kafr El-Sheikh Governorate Egypt. *Journal of the Egyptian Society of Parasitology* **40**, 295–302.
- He YC, Li S, Li XR and Qian ZB (2016) Serological survey of animal toxoplasmosis in Zhangye city of Gansu Province. *China Animal Health Inspection* **33**, 12–13 (in Chinese).
- Hiob L, Koethe M, Schares G, Goroll T, Dauschies A and Bangoura B (2017) Experimental *Toxoplasma gondii* and *Eimeria tenella* co-infection in chickens. *Parasitology Research* **116**, 3189–3203.
- Holsback L, Pena HFJ, Ragozo A, Lopes EG, Gennari SM and Soares RM (2012) Serologic and molecular diagnostic and bioassay in mice for detection of *Toxoplasma gondii* in free ranges chickens from Pantanal of Mato Grosso do Sul. *Pesquisa Veterinária Brasileira* **32**, 721–726.
- Hotop A, Buschtöns S, Bangoura B, Zöller B, Koethe M, Spekter-Bosker K, Hotop SK, Tenter AM, Däubener W, Straubinger RK and Groß U (2014) Humoral immune responses in chickens and turkeys after infection with *Toxoplasma gondii* by using recombinant antigens. *Parasitology Research* **113**, 1473–1480.
- Ibrahim HM, Abdel-Ghaffar F, Osman GY, El-Shourbagy SH, Nishikawa Y and Khattab RA (2016) Prevalence of *Toxoplasma gondii* in chicken samples from delta of Egypt using ELISA, histopathology and immunohistochemistry. *Journal of Parasitic Diseases* **40**, 485–490.
- Iqbal A, Janecko N, Pollari F and Dixon B (2018) Prevalence and molecular characterization of *Toxoplasma gondii* DNA in retail fresh meats in Canada. *Food and Waterborne Parasitology* **13**, e00031.
- Khademi SZ, Ghaffarifar F, Dalimi A, Davoodian P and Abdoli A (2018) Molecular detection and genotype identification of *Toxoplasma gondii* in domestic and industrial eggs. *Journal of Food Safety* **38**, e12534.
- Khademvatan S, Saki J, Yousefi E and Abdizadeh R (2013) Detection and genotyping of *Toxoplasma gondii* strains isolated from birds in the southwest of Iran. *British Poultry Science* **54**, 76–80.
- Khan F, Rooman M, Rehman HU, Rab A, Khan A, Rehman AU, Khan A, Ullah A, Ali M and Ahmad A (2018) Prevalence of *Toxoplasma gondii* infection in domestic animals in District Bannu Khyber Pakhtunkhwa (KP), Pakistan. *International Journal of Biomolecules and Biomedicine* **7**, 1–6.
- Khan MB, Khan S, Rafiq K, Khan SN, Attaullah S and Ali I (2020) Molecular identification of *Toxoplasma gondii* in domesticated and broiler chickens (*Gallus domesticus*) that possibly augment the pool of human toxoplasmosis. *PLoS ONE* **15**, e0232026.
- Lei CH, Cai YQ, Bao ZZ, Bian SS and Gao D (2015) Serological investigation of *Toxoplasma gondii* infection of free-range chickens and sparrows in a free-range chicken farm. *Heilongjiang Animal Husbandry and Veterinary Medicine* **2**, 71–72 (in Chinese).
- Li JN (2015) Analysis of *Toxoplasma gondii* infection and risk factors in Xinxiang area (Master's thesis). Xinxiang Medicine College. 1–49 (in Chinese).
- Lindström I, Sundar N, Lindh J, Kironde F, Kabasa JD, Kwok OCH, Dubey JP and Smith JE (2008) Isolation and genotyping of *Toxoplasma gondii* from Ugandan chickens reveals frequent multiple infections. *Parasitology* **135**, 39–45.
- Liu RZ, Chen ZQ, Zhang CF, Zhong WC and Chen MX (2013) Serological investigation of *Toxoplasma gondii* infection in some areas of Guangdong Province. *Poultry Husbandry and Disease Control*, 7–9 (in Chinese).
- Liu XC, He Y, Han DG, Zhang ZC, Li K, Wang S, Xu LX, Yan RF and Li XR (2017) Detection of *Toxoplasma gondii* in chicken and soil of chicken farms in Nanjing region, China. *Infectious Diseases of Poverty* **6**, 62.
- Liu XY, Wang ZD, El-Ashram S and Liu Q (2019) *Toxoplasma gondii* oocyst-driven infection in pigs, chickens and humans in northeastern China. *BMC Veterinary Research* **15**, 366.
- Long X (2013) Serological investigation of *Toxoplasma gondii* in Jingzhou city (Master's thesis). Yangtze University. 1–49 (in Chinese).
- Lopes CS, Franco PS, Silva NM, Silva DAO, Ferro EAV, Pena HFJ, Soares RM, Gennari SM and Mineo JR (2016) Phenotypic and genotypic characterization of two *Toxoplasma gondii* isolates in free-range chickens from Uberlândia, Brazil. *Epidemiology and Infection* **144**, 1865–1875.
- Luo H, Li K, Shahzad M, Zhang H, Lan Y and Xiong X (2017) Seroprevalence of *Toxoplasma gondii* infection in wild boars, wild rabbits, and wild chickens in Hubei Province, China. *Korean Journal of Parasitology* **55**, 85–88.
- Magalhães FJR, da Silva JG, Ribeiro-Andrade M, Pinheiro Júnior JW and Mota RA (2016) High prevalence of toxoplasmosis in free-range chicken of the Fernando de Noronha Archipelago, Brazil. *Acta Tropica* **159**, 58–61.

- Mahami-Oskouei M, Moradi M, Fallah E, Hamidi F and Asl Rahnamaye Akbari N (2017) Molecular detection and genotyping of *Toxoplasma gondii* in chicken, beef, and lamb meat consumed in Northwestern Iran. *Iranian Journal of Parasitology* **12**, 38–45.
- Mahmood ZU, Zahid M, Sthanadar AA, Shah M and Hussain A (2014) Seroprevalence of *Toxoplasma gondii* infection in *Gallus domesticus* of district Mardan, Khyber Pakhtunkhwa, Pakistan. *Pakistan Journal of Zoology* **46**, 1705–1710.
- Maksimov P, Basso W, Zerweck J, Schutkowski M, Reimer U, Maksimov A, Conraths FJ and Schares G (2018) Analysis of *Toxoplasma gondii* clonal type-specific antibody reactions in experimentally infected turkeys and chickens. *International Journal for Parasitology* **48**, 845–856.
- Marques JM, Isbrecht FB, Lucas TM, Guerra IMP, Dalmolin A, da Silva RC, Langoni H and da Silva AV (2009) Detecção de anticorpos anti-*Toxoplasma gondii* em animais de uma comunidade rural do Mato Grosso do Sul, Brasil. *Semina: Ciências Agrárias. Londrina* **30**, 889–898.
- Matsuo K, Kamai R, Uetsu H, Goto H, Takashima Y and Nagamune K (2014) Seroprevalence of *Toxoplasma gondii* infection in cattle, horses, pigs and chickens in Japan. *Parasitology International* **63**, 638–639.
- Millar PR, Alves FMX, Teixeira VQ, Vicente RT, Menezes EM, Sobreiro LG, de Almeida Pereira VL and Amendoeira MRR (2012) Occurrence of infection with *Toxoplasma gondii* and factors associated with transmission in broiler chickens and laying hens in different raising systems. *Pesquisa Veterinária Brasileira* **32**, 231–236.
- Mohammed AA and Abdullah SHH (2013). Diagnostic study of toxoplasmosis in domestic chickens in Sulaimani Province. *Al-Qadisiya Journal of Veterinary Medicine Sciences* **12**, 63–69.
- Moré G, Maksimov P, Pardini L, Herrmann DC, Bacigalupe D, Maksimov A, Basso W, Conraths FJ, Schares G and Venturini MC (2012) *Toxoplasma gondii* infection in sentinel and free-range chickens from Argentina. *Veterinary Parasitology* **184**, 116–121.
- Mose JM, Kagira JM, Karanja SM, Ngotho M, Kamau DM, Njuguna AN and Maina NW (2016) Detection of natural *Toxoplasma gondii* infection in chicken in Thika Region of Kenya using nested polymerase chain reaction. *BioMed Research International* **2016**, 7589278.
- Mose JM, Kamau DM, Kagira JM, Maina N, Ngotho M, Njuguna A and Karanja SM (2017) Development of neurological mouse model for toxoplasmosis using *Toxoplasma gondii* isolated from chicken in Kenya. *Pathology Research International* **2017**, 4302459.
- Pardini L, Moré G, Rudzinski M, Gos ML, Campero LM, Meyer A, Bernstein M, Unzaga JM and Venturini MC (2016) *Toxoplasma gondii* isolates from chickens in an area with human toxoplasmic retinochoroiditis. *Experimental Parasitology* **166**, 16–20.
- Pena HFJ, Vitaliano SN, Beltrame MAV, Pereira FEL, Gennari SM and Soares RM (2013) PCR-RFLP genotyping of *Toxoplasma gondii* from chickens from Espírito Santo state, Southeast region, Brazil: new genotypes and a new SAG3 marker allele. *Veterinary Parasitology* **192**, 111–117.
- Pena HFJ, Alves BF, Soares HS, Oliveira S, Ferreira MN, Bricarello PA, Machado TMP, Castro BBP and Gennari SM (2018) Free-range chickens from Santa Catarina state, southern Brazil, as asymptomatic intermediate hosts for *Toxoplasma gondii* clonal type I and typical Brazilian genotypes. *Veterinary Parasitology: Regional Studies and Reports* **13**, 55–59.
- Rajendran C, Su C and Dubey JP (2012) Molecular genotyping of *Toxoplasma gondii* from Central and South America revealed high diversity within and between populations. *Infection, Genetics and Evolution* **12**, 359–368.
- Ribeiro-Andrade M, de Crasto Souza Carvalho J, Amorim da Silva R, da Conceição Carvalho M, Nascimento Porto WJ and Mota RA (2019) Inter- and intra-genotype differences in induced cystogenesis of recombinant strains of *Toxoplasma gondii* isolated from chicken and pigs. *Experimental Parasitology* **207**, 107775.
- Rocha DS, Nilsson MG, Maciel BM, Pena HFJ, Alves BF, Silva AV, Gondim LFP and Albuquerque GR (2018) Genetic diversity of *Toxoplasma gondii* isolates from free-range chickens in Bahia, Brazil. *Journal of Parasitology* **104**, 377–382.
- Rodrigues FT, Moreira FA, Coutinho T, Dubey JP, Cardoso L and Lopes AP (2019) Antibodies to *Toxoplasma gondii* in slaughtered free-range and broiler chickens. *Veterinary Parasitology* **271**, 51–53.
- Ruiz A and Frenkel JK (1980) Intermediate and transport hosts of *Toxoplasma gondii* in Costa Rica. *American Journal of Tropical Medicine and Hygiene* **29**, 1161–1166.
- Saichua P, Jumnainsong A, Tantrawatpan C, Kiatsopit N, Kopolrat K, Suwannatrai A and Sithithaworn P (2017) Seroprevalence of *Toxoplasma gondii* in free range chickens (*Gallus domesticus*) in Khon Kaen province, Thailand. *Tropical Biomedicine* **34**, 419–424.
- Sarr A, Galal I, Boumediene F, Hamidović A, Dardé ML, Diallo M, Sow A, Niang Y, Cuny T and Mercier A (2020) Seroprevalence and risk factors of *Toxoplasma gondii* infection in free-range chickens in Senegal, West Africa. *Vector-Borne and Zoonotic Diseases* **20**, 15–21.
- Schares G, Bangoura B, Randau F, Goroll T, Ludewig M, Maksimov P, Matzkeit B, Sens M, Bärwald A, Conraths FJ, Opsteegh M and van der Giessen J (2017a) High seroprevalence of *Toxoplasma gondii* and probability of detecting tissue cysts in backyard laying hens compared with hens from large free-range farms. *International Journal for Parasitology* **47**, 765–777.
- Schares G, Herrmann DC, Maksimov P, Matzkeit B, Conraths FJ, Moré G, Preisinger R and Weigend S (2017b) Chicken line-dependent mortality after experimental infection with three type IIxIII recombinant *Toxoplasma gondii* clones. *Experimental Parasitology* **180**, 101–111.
- Schares G, Koethe M, Bangoura B, Geuthner AC, Randau F, Ludewig M, Maksimov P, Sens M, Bärwald A, Conraths FJ, Villena I, Aubert D, Opsteegh M and van der Giessen J (2018) *Toxoplasma gondii* infections in chickens – performance of various antibody detection techniques in serum and meat juice relative to bioassay and DNA detection methods. *International Journal for Parasitology* **48**, 751–762.
- Shen B (2010) *Investigation on Toxoplasma gondii infection in chickens in some areas in China* (Master's thesis). Nanjing Agricultural University. 1–81 (in Chinese).
- Shwab EK, Zhu XQ, Majumdar D, Pena HFJ, Gennari SM, Dubey JP and Su C (2014) Geographical patterns of *Toxoplasma gondii* genetic diversity revealed by multilocus PCR-RFLP genotyping. *Parasitology* **141**, 453–461.
- Silva LA, Andrade RO, Carneiro ACAV and Vitor RWA (2014) Overlapping *Toxoplasma gondii* genotypes circulating in domestic animals and humans in southeastern Brazil. *PLoS ONE* **9**, e90237.
- Soares RM, Silveira LH, da Silva AV, Ragozo A, Galli S, Lopes EG, Gennari SM and Pena HFJ (2011) Genotyping of *Toxoplasma gondii* isolates from free range chickens in the Pantanal area of Brazil. *Veterinary Parasitology* **178**, 29–34.
- Sousa IC, Pena HFJ, Santos LS, Gennari SM and Costa FN (2016) First isolation and genotyping of *Toxoplasma gondii* from free-range chickens on São Luis Island, Maranhão state, Brazil, with a new genotype described. *Veterinary Parasitology* **223**, 159–164.
- Su C and Dubey JP (2020) Isolation and genotyping of *Toxoplasma gondii* strains. *Methods Molecular Biology* **2071**, 49–80.
- Sun P (2018) *Investigation and analysis of Toxoplasma gondii infection in chicken products in Shandong Province* (Master's thesis). Shandong Agriculture University. 1–56 (in Chinese).
- Sun X, Wang Z, Li J, Wei F and Liu Q (2015) Evaluation of an indirect ELISA using recombinant granule antigen GRA1, GRA7 and soluble antigens for serodiagnosis of *Toxoplasma gondii* infection in chickens. *Research in Veterinary Science* **100**, 161–164.
- Tagwireyi WM, Etter E and Neves L (2019) Seroprevalence and associated risk factors of *Toxoplasma gondii* infection in domestic animals in south-eastern South Africa. *Onderstepoort Journal of Veterinary Research* **86**, a1688.
- Tang X, Yin G, Qin M, Tao G, Suo J, Liu X and Suo X (2016) Transgenic *Eimeria tenella* as a vaccine vehicle: expressing TgSAG1 elicits protective immunity against *Toxoplasma gondii* infections in chickens and mice. *Scientific Reports* **6**, 29379.
- Tian PR and Cui P (2010) Investigation on *Toxoplasma gondii* infection in chickens in Zhangjiaikou city. *Animal Husbandry and Feed Science* **31**, 172–173 (in Chinese).
- Tilahun G, Tiao N, Ferreira LR, Choudhary S, Oliveira S, Verma SK, Kwok OCH, Molla B, Saville WJA, Medhin G, Kassa T, Aleme H, Gebreyes WA, Su C and Dubey JP (2013) Prevalence of *Toxoplasma gondii* from free-range chickens (*Gallus domesticus*) from Addis Ababa, Ethiopia. *Journal of Parasitology* **99**, 740–741.
- Travisani N, Barros LD, Vieira-Neto A, Sartor AA, Souza AP, Garcia JL and Moura AB (2017) Genotyping of *Toxoplasma gondii* isolates from naturally infected *Gallus domesticus* in Santa Catarina state, Brazil. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* **69**, 139–145.
- Velmurugan GV, Dubey JP and Su C (2008) Genotyping studies of *Toxoplasma gondii* isolates from Africa revealed that the archetypal clonal

- lineages predominate as in North America and Europe. *Veterinary Parasitology* **155**, 314–318.
- Verma SK, Ajzenberg D, Rivera-Sanchez A, Su C and Dubey JP (2015) Genetic characterization of *Toxoplasma gondii* isolates from Portugal, Austria, and Israel reveals higher genetic variability within the type II lineage. *Parasitology* **142**, 948–957.
- Vieira FEG, Sasse JP, Minutti AF, Miura AC, de Barros LD, Cardim ST, Martins TA, de Seixas M, Yamamura MI, Su C and Garcia JL (2018) *Toxoplasma gondii*: prevalence and characterization of new genotypes in free-range chickens from south Brazil. *Parasitology Research* **117**, 681–688.
- Vielmo A, Pena HFJ, Panziera W, Bianchi RM, De Lorenzo C, Oliveira S, Alves BF, Gennari SM, Pavarini SP, de Barros CSL and Driemeier D (2019) Outbreak of toxoplasmosis in a flock of domestic chickens (*Gallus gallus domesticus*) and guinea fowl (*Numida meleagris*). *Parasitology Research* **118**, 991–997.
- Vismarra A, Mangia C, Barilli E, Brindani F, Bacci C and Kramer L (2016) Meat juice serology for *Toxoplasma gondii* infection in chickens. *Italian Journal of Food Safety* **5**, 5586.
- Vitaliano SN, de Mendonça GM, de Sandres FAM, Camargo JSAA, de Tarso P, Basano SA, Silva JCD, de Souza VKG, Cartonilho G, de Almeida ATS, Gennari SM and Camargo LMA (2015) Epidemiological aspects of *Toxoplasma gondii* infection in riverside communities in the Southern Brazilian Amazon. *Revista da Sociedade Brasileira de Medicina Tropical* **48**, 301–306.
- Wang LF (2018) The oocyst wall protein TgOWP8 of *Toxoplasma gondii* and application in detection of antibodies in serum of pigs and chickens (Master's thesis). Jilin Agriculture University, 1–54 (in Chinese).
- Wang L, Cheng HW, Huang KQ, Xu YH, Li YN, Du J, Yu L, Luo QL, Wei W, Jiang L and Shen JL (2013) *Toxoplasma gondii* prevalence in food animals and rodents in different regions of China: isolation, genotyping and mouse pathogenicity. *Parasites & Vectors* **6**, 273.
- Wang DW, Han XH, Mu MY, Yuan GM, Zhang GX, He JB, Yang N and Li HK (2014a) Epidemiological survey of toxoplasmosis in some animals from northeastern China. *Heilongjiang Animal Husbandry and Veterinary Medicine* 129–131.
- Wang S, Zhao G, Wang W, Xie Q, Zhang M, Yuan C, Hassan IA, Liu X, Xu L, Yan R, Song X and Li X (2014b) Pathogenicity of two *Toxoplasma gondii* strains in chickens of different ages infected via intraperitoneal injection. *Avian Pathology* **43**, 91–95.
- Wang S, Zhao GW, Wang W, Zhang ZC, Shen B, Hassan IA, Xie Q, Yan RF, Song XK, Xu LX and Li XR (2015) Pathogenicity of five strains of *Toxoplasma gondii* from different animals to chickens. *Korean Journal of Parasitology* **53**, 155–162.
- Wang M, Ye Q, Zhang NZ and Zhang DL (2016) Seroprevalence of *Toxoplasma gondii* infection in food-producing animals in Northwest China. *Chinese Journal of Zoonoses* **32**, 608–612.
- Wang R, Zhao N, Zhang H, Wang F, Li H, Liu Y, Zhao X and Zhang X (2020) Prevalence of *Toxoplasma gondii* infections in chicken hearts from farmers' markets and supermarkets in the Tai'an Region of China. *Journal of Food Protection* **83**, 338–341.
- Wen J, Zou J, Huang X, Wen H, Tselmeg, Suo X and Liu, X (2019) Identification of candidate antigens by 2-DE immunoblotting for diagnosis of *Toxoplasma gondii* infection in chickens and rabbits. *Experimental Parasitology* **204**, 107723.
- Wu SC, Zhao DZ, Sun FL, Zhao Y, Xue SJ and Xu YD (2018) Preliminary investigation on the serology of *Toxoplasma gondii* in chicken from Yanbian. *Heilongjiang Animal Science and Veterinary Medicine* **22**, 103–104 (in Chinese).
- Xu P, Song X, Wang W, Wang F, Cao L and Liu Q (2012) Seroprevalence of *Toxoplasma gondii* infection in chickens in Jinzhou, northeastern China. *Journal of Parasitology* **98**, 1300–1301.
- Xu Y, Wang FY, Liu XY, Wei F and Liu Q (2014) A modified agglutination test for diagnosing toxoplasmosis in chicken. *Chinese Journal of Veterinary Medicine* **34**, 1781, 1782, 1789 (in Chinese).
- Yan C, Yue CL, Yuan ZG, He Y, Yin CC, Lin RQ, Dubey JP and Zhu XG (2009) *Toxoplasma gondii* infection in domestic ducks, free-range and caged chickens in southern China. *Veterinary Parasitology* **165**, 337–340.
- Yan C, Yue CL, Yuan ZG, Lin RQ, He Y, Yin CC, Xu MJ, Song HQ and Zhu XQ (2010) Molecular and serological diagnosis of *Toxoplasma gondii* infection in experimentally infected chickens. *Veterinary Parasitology* **173**, 179–183.
- Yang N, Mu MY, Li HK and He JB (2012a) Epidemiological survey of toxoplasmosis in chickens from northeastern China. Proceedings of the 16th symposium of poultry epidemiology branch of Chinese animal husbandry and veterinary association. Beijing, p. 184 (in Chinese).
- Yang N, Mu MY, Li HK, Long M and He JB (2012b) Seroprevalence of *Toxoplasma gondii* infection in slaughtered chickens, ducks, and geese in Shenyang, northeastern China. *Parasites & Vectors* **5**, 237.
- Yin ZW (2019) *Serological investigation of four diseases in poultry in Jilin Province* (Master's thesis). Jilin Agriculture University, 1–42 (in Chinese).
- Ying Y, Verma SK, Kwok OCH, Alibana F, McLeod R, Su C, Dubey JP and Pradhan AK (2017) Prevalence and genetic characterization of *Toxoplasma gondii* in free-range chickens from grocery stores and farms in Maryland, Ohio and Massachusetts, USA. *Parasitology Research* **116**, 1591–1595.
- Zhang R, Thabet A, Hiob L, Zheng W, Dausgies A and Bangoura B (2018) Mutual interactions of the apicomplexan parasites *Toxoplasma gondii* and *Eimeria tenella* with cultured poultry macrophages. *Parasites & Vectors* **11**, 453.
- Zhao GW, Shen B, Xie Q, Xu LX, Yan RF, Song XK, Adam HI and Li XR (2012a) Isolation and molecular characterization of *Toxoplasma gondii* from chickens in China. *Journal of Integrative Agriculture* **11**, 1347–1353.
- Zhao G, Shen B, Xie Q, Xu LX, Yan RF, Song XK, Hassan IA and Li XR (2012b) Detection of *Toxoplasma gondii* in free-range chickens in China based on circulating antigens and antibodies. *Veterinary Parasitology* **185**, 72–77.
- Zhu J, Shen MH, Chen LE, Cao XY, Sun WM and Jin YC (2015) Serological survey of *Toxoplasma gondii* infection in poultry in Songjiang District. *Shanghai Journal of Animal Husbandry and Veterinary Medicine* **6**, 32–33 (in Chinese).
- Zou J, Huang XX, Yin GW, Ding Y, Liu XY, Wang H, Chen QJ and Suo X (2011) Evaluation of *Toxoplasma gondii* as a live vaccine vector in susceptible and resistant hosts. *Parasites & Vectors* **4**, 168.
- Zou Y, Nie LB, Zhang NZ, Zou FC, Zhu XQ and Cong W (2017) First genetic characterization of *Toxoplasma gondii* infection in poultry meat intended for human consumption in eastern China. *Infection, Genetics and Evolution* **55**, 172–174.