

# Epidemiological review of toxoplasmosis in humans and animals in Romania

J. P. DUBEY<sup>1\*</sup>, I. HOTEA<sup>2\*</sup>, T. R. OLARIU<sup>3</sup>, J. L. JONES<sup>4</sup> and G. DĂRĂBUȘ<sup>2</sup>

<sup>1</sup> United States Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, Animal Parasitic Diseases Laboratory, Building 1001, BARC-East, Beltsville, MD 20705-2350, USA

<sup>2</sup> Department of Parasitology, Faculty of Veterinary Medicine, Banat's University of Agricultural Sciences and Veterinary Medicine, 119, Aradului Street, 300645 Timisoara, Timis, Romania

<sup>3</sup> Department of Parasitology, Victor Babes University of Medicine and Pharmacy, P-ta Murgu Eftimie, 2, 300041 Timisoara, Timis, Romania

<sup>4</sup> Parasitic Diseases Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Building 24, 1600 Clifton Road, NE, Mailstop A-06, Atlanta, GA30333, USA

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## SUMMARY

Infections by the protozoan parasite *Toxoplasma gondii* are widely prevalent in humans and other animals worldwide. However, information from eastern European countries is sketchy. In many eastern European countries, including Romania, it has been assumed that chronic *T. gondii* infection is a common cause of infertility and abortion. For this reason, many women in Romania with these problems were needlessly tested for *T. gondii* infection. Most papers on toxoplasmosis in Romania were published in Romanian in local journals and often not available to scientists in other countries. Currently, the rate of congenital infection in Romania is largely unknown. In addition, there is little information on genetic characteristics of *T. gondii* or prevalence in animals and humans in Romania. In the present paper we review prevalence, clinical spectrum and epidemiology of *T. gondii* in humans and animals in Romania. This knowledge should be useful to biologists, public health workers, veterinarians and physicians.

Key words: *Toxoplasma gondii*, Romania, toxoplasmosis, humans, animals, clinical, congenital.

## INTRODUCTION AND HISTORY

The parasite *Toxoplasma gondii* and the disease it causes, toxoplasmosis, were first noted in 1908 in the rodent *Ctenodactylus gundi* in Tunisia by Nicolle and Mancaeux (1908, 1909), and in the domestic rabbit (*Oryctolagus cuniculus*) in Brazil by Splendore (1908). Clinical disease was first recognized in Italy in a domestic animal, a dog, by Mello (1910). The first proven case of congenital toxoplasmosis was described in an infant in the USA by Wolf *et al.* (1939).

The discovery of a novel and specific serologic test, the dye test, by Sabin and Feldman (1948) made it possible to conduct population-based surveys for this parasite. Soon it became clear that *T. gondii* infections are common in humans and animals and clinical disease is relatively uncommon.

The earliest publication on toxoplasmosis in Romania we found is that of Dragomir (1956) who isolated viable *T. gondii* from a human infant. At about the same time, Radacovici and Atanasiu (1959), Lupașcu *et al.* (1963), Elias and Porsche (1961),

Elias *et al.* (1963a, b), Elias (1966) and Elias and Budiu (1973) reported on toxoplasmosis in humans and animals in Romania. Since then, there have been many reports, mostly serological surveys in women with gynaecological problems. In the present paper we review prevalence, clinical spectrum and epidemiology of *T. gondii* in humans and animals in Romania.

## METHODS FOR PRESENT REVIEW

Romania has a human population of >19 million, and joined the European Union in 2007. The country is divided into 8 regions (Fig. 1). We have used abbreviated names of these regions in the following review; full names with human populations are shown in Fig. 1. Our initial search of the PUBMED database indicated references to only 25 papers on toxoplasmosis in humans and animals from Romania. Subsequently, we found numerous papers, mostly in Romanian journals. In the present review we attempted to incorporate all published reports available to us on natural *T. gondii* infections. We consulted original papers when possible. Papers published as abstracts, at symposia and conferences, and reviews, or papers we could not access are listed as supplementary information (in Appendix online – in

\* Corresponding authors: USDA, ARS, APDL, BARC-East, Building 1001, Beltsville, MD 20705, USA. E-mail: jitender.dubey@ars.usda.gov; USAMVB Timisoara, 119, Aradului Street, 300645, Timisoara, Romania. E-mail: hotea\_ionela@yahoo.com

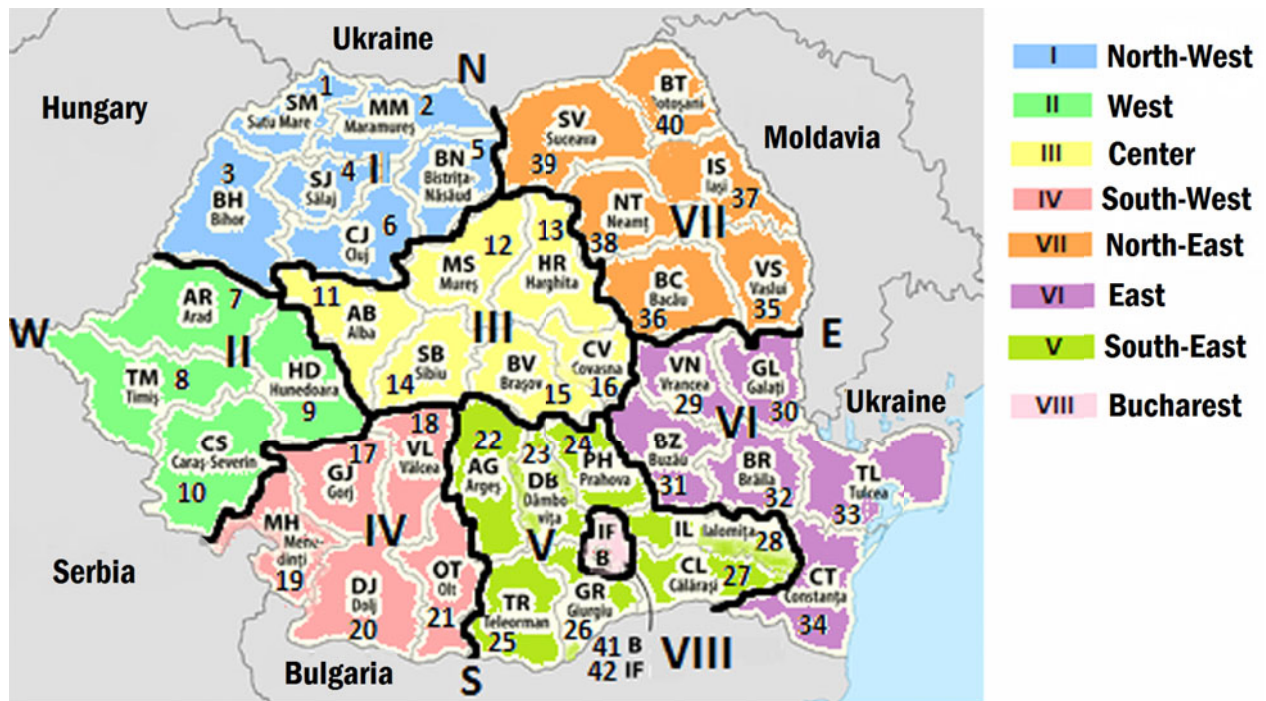


Fig. 1. Map of Romania with 8 regions and distribution of human population. Figures in parentheses are millions of people (in 2011) and % of the total population. Total population of Romania – 19 042 936. **I** Maramures-Crisana (2·49; 13·1%); **II** Banat (1·73; 9·08%); **III** Transilvania (2·25; 11·82%); **IV** Oltenia (1·98; 10·39%); **V** Muntenia (2·99; 15·75%); **VI** Moldova-Tulcea (2·4; 12·6%); **VII** Bucovina-Moldova (3·15; 16·53%); **VIII** Bucharest – capital of Romania (2·04; 10·72%).

Online version only). The main objectives of the review are to summarize research accomplished on toxoplasmosis in Romania, suggest areas for future research, and to encourage international collaboration.

Detailed historical, serological, parasitological and clinical information on *T. gondii* infections in humans and other animals are summarized in the tables throughout the review. Different serological techniques used in Romanian studies are listed in Table 1. Cut-off values for serological tests are listed wherever the authors provided the information. Details of in-house tests are not listed in Table 1 or any subsequent tables. Superscripts in the tables and text refer to details of the serological tests provided in Table 1.

The finding of *T. gondii* antibodies indicates exposure to the parasite. The sensitivity and specificity of different serological tests used to detect *T. gondii* antibodies varies a lot with the test used, serum dilution and the stage of infection. The skin test (dermal hypersensitivity), one of the first tests used to detect *T. gondii* exposure, is very insensitive, and is rarely used now. The Sabin–Feldman dye test is the most sensitive and specific test for human toxoplasmosis but it is rarely used now because it requires the use of live parasites and a complement-like factor from human serum; moreover, the test does not work with sera of some animal species. The indirect fluorescent antibody test (IFAT) and the modified agglutination test (MAT) use whole, killed

tachyzoites, and the results are comparable with those obtained with the dye test, especially at serum dilution of 1:64 or higher. There are several ELISAs developed to detect *T. gondii* exposure and some of them are commercially available (Table 1). Some serological tests can distinguish class and type of antibodies (IgM, IgA, IgE, avidity); we have listed them where this information was provided. We would like to emphasize that the detection of antibodies only indicates exposure and the definitive evidence of infection requires demonstration of the parasite.

TOXOPLASMOSES IN HUMANS

Prevalence of *T. gondii* infection

There is little information concerning *T. gondii* prevalence in the general human population in Romania. Most serological surveys are based on convenience samples, except a recent study by Coroiu *et al.* (2009) who tested 1155 sera based on stratified sampling from the general population in north-west and central Romania with a total population of 4·6 million in 11 counties. Sera were tested for *T. gondii* IgG antibodies by two commercial tests (ELISA<sup>13</sup>, LAT<sup>2</sup>) with similar results. Antibodies to *T. gondii* were found in 686 (59·4%) of 1155 sera; seropositivity varied from 44·9–70·2% depending on the county sampled; prevalence in different counties

Table 1. Details of serological tests used for the detection of antibodies to *T. gondii* in animals and humans in Romania

Test abbreviation	Antigen	Cut-off	Manufacturer <sup>a</sup>	Reference/citation in the present review
<b>Latex agglutination, LAT</b> Pastorex Toxo Test (LAT <sup>1</sup> ) Sanofi Pasteur kit (LAT <sup>2</sup> )	Soluble	Not stated	Bio-Rad, Marnes-la-Coquette, France <a href="http://www.bio-rad.com">www.bio-rad.com</a> Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France (Note, these companies have merged into Biorad)	Tables 2 and 4 Table 4
<b>Modified agglutination, MAT</b> Toxoscreen DA Direct agglutination, DAT	Formalin-treated whole tachyzoites	1:40	Biomérieux, Craponne, France <a href="http://www.biomerieux.com">www.biomerieux.com</a>	Table 4
<b>ISAGA (Immunosorbent Agglutination Assay test)</b>	Whole tachyzoites-killed	Not stated	Not stated	Table 2
<b>Indirect haemagglutination, IHAT</b>	Soluble	1:64	In-house	Table 5
<b>Sabin Feldman dye test</b>	Live tachyzoites	1:4	In-house	Tables 2, 3 and 5
<b>Indirect fluorescent antibody, IFAT</b>	Whole acetone-fixed tachyzoites	1:32	Cantacuzino Institute, Bucuresti, Romania <a href="http://www.cantacuzino.ro">www.cantacuzino.ro</a>	Tables 2 and 4
<b>Skin test</b>	Soluble		In-house	Table 2
<b>Complement fixation (CFT)</b>			In-house	Table 5
<b>Enzyme linked immunosorbent assay (ELISA)</b>				
1. ELISA Immuno Comb Toxo	Solid phase-slide test	1:32	Biogal Galed Labs, Kibbutz Galed, Israel <a href="http://www.biogal.co.il">www.biogal.co.il</a>	Tables 4 and 5
2. ELISA ID Screen Toxoplasmosis Indirect Multi-species)	P 30, recombinant	1:32	ID.VET Innovative Diagnostics, Montpellier, France <a href="http://www.id-vet.com">www.id-vet.com</a>	Tables 4 and 5
3. ELISA Captia <i>Toxoplasma gondii</i> IgG	Inactivated	Not stated	Trinity Biotech P.L.C., Bray, Co Wicklow, Ireland <a href="http://www.trinitybiotech.com">www.trinitybiotech.com</a>	Table 1
4. ELISA ETI-TOXO-G	Solid	Not stated	DiaSorin S.P.A., Saluggia, Vercelli, Italy <a href="http://www.diasorin.com">www.diasorin.com</a>	Table 2
5. ELISA Platelia TOXO IgG	Whole tachyzoites	Not stated	Bio-Rad, Marnes-la-Coquette, France <a href="http://www.bio-rad.com">www.bio-rad.com</a>	Table 2
6. TOXO IgG Detect ELISA Kit	Inactivated	> 10 UI mL <sup>-1</sup>	BioKit, Barcelona, Spain <a href="http://www.biokit.com">www.biokit.com</a>	Table 2
7. ELISA TOXO IgG		Not stated	Bios Gmbh Labordianstik, München Bayern, Germany <a href="http://www.pasteur.ro">www.pasteur.ro</a>	Text
8. ELISA Eli-Tox-P	Soluble	Not stated	Pasteur Institute, Bucuresti, Romania <a href="http://www.pasteur.ro">www.pasteur.ro</a>	Table 5
9. ELISA TOXO IgG	Whole tachyzoites-formalin-fixed	1:50	SafePath Laboratories L.L.C., Carlsbad, California, USA <a href="http://www.safepath.com">www.safepath.com</a> (currently the company bought by Biorad)	Table 5
10. ELISA Chekit Toxotest	Inactivated	Not stated	Idexx-Bommeli Laboratories, Westbrook, Maine, USA <a href="http://www.idexx.com">www.idexx.com</a>	Tables 3 and 5
11. ELISA Pourquier Toxo	Not stated	Not stated	Institut Pourquier S.A., Montpellier, France	Table 3
12. ELISA Eli-Tox-O	Soluble	Not stated	Pasteur Institute, Bucuresti, Romania <a href="http://www.pasteur.ro">www.pasteur.ro</a>	Tables 5 and 6
13. ELISA IgG, IgM Dia Sorin kit	Solid	Not stated	DiaSorin S.P.A., Saluggia, Vercelli, Italy <a href="http://www.diasorin.com">www.diasorin.com</a>	Table 2
14. MEIA (Microparticle Enzyme Immunoassay, AxSYM Toxo IgG and IgM)	Soluble	≥ 3 UI mL <sup>-1</sup>	Abbott Laboratories, Illinois, USA <a href="http://www.abbott.com">www.abbott.com</a>	Text

<sup>a</sup> General distributor: SC Aspius SRL, no. 25/21, Aurel Vlaicu street, 310147, Arad, Romania, phone: +40 724574943, E-mail: [contact@aspius.ro](mailto:contact@aspius.ro)

were as follows: Alba 59 (70.2%) of 84, Bihor 104 (65.8%) of 158, Bistrița-Năsăud 46 (64.7%) of 71, Cluj 80 (44.9%) of 178, Covasna 34 (53.1%) of 64, Harghita 26 (36.6%) of 71, Maramureș 77 (64.7%) of 119, Mureș 68 (61.8%) of 110, Satu Mare 75 (63.0%) of 119, Sălaj 63 (67.7%) of 93, and Sibiu 55 (62.5%) of 88. The highest (70.2%) seropositivity was in people from Alba, and the lowest (36.6%) in Harghita county. Prevalence in males, 279 (60.3%) of 462, was similar to that in females, 408 (58.8%) of 693, and slightly lower in the urban population, 264 (55.1%) of 479, than the rural population, 363 (63.6%) of 570. Prevalences by age were: 46 (24.3%) of 189 <14 years, 26 (43.3%) of 60–15 to 19 years, 59 (49.7%) of 120–20 to 29 years, 68 (55.7%) of 122–30 to 39 years, and 191 (71.5%) of 267 at 40 years or older. In this population, prevalence in women of fertile age (defined in this paper as 16–35 years) was high (51.4%). It is of interest that 6 of 68 children 1–9 years old were seropositive. The authors tabulated results for each category in each of 11 counties. Of the total of 687 seropositives of 1155 persons, only 1 had IgM antibodies. To our knowledge this is the only population-based survey for *T. gondii* in Romania. Unfortunately, risk assessment data were limited. There were several other studies with <100 people (Antoniou *et al.* 2005; Teodorescu *et al.* 2006; Csep, 2010a).

There are other surveys of *T. gondii* infection in humans tested for various reasons. Surveys in women of childbearing age or in those who were tested mainly because of gynaecological problems are given in Table 2. In general, seroprevalence was higher in women with gynaecological problems than women in the general population. Data from other patients or the general population are given in Table 3. In general, seroprevalence was higher in persons from rural than urban areas, but data are limited.

#### Clinical toxoplasmosis

**Congenital.** Although *T. gondii* can sometimes cause abortion in women, there is no evidence that it causes habitual abortion (Dubey and Beattie, 1988). Unfortunately, in many eastern European countries and Asia it has been assumed that chronic *T. gondii* infection is a common cause of infertility and abortion. For this reason, many women in Romania with these problems were tested for *T. gondii* infection (Table 2). In addition to those listed in Table 2, there are other reports of *T. gondii* testing of women with pregnancy problems (Neagoe *et al.* 2007). However, the relationship between *T. gondii* infection and pregnancy problems cannot be established by serological testing alone.

Little is known of congenital toxoplasmosis in Romania. An estimate of the incidence of clinically manifest prenatal toxoplasmosis may be obtained in

two ways (Dubey and Beattie, 1988). First from reports of observed cases, and second from calculations based on the infection rates during pregnancy and follow-up of live born infants. Stroia and Ungureanu (2007) reported a 4-month-old child with hydrocephalus, cerebral calcification, and IgG and IgM seropositivity to *T. gondii* but details are scanty. The authors rightly recognized that these symptoms also occur in other diseases. Csep (2010c) diagnosed 9 cases of congenital toxoplasmosis, but again details are sketchy. Panaitescu *et al.* (1995b) reported a very high rate of seroconversion in 96 (19.7%) of 485 women in Bucharest. The seroconverted women were followed clinically until delivery but it is not clear from the results how many of these mothers delivered congenitally infected children.

Crucerescu and Lovin (2001) tested cord blood of 1226 newborns for *T. gondii* antibodies. Antibodies to *T. gondii* were detected in 546 (44.5%) children. Out of these, 9 children were considered at risk based on differential serology and followed for 1 year. One of these 9 children had persistent *T. gondii* antibodies after 1 year; whether this child became symptomatic is unknown. Thus, in this select population from eastern Romania, the congenital transmission rate was 1 for 1226 live-born children.

Antibodies to *T. gondii* were sought by several investigators in children suspected to have congenital toxoplasmosis and their mothers, but the results were not conclusive to establish definitive diagnosis (Elias *et al.* 1963b; Elias, 1966; Georgescu, 1976; Proca-Cioban *et al.* 1981; Junie and Coroiu, 1995; Panaitescu *et al.* 1995a; Junie *et al.* 2002; Costache *et al.* 2004, 2008a,b, 2010; Teodorescu *et al.* 2006; Barabás-Hajdu *et al.* 2007; Lazăr and Barbu, 2007; Neagoe *et al.* 2007; Oprea *et al.* 2007; Rugină *et al.* 2007; Stroia and Ungureanu, 2007; Mării *et al.* 2008; Csep, 2010a, b, c). Three studies from Cluj reported *T. gondii* antibodies in about one third of malformed children (116 [29.4%] of 394, Junie and Coroiu, 1995; 73 [28.9%] of 253, Junie *et al.* 2000, 2002; 9 [32.1%] of 28). Overall, from the evidence presented in these studies it is difficult to estimate the rate of congenital toxoplasmosis.

**Ocular.** There are several reports of serological and clinical examinations of patients suspected of ocular toxoplasmosis in Romania (Panaitescu *et al.* 1978; Proca-Cioban *et al.* 1981; Junie and Coroiu, 1995; Crucerescu, 1998; Crețu *et al.* 2000, 2007; Lazăr *et al.* 2002; Costache *et al.* 2004; Dogan and Farah, 2004; Radbea *et al.* 2006; Siloși *et al.* 2006; Jurja, 2007; Teodorescu *et al.* 2008). Most of these studies are retrospective. Proca-Cioban *et al.* (1981) determined IFAT antibodies in 1712 children (3–19 years old) with neurological manifestations and 338 children with ocular diseases; 144 (8.4%) of 1712 with neurological signs and 36 (10.6%) of 338 with ocular disease were seropositive. The authors did not



Table 2. Reports of *T. gondii* antibodies in pregnant and childbearing age women tested in hospitals or private clinics in Romania

Year	Population	Area on the map	No. tested	Test	No. positive (%)	Additional serological tests	Reference
Not given	Pregnant or post-partum	II-8	552	Dye test, skin test	200 (36.3)	None	Elias (1966)
	Women with gynaecological problems		142		107 (75.4)		
1976–1977	Pregnant women	VII-37	1050	IFAT, 1:40	425 (40.4)	None	Mihai <i>et al.</i> (1978)
Not given	Women with abnormal pregnancies	VIII-41	2663	IFAT, 1:40	948 (35.6)	None	Panaitescu <i>et al.</i> (1978)
	Women in the first quarter of pregnancy, 29 abortions		421		82 (19.4)		
	Post-partum women		1688		64 (3.7)		
2002	General population	II-8	580	MEIA IgG <sup>14</sup>	300 (51.7)	IgM	Boer <i>et al.</i> (2002)
1996–1999	Healthy women of child bearing age	VII-37	810	IFAT IgG,	335 (41.3)	IgM, ISAGA, IgA	Crucerescu and Lovin (2001)
	Women with abortions		378	titre not given	155 (41.0)		
2003	Pregnant women	I-6	165	ELISA IgG,	60 (36.3)	IgM	Costache <i>et al.</i> (2004)
	Women with gynaecological disorders of childbearing age		462	kit used not given ELISA IgG	185 (40.0)		
Not given	Pregnant women	IV-20	50	ELISA <sup>2</sup> IgM	12 (24.0)	IgM	Silosi <i>et al.</i> (2006)
2000–2005	Pregnant women	III-12	277	ELISA <sup>13</sup> IgG	102 (36.8)	IgM	Barabás-Hajdu <i>et al.</i> (2007)
	Others		1982		509 (25.7)		
	Mothers of newborn		53		28 (52.8)		
2001–2006	Abnormal pregnancy women	VIII-41	148	ELISA <sup>5</sup> IgG	80 (54.0)	IgM	Teodorescu <i>et al.</i> (2007)
	Normal pregnancy women		100	ELISA <sup>5</sup> IgG	12 (12.0)		
2006 <sup>a</sup>	Women of childbearing age	II-8	184	LAT <sup>2</sup>	106 (57.6)	IgM	Olariu <i>et al.</i> (2008)
2007–2008	Pregnant women	II-8	139	ELISA <sup>13</sup> IgG	22 (15.8)	IgM,	Mederle <i>et al.</i> (2008)
2005–2007	Pregnant women	I-6	510	ELISA <sup>4</sup> IgG	199 (39.0)	IgM, IgA, avidity	Costache <i>et al.</i> (2008)
2007	Retrospective study of mothers with live newborns	I-6	90	ELISA IgG,	37 (41.1)	IgM	Mării <i>et al.</i> (2008)
				kit not given			
2007–2009	Pregnant women and childbearing age, 30% abortion	I-3	107	ELISA IgG <sup>6</sup>	35 (32.7)	IgM, IgA	Csep (2010b, c)
2006–2009	Mothers of neonates with congenital abnormalities	I-6	280	ELISA <sup>4</sup>	148 (52.8)	IgM, IgA	Costache <i>et al.</i> (2010)
Not given	Pregnant women	II-8	660	ELISA IgG <sup>7</sup>	308 (46.6)	None	Navolan <i>et al.</i> (2012)

<sup>a</sup> Personal communication to J. P. Dubey, April 2013.

Table 3. Occurrence of *Toxoplasma gondii* antibodies in sera of humans from various sources in Romania and correlates of infection

No. of persons	Area/region	Test, titre	Sources/groups	Main findings/comments	Reference
326	VIII-41	Dye test, titre not given	Clinical status, age	Overall, 60 (18.4%) of 326 persons seropositive; 25 (16.3%) of 153 children, 16 (20.5%) of 78 mothers, 10 (24.4%) of 41 with ocular diseases. Viable <i>T. gondii</i> isolated from 3 congenitally infected children.	Radacovici and Atanasia (1959)
869	VIII-41, II-8	Skin test	Occupation, clinical status	149 (17.1%) of 869 people positive. Slaughterhouse workers 40 (18.1%) of 221, 82 (22.8%) of 359 persons with mental disorders, 13 (32.5%) of 40 mothers with gynaecological problems, 11 (55%) of 20 patients with ocular diseases, 3 (1.4%) of 210 students, and 0 of 19 healthy adults.	Lupaşcu <i>et al.</i> (1963)
969	II-8	Dye test 1:4, skin test	Occupation, clinical status	193 (31.8%) of 607 persons positive, 109 (76.8%) of 142 women with premature birth, 228 (41.2%) of 553 children with neuropsychiatric disorders, 15 (12.6%) of 118 normal children, Seropositivity higher in slaughterhouse workers and other occupational groups. These papers are confusing because the sources of samples and persons in each category tested are not stated. Elias and Porsche (1961) stated that out of 507 healthy people the dye test positivity rates were: 7.4% in 0–10 year olds, 9.0% in 11–20 year olds, 15.2% in 21–30 year olds, 20.6% in 31–40 year olds, 16.0% in 41–50 year olds, and 33.6% in 51–60 year olds but the number of persons in each age group was not given	(Elias and Porsche, 1961; Elias <i>et al.</i> 1963a, b; Elias, 1966; Elias and Pucă, 1967)
304	VIII-42	Dye test	Age	41 (13.4%) of 304 seropositive; 0 of 39 <4 years old, 8 (7.3%) of 109 5–9 years old, 20 (16.6%) of 120 10–15 years old, 10 (27.7%) of 36 >15 years old	Georgescu (1976)
280	VIII-41	Skin test	Clinical status, occupation	123 (44.1%) of 280 person suspected of congenital and acute toxoplasmosis positive, 42 (41.2) of 102 deaf-mute, 7 (33.3%) of 21 with ocular disease, 43 (84.6%) of 57 livestock workers. Data on control group tested not clear	Toma <i>et al.</i> (1967)
231	VII-37	IFAT	From 3 localities, general population	131 (56.7%) of 231 from 3 localities positive: 51 (100%) of 51, 27 (71%) of 38 and 53 (37%) of 142. Seropositivity followed for 5 years; remained stable in 92, 85 and 29% in the 3 localities	Mihai <i>et al.</i> (1976, 1978)
7184	VIII-41	IFAT, 1:40	Clinical status, occupation	948 (35.6%) of 2663 women with abnormal pregnancies, <b>64 (3.7%) of 1688 women from the general population</b> , 171 (16.8%) of 1012 livestock workers, 136 (27.5%) of 493 ocular patients, and 101 (11.1%) of 907 miscellaneous persons	Panaitescu <i>et al.</i> (1978)
500	VIII-41	ELISA, in-house	Ocular patients	<b>Retrospective study of 1/3rd cases of toxoplasmosis in one teaching hospital. Patients 0–76 years old. Urban 322 (64.4%), rural 178 (34.6%), males 256 (51.4%), females 243 (48.6%), 98 (19.6%) contact with cats, 221 (44.2%) eating undercooked meat.</b>	Creţu <i>et al.</i> (2007)
63	VIII-41	ELISA, in-house	Lymphadenitis patients.	Retrospective study of patients in one hospital 1999–2005. 45 (72%) cases in 10–35 years old, 44 (71%) urban, no gender difference	Codreanu and Radulescu (2007)
148	VIII-41	ELISA <sup>5</sup> IgG	Pregnant	Higher seroprevalence in mothers that ate undercooked meat and had greater contact with soil, but details not given.	Teodorescu <i>et al.</i> (2007)
184	II-8	LAT <sup>1</sup>	Consecutive women tested at one hospital	Retrospective study. 106 (57.6%) of 184 positive: 6 (33.3%) of 18 14–19 years old, 36 (53.7%) of 67 20–29 years old, 39 (60.9%) of 64 30–39 years old, and 25 (71.4%) of 35 40–45 years old. 50 (48.1%) of 104 women from urban and 56 (70.0%) of 80 rural area. <b>Higher prevalence in rural and older women.</b>	Olariu <i>et al.</i> (2008)

510	I-6	ELISA <sup>5</sup>	<b>Prospective study</b> of pregnant women	Seroprevalence increase with age (39.7% in 78 of 196 in 20–26 year, 42.9% in 121 of 282 in 27–33 year), acute toxoplasmosis leading to abortion decrease with trimester (66.6% in 12 of 18 in 1st, 27.7% in 5 of 18 in 2nd, 5.5% in 1 of 18 in 3rd trimester)	Costache <i>et al.</i> (2008b)
280 <sup>a</sup>	I-6	ELISA <sup>5</sup>	<b>Prospective study</b> of pregnant women	<b>Seroprevalence increase with age</b> (42.8% in 12 of 28 in 16–20 years old, 52.1% in 96 of 184 in 21–30 years old) parity (1 pregnancy – 46.9% IgG + in 60 of 128, 2 pr – 52.8% IgG + in 38 of 72, 3 pr – 63.9% IgG + in 23 of 36; 4 pr. – 64.3% IgG + in 18 of 28; >4 pr. – 75% IgG + in 6 of 8) and rural (20.6% (+) in 24 of 116 living in rural area vs. 5.7% (+) in 8 of 140 urban area) <sup>b</sup>	Costache <i>et al.</i> (2010)

<sup>a</sup> Personal communication J.P.D – March 2013.

<sup>b</sup> Selected from 15 896 births between 2006–2009 because of clinical suspicion of toxoplasmosis in newborn (personal communication to J. P. D March, 2013). In bold = noteworthy data.

provide any data but stated that seroprevalence in the normal population (presumably children) was 3.7% (Proca-Cioban *et al.* 1981). Dogan and Farah (2004) diagnosed ocular toxoplasmosis in 21 (21.9%) of 96 cases of uveitis in children in a hospital in Bucharest from 1993–2002. Similarly, Teodorescu *et al.* (2008) reported 90 (66.7%) cases of ocular toxoplasmosis among all 135 hospitalized cases of toxoplasmosis (acquired or congenital) in other hospitals in Bucharest from 2000–2005. Crețu *et al.* (2007) retrospectively examined records of 500 patients diagnosed with ocular toxoplasmosis during 1995–2005 in Colentina Teaching Hospital in Bucharest. Concurrent infections (250 toxocariasis, 129 other infections including tuberculosis and syphilis) were associated in two thirds of cases. The diagnosis was based on serology and clinical findings. Patients were 0–76 years old (median 44 months), 20 case-patients (4%) of them were considered to have postnatally acquired toxoplasmosis, based on onset of clinical symptoms and differential serologic (avidity, IgM) testing. Chorioretinitis, present in 410 cases (82%), was the main finding and was unilateral in 298 cases (59.6%), affected both eyes in 112 cases (22.4%), and cataracts were seen in 74 cases (14.8%) of 500 persons. They described onset and progression of lesions, and attempted to determine risk factors. The authors stated that these cases analysed were one-third of cases seen in this hospital. It is not known if more eye patients sought diagnosis at this facility. Overall, toxoplasmic ophthalmitis is considered common in the Romanian population (Lazăr *et al.* 2002) but there is no information on prevalence of ocular disease in the general population. Therefore, we are unable to compare these findings with those from other countries in Europe or America.

*Lymphadenitis.* Cervical lymphadenopathy is the most common sign of acquired toxoplasmosis. Demonstration of *T. gondii* DNA or live parasites in biopsy is one way to confirm diagnosis. Presumptive diagnosis may be made based on symptoms and serological tests for acute toxoplasmosis. There have been several reports of serological examination of patients with lymphadenopathy (Ștefănoiu *et al.* 1983; Crucerescu and Lovin, 2002; Costache *et al.* 2004; Codreanu and Rădulescu, 2007; Dumitru *et al.* 2007; Ghinea *et al.* 2007; Siloși *et al.* 2007). Crucerescu and Lovin (2001) reported that 117 (34.1%) of 343 lymphadenitis patients were seropositive of which 44 (12.8%) had acute acquired infection based on IgG avidity. Ștefănoiu *et al.* (1983) reported IFAT antibodies in 297 (28.6%) of 1038 patients with lymphadenopathy; histological examination of biopsy of lymph nodes in 19 cases revealed reactive adenopathy but *T. gondii* was not found.

*Human immunodeficiency virus (HIV).* The HIV epidemic in the 1980s brought recognition of cerebral

toxoplasmosis in adults but these cases were not reported until 2000 in Romania. Encephalitis is the predominant presentation of clinical toxoplasmosis in HIV-infected patients. Although computer tomography (CT) and serological examination are useful, definitive diagnosis can only be made postmortem or by biopsy examination. Other conditions including lymphomas can mimic toxoplasmosis, and the determination of type of immunoglobulin and the magnitude of *T. gondii* titre are not helpful in differential diagnosis. In most HIV-infected patients clinical toxoplasmosis is a reactivation of a chronic infection, and most of these patients have *T. gondii* antibodies.

There are several reports of toxoplasmosis in HIV-infected patients in Romania (Colţan *et al.* 2000; Codarcea *et al.* 2000; Cambrea *et al.* 2007). Crucerescu and Lovin (2001) found *T. gondii* antibodies in 35 (34.3%) of 102 HIV-infected patients; 11 (2 adults, 9 children) had encephalitis, and 2 patients died despite therapy. A series of cases were reported in 2007 (Cambrea *et al.* 2007; Codarcea *et al.* 2007; Erscoiu *et al.* 2007; Marcaş *et al.* 2007; Oprea *et al.* 2007). A striking observation is that most of these cases were in young persons. Out of 34 teenage patients with cerebral toxoplasmosis, 16 died despite therapy (Cambrea *et al.* 2007). To our knowledge, none of the cases mentioned above were confirmed by biopsy or postmortem examination.

#### *Toxoplasma gondii* isolation from human samples

Dragomir (1956) attempted isolation of *T. gondii* from 3 cases of toxoplasmosis (details of patients not given) by bioassay in mice. For this, the ventricular fluid was centrifuged; the sediment was suspended in 2 mL of saline and inoculated intraperitoneally (i.p.) into 2 white mice. The first mouse was killed 4 days post-inoculation (p.i.) and tachyzoites were found in the peritoneal fluid. The parasite was maintained by serial passage in mice, and the strain became virulent for mice after 4 passages. The photographs provided in this paper leave no doubt about the first isolation of *T. gondii* in Romania.

Rodacovici and Atanasiu (1959) attempted isolation of *T. gondii* from 57 congenitally infected children and 6 adults by bioassays in mice. They isolated viable *T. gondii* from 3 cases of fatal toxoplasmosis in children. They found *T. gondii* in tissues of another 11 congenitally infected children and 1 adult with acquired toxoplasmosis but were unable to isolate viable *T. gondii*.

Elias *et al.* (1963b) attempted to isolate *T. gondii* from tissues of 7 infants with malformations by bioassays in mice. Viable *T. gondii* was isolated from 1 infant but details are sketchy.

Recently, Costache *et al.* (2013) isolated viable *T. gondii* from the cerebrospinal fluid (CSF) of a 32-week gestational age girl born prematurely but naturally to a mother who had serological evidence of recently acquired *T. gondii* infection during pregnancy (the mother seroconverted between 2 and 6 months of gestation). The girl had gross evidence of hydrocephalus and microphthalmia of the left eye. Ophthalmoscopic examination revealed acute central chorioretinitis of the right eye, retinal detachment and anterior and posterior uveitis of the left eye. The CSF was collected from the girl 4 days after birth, and examined for *T. gondii* infection. *Toxoplasma gondii* DNA was demonstrated directly in the CSF, and viable parasite isolated by bioassay in outbred mice. For bioassay, the CSF was centrifuged, the sediment suspended in isotonic saline, and inoculated i.p. into 3 outbred white mice. The inoculated mice remained asymptomatic; *T. gondii* tissue cysts were demonstrated in the brains of mice killed 4 weeks p.i. This *T. gondii* strain was designated as ROU-H-001 and cryopreserved. Genotyping with 15 microsatellite markers revealed that it is a Type II strain (Costache *et al.* 2013). These findings are noteworthy because it is the first genotyping of a viable isolate of *T. gondii* from Romania from any host.

#### *Epidemiology of human toxoplasmosis*

To our knowledge there are no statistically well-controlled epidemiological studies in Romania. Epidemiological data were collected mostly retrospectively, without calculation of statistical significance; we have summarized them in Table 3. Most studies revealed an increase of seroprevalence with age and rural living.

#### TOXOPLASMOSIS IN ANIMALS

##### *Cats*

*Serological prevalence and risk factors.* *Toxoplasma gondii* antibodies were detected in 30–80% of cats in small surveys involving 20–62 cats (Table 4). Györke *et al.* (2011) made an extensive investigation using serum samples from 236 house cats from three regions (Center-III, Southwest-IV and Northwest-I). Several aspects of this study are noteworthy. The sample size was adequate to study risk factors, prevalence was determined using 6 serological tests (MAT, IFAT, 4 ELISAs) and results could be compared directly with a previously published study from the Netherlands (Opsteegh *et al.* 2012); the MAT and ELISA-RIVM were performed in the Netherlands by Opsteegh *et al.* (2012).

In the Györke *et al.* (2011) study, using a commercial ELISA<sup>2</sup>, 111 (47%) of 236 cats were seropositive. Of these 236 sera, 203 sera were



Table 4. Serological surveys of *Toxoplasma gondii* in domestic cats in Romania

Year of survey	Area, region on map	Source of sera	No. tested	Test, titre	No. positive (%)	Correlates of infection available	Reference
No data	II-8	Pets	13	Dye test, 4	4 (30.7)	No data	Elias (1966)
2002–2004	VIII-41	Pets, VC	20	ELISA <sup>1</sup>	11 (55.0)	No data	Antoniu <i>et al.</i> (2005)
2006–2007	VIII-41	Pets	94	ELISA <sup>1</sup>	42 (44.6)	No data	Petriceanu <i>et al.</i> (2007)
2007	I-6	Pets	50	ELISA <sup>1</sup>	31 (62.0)	a, b, c, d	Titilincu <i>et al.</i> (2008b)
2008	VIII-41	No data	42	LAT <sup>2</sup> , 32 IFAT	21 (42.0) 20 (47.6)	a, b, c, d	Antoniu <i>et al.</i> (2007, 2008)
2007–2010	I-6	Pets	236	ELISA <sup>1</sup>	23 (54.7)	a, b, c, d	Györke <i>et al.</i> (2011)
2008–2009	II-7	Pets, urban, rural	36	Several methods (see text) ELISA <sup>2</sup>	111 (47.0) 29 (80.5)	a, b, c, d	Hotea <i>et al.</i> (2009c); Dărăbuş <i>et al.</i> (2011b)
2008–2009	II-9	Pets, urban, rural	42	ELISA <sup>2</sup>	25 (59.5)	a, b, c, d	Hotea <i>et al.</i> (2009a)
2008–2010	II-10	Pets	62	ELISA <sup>2</sup>	48 (77.4)	a, b, c, d	Hotea <i>et al.</i> (2012)

VC = veterinary clinics.  
a = age, b = sex, c = diet, d = habitat.

comparatively tested by 6 tests. Seropositivity varied from 46.7% to 60.5%, depending on the test. It is of interest that results for 147 tests (87 positive, 60 negative) of the 203 sera were the same in all 6 tests. Overall, the IDVet ELISA<sup>2</sup> gave most concordant results. Based on the IDVet test age, breed, diet, outdoor access, location and the source of sera affected the *T. gondii* seropositivity. Seroprevalence increased with age and indoor/outdoor living: outdoors (85 [59%] of 144) *vs* indoors (19 [26.8%] of 71). Somewhat similar conclusions were obtained in other references listed in Table 4 although the number of cats were too small for a valid comparison. These results were expected because most cats acquire *T. gondii* infection post-natally, soon after weaning when cats begin to hunt for food (Dubey and Beattie, 1988). Most purebred cats are kept indoors and fed processed diets by their economically advantaged owners.

*Prevalence of T. gondii-like oocysts in cat feces.*  
*Toxoplasma gondii*-like oocysts were found in feces of 18 of 300 cats from Bucharest by Pop *et al.* (1986), 5 of 414 cats from Transylvania by Mircean *et al.* (2010) and 1 of 62 cats from Caraş-Severin by Hotea *et al.* (2012). Additionally, oocysts were not found in feces of 63 cats from Cluj and Dolj (Titilincu *et al.* 2008b) and 36 cats from Arad county (Hotea *et al.* 2009c; Dărăbuş *et al.* 2011b – both papers refer to the same data). These results are based on microscopical examination, and not definitive. *Toxoplasma gondii*-like oocysts in cat feces include *T. gondii*, *Hammondia* spp., *Neospora caninum* and *Besnoitia* spp., and these oocysts cannot be diagnosed without bioassays or DNA identification (Dubey, 2010).

*Clinical toxoplasmosis.* Diagnosis of clinical toxoplasmosis in cats is difficult without postmortem examination. At present there is no confirmed report of clinical toxoplasmosis in cats in Romania. Georgescu *et al.* (2009) reported clinical signs of encephalitis and ophthalmitis in a cat (age or type of cat not stated) in Bucharest. The diagnosis was based on finding positive IgM and IgG antibodies to *T. gondii* (titre or the test performed were not given), and positive response to clindamycin therapy. The diagnosis is at best presumptive because IgM antibodies can persist in asymptomatic cats for months (Dubey, 2010).

*Isolation of viable T. gondii.* *Toxoplasma gondii* was isolated from 12 (4%) of 300 tissues of cats from Bucharest by bioassay in mice. The data are only indicative because tissues from 3 cats were pooled and 100 pools were bioassayed (Pop *et al.* 1986).

Table 5. Surveys for *T. gondii* antibodies in sheep in Romania

Year	Area	Source of sera	No. tested	Test	No. positive (%)	Reference
No data	II-8	No data	635	Dye test	340 (53.5)	Elias (1966)
No data	III-16	Farms, sheep age 3-5-6 year	192	IFAT	88 (45.8)	Sharma (1980)
	V-25		249	IHAT	75 (39.0)	
	VIII-41		61	IFAT	77(30.9)	
				IHAT	69 (27.7)	
No data	VIII-41	No data	222	IFAT	20 (32.7)	
			106	IHAT	12 (19.7)	
				CFT	38 (17.0)	Medrea and Constantinescu (1991)
2005-2006	VII-37	Farms, rams and ewes, all ages	572	IHAT	9 (8.5)	
				ELISA <sup>3</sup>	394 (68.8)	Bondoc <i>et al.</i> (2007)
2008	VIII-41	Abattoir, sheep all ages	140	ELISA <sup>4</sup>	39 (27.8)	Militaru <i>et al.</i> (2008)
2006-2007	VIII-41	Not given	148	ELISA <sup>1</sup>	105 (70.9%)	Petriceanu <i>et al.</i> (2007)
2008	I-2	Abattoir, sheep age 2-8 year	51, 2-3 year	ELISA <sup>1</sup>	23 (45.0)	Iovu <i>et al.</i> (2008a, b, c)
	I-3		54, 7-8 year		25 (46.3)	
2008	II-7	5 farms, ewes age 3-4 year	250	ELISA <sup>1</sup>	106 (42.4)	Hotea <i>et al.</i> (2009b)
2009	II-8	Abattoir, lambs age 35-55 days	200	ELISA <sup>1</sup>	13 (6.5)	Hotea <i>et al.</i> (2011a)
2008-2009	II-8	5 farms, sheep age 2-5 year	600	ELISA <sup>1</sup>	218 (36.3)	Dărăbuş <i>et al.</i> (2011b)
2008-2010	II-8	15 farms, sheep age 3-5 year	750	ELISA <sup>1</sup>	493 (65.7)	Hotea <i>et al.</i> (2011b)
2011	VIII-41	Farms, ewes all ages	200	IFAT, titre not given	44 (22.0)	Chiţimia <i>et al.</i> (2011)
2008-2010	II-10	Farms, sheep age 3-5 year	450	ELISA <sup>1</sup>	276 (61.3)	Hotea <i>et al.</i> (2012)
2008-2010	I-6	Backyards, all ages	239	ELISA <sup>1</sup>	138 (57.7)	Balea <i>et al.</i> (2012)

Sheep

A significant ovine population in Romania has been exposed to *T. gondii* infection (Table 5). Seroprevalence varied with the region, age and the serological methods. Elias (1966) found 53% (340 of 635) seropositivity in sheep tested by the dye test, however, most (192) of the sera had only low titres of 4 and 16; significance of these low dye test titres is unknown. Also, sheep sera should be inactivated at 60 °C to inactivate the ovine complement (Dubey and Beattie, 1988). Sharma (1980) found more variability with the IHAT vs IFAT; he found excellent correlation between IFAT and the dye test in 100 sera. Hotea *et al.* (2011a) found that only 13 (6.5%) of 200, 35-55-day-old lambs slaughtered for Easter were seropositive; it is likely that some of these lambs had colostrally acquired antibodies. Several surveys listed in Table 5 used different ELISAs and there are no data on their specificity and sensitivity based on isolation of *T. gondii* from asymptomatic sheep. Ştirbu-Teofănescu *et al.* (2005) found good correlation between an in-house ELISA and IFAT, and Titilincu *et al.* (2008a, 2009) found good correlation between MAT and 2 commercial ELISAs and an in-house ELISA.

*Toxoplasma gondii* is an important cause of ovine abortion worldwide (Dubey, 2010), but there is no definitive information on this subject in Romania. Elias *et al.* (1963a) and Elias (1966) found higher

seropositivity in 80 (51.9%) of 154 ewes that aborted vs 61 (44.5%) of 137 healthy sheep from a flock that experienced a storm of abortions (Elias *et al.* 1963a, b); it is not clear to us if both papers relate to the same farms or different farms. They were unable to isolate viable *T. gondii* from aborted fetuses but details are sketchy. Pyrimethamine treatment of 20 ewes with high antibody titres prevented abortion but again details are sketchy. Medrea and Constantinescu (1991) also reported higher *T. gondii* seropositivity in sheep with neonatal losses but exact figures are not clearly stated.

Goats

Iovu *et al.* (2012) studied in-depth epidemiology of toxoplasmosis in dairy goats from Romania. They tested 735 goats from 4 areas of Romania. Goat sera were tested for *T. gondii* IgG antibodies by ELISA<sup>12</sup>. Seroprevalence varied from 20-84%, depending on the sampling; antibodies were found in 8 (20.0%) of 40 goats from Muntenia, 144 (39.2%) of 367 goats from Transylvania, 194 (69.8%) of 278 goats from Crişana and 42 (84.0%) of 50 goats from Maramureş (for regions see Fig. 1). As expected, seroprevalence was higher in backyard-raised goats, 58 (79.5%) of 73, than in goats raised on farms, 330 (49.8%) of 662; and in adults, 386 (55.8%) of 692, vs kids, 2 (4.7%) of 43. The goat-kids tested were 2 months old and might

still have colostrally acquired antibodies. This paper included results reported by Titilincu *et al.* (2008c) and Balea *et al.* (2012) (personal communication to J.P. Dubey, February 2013). Results indicated that most goats acquire infection postnatally by ingesting food or water contaminated with oocysts. This research is significant because the survey was made on dairy goats; *T. gondii* can be transmitted to humans via goat milk (Dubey, 2010).

### Pigs

Seroprevalence varied with the type of pigs surveyed; <3% of fattening pigs (<8 months) were seropositive compared with higher seropositivity in older pigs (Table 6). An extremely high rate of seropositivity, 49 (94%) of 52, was found in wild pigs (Hotea *et al.* 2010c). As expected, pigs housed indoors under intensive management were not exposed to *T. gondii* infection, compared with those housed outdoors, except in the survey reported by Iovu *et al.* (2008b) (Table 6). Iovu *et al.* (2008b) found good correlation between 2 commercial ELISAs<sup>2,9</sup>. Paștiu *et al.* (2013) using IFAT cut-off of 1:32 found antibodies in 24 (16%) of 150 wild boars, 0 of 660 fattening pigs, and 783 (30.5%) of 2564 backyard pigs. The prevalence in backyard pigs varied from 13.3 to 60%. It was remarkable that none of the 200 sows from one establishment were seropositive compared with 46 (26.9%) of 171 sows from another establishment, although both farms used intensive management. The data on pigs reported by Balea *et al.* (2012) were included in Paștiu *et al.* (2013) (personal communication to J. P. D.).

Little is known of clinical toxoplasmosis in pigs in Romania. Iovu *et al.* (2010) examined fetal tissues and fluids from 32 sow abortions and did not find *T. gondii* DNA in abortus.

### Miscellaneous animals

Little is known of *T. gondii* infection in cattle in Romania. Elias (1966) found dye test antibodies in 31 (18.9%) of 164 cattle. Medrea and Constantinescu (1991) reported seropositivity in 84 (12.6%) of 667 cattle by the complement fixation test.

Antibodies to *T. gondii* were found in 104 (51.4%) of 202 domestic dogs by Elias (1966), and in 14 (25.0%) of 56 of stray dogs from Cluj-Napoca by Cozma *et al.* (2007) using 1:100 serum dilution in the IFAT.

Elias (1966) found dye test antibodies in 834 (45.3%) of 1840 rabbits, 21 (26.2%) of 80 hamsters and 3 (10.3%) of 29 rats. Dărăbuș *et al.* (2011b) detected *T. gondii* antibodies by ELISA<sup>4</sup> in 19 (73.1%) of 26 animals in a zoo, including 1 of 1 *Felis catus*, 2 of 2 *Felis sylvestris*, 3 of 3 *Panthera leo*, 2 of 5 *Capra aegagrus*, 2 of 3 *Capreolus capreolus*, 1 of 1

*Lama guanicoe*, 2 of 2 *Rangifer tarandus*, 1 of 3 *Equus caballus*, 4 of 4 *Procyon lotor* and 1 of 1 *Ursus arctos*.

Păstârnac (2009) discussed a large outbreak of toxoplasmosis in minks – these results need confirmation using verifiable methods.

Gheoca *et al.* (2009) reported unusual findings that need verification. They found *T. gondii*-like oocysts in feces of 4 of 6 rodents and cysts in tissues of 3 rodents. They discuss possible spread of *T. gondii* by rodent feces. In our opinion the cysts illustrated appear to be pollen grains and there is no evidence that *T. gondii* is transmitted via rodents other than by carnivorousism.

### Isolation of viable *T. gondii* from food animals

Pop *et al.* (1989) bioassayed diaphragms of 740 pigs, 910 cattle and 1340 sheep from slaughterhouses. Five grams of muscle from each of 10 animals were pooled by species, digested in acidic pepsin, and inoculated intraperitoneally into 6 mice. Viable *T. gondii* was isolated from 7 (9.5%) of 74 swine pools, 9 (9.9%) of 91 beef pools, and 11 (8.2%) of 134 mutton pools. In 19 (70.4%) of 27 positive samples tachyzoites were found in the peritoneal exudates, and tissue cysts were found in 24 groups of positive mice. One isolate from beef and 1 isolate from pork were virulent for mice. The isolation of *T. gondii* from approximately 10% of beef samples is unusual. Whether beef samples were contaminated with pork or lamb will never be known. It is also unfortunate that the samples were pooled, and there is no archived material for verification. *Toxoplasma gondii* has been rarely isolated from beef in other attempts worldwide and the role of beef in the epidemiology of toxoplasmosis needs investigation (Dubey and Beattie, 1988; Dubey, 2010).

Sharma (1980) isolated viable *T. gondii* tissues from 2 (15.4%) of 13 serologically positive sheep and 1 sheep not serologically examined. All 3 isolates were non-pathogenic for mice; these isolates were not cryopreserved. Recently, Turcitu *et al.* (2012) detected *T. gondii* genomic DNA in 17 (18.4%) of brain homogenate samples from 92 sheep as part of an investigation on scrapie.

### PERSPECTIVE

During the preparation of this review it became clear that most of the research on toxoplasmosis conducted in Romania was probably not read/known to scientists in other countries. As stated earlier our initial search of the PUBMED database indicated references to only 25 papers on toxoplasmosis in humans and animals from Romania. Here, we have listed all >150 papers that we could find and summarized the current status of research on toxoplasmosis. There is a great need to establish a central facility for

Table 6. Surveys of *Toxoplasma gondii* antibodies in pigs in Romania

Year	Area, region map	Source of sera	Type	No. tested	Test, titre	No. positive (%)	Reference
No data	II-8	Not given	Not given	395	Dye test	99 (25.0)	Elias (1966)
2005	VII-37	Backyard	All ages	190	ELISA <sup>3</sup>	63 (33.2)	Bondoc <i>et al.</i> (2007)
2006				190		167 (87.9)	
2006–2007	VIII-41	Intensive	Sows	74	ELISA <sup>1</sup>	72 (97.2)	Petriceanu <i>et al.</i> (2007)
No data	I-6	Intensive	Sows	86	ELISA <sup>3</sup>	34 (39.5)	Iovu <i>et al.</i> (2008b)
				94	ELISA <sup>4</sup>	31 (33.0)	
2008	VIII-41	Abattoir	All ages	265	ELISA <sup>1</sup>	165 (62.2)	Știrbu-Teofănescu <i>et al.</i> (2008)
2007–2009	I-1	Intensive farms	Fattening pigs	47	ELISA <sup>4</sup>	0	Iovu <i>et al.</i> (2009)
	II-7		Fattening pigs	47		1 (2.1)	
	III-14		Fattening pigs	94		0	
	I-2		Fattening pigs	94		0	
	I-1		Sows	200		1 (0.5)	
	I-6		Sows	85		42 (49.4)	
2008–2009	II-8	Extensive farms	4 months–2 years	1600	ELISA <sup>4</sup>	351 (21.9)	Hotea <i>et al.</i> (2010b)
2008–2009	II-8	Intensive farms	All ages	1700	ELISA <sup>4</sup>	15 (0.8)	Hotea <i>et al.</i> (2010a)
			Boars	100		3 (3.0)	
			Sows	200		12 (6.0)	
		Semi-intensive farms	All ages	400		6 (1.5)	
2008–2009	II-8	Hunted	Wild boars	52	ELISA <sup>4</sup>	49 (94.2)	Hotea <i>et al.</i> (2010c)
2009–2010	II-7	Extensive farms	All ages	700	ELISA <sup>4</sup>	113 (16.2)	Hotea <i>et al.</i> (2011c)
2008–2010		Back yard			IFAT (in house), 32		Paștiu <i>et al.</i> (2013)
	III-15			348		47 (13.5)	
	III-13			384		53 (13.8)	
	III-12			457		138 (30.2)	
	II-6			434		204 (47.0)	
	I-4			141		42 (29.8)	
	I-1			276		15 (5.4)	
	I-2			432		262 (60.6)	
	II-6		Fattening	660		0	
	II-6	Intensive	Sows	171		46 (26.9)	
	I-1			200		0	
	Central region	Hunting	Wild boars	150		24 (16.0)	



toxoplasmosis research and to conduct a national surveillance study using a statistically valid survey for prevalence. There are adequately trained scientists in Romania to conduct this research but there is a need for financial support from European funding agency/agencies. The present research was conducted mostly using commercial kits, which are quite expensive for survey research. Little is known regarding the mortality and morbidity of toxoplasmosis in humans and animals in Romania. Until now, nothing was known of the genetic diversity of *T. gondii* in Romania, although some progress has been made recently in this direction and the first viable isolate of *T. gondii* from a congenitally infected child has been genotyped (Type II, as in children in France) and deposited in an international reference centre in France (Costache *et al.* 2013). Toxoplasmosis is an important cause of abortion in sheep and goats in many countries but nothing is known of this in Romania. Romania is a major country exporting mutton and other sheep products to Europe and Arab countries. Until the 1990s Romania was a socialist country with little contact with non-Soviet-block countries. International research collaborations are needed for total assimilation of Romania in the western world. There is little information concerning the presence of viable *T. gondii* in food animals in Romania. For this, bioassays for viable *T. gondii* are needed because determination of parasite DNA and antibodies only indicate exposure and not the live parasite presence.

#### SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit <http://dx.doi.org/S0031182013001509>.

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#### REFERENCES

- \*Antoniou, S., Moldoveanu, D. and Ionescu, V. (2005). Anthrozoological aspects in toxoplasmosis. *Revista Romana de Medicina Veterinara* **15**, 135–140.
- \*Antoniou, S., Știrbu-Teofănescu, B. and Militaru, D. (2007). The paraclinical diagnosis of toxoplasmosis in cats. *Revista Romana de Parazitologie* **17**, 15–18.
- \*Antoniou, S., Teofănescu, B. Ș., Militaru, D. and Căpitanu, G. (2008). Feline toxoplasmosis diagnosis using indirect immunofluorescence reaction and ELISA. *Revista Romana de Medicina Veterinara* **18**, 250–254.
- Balea, A., Pastiu, A. I., Györke, A., Mircean, V. and Cozma, V. (2012). The dynamics of anti-*Toxoplasma gondii* antibodies in small ruminants and pigs from Cluj County, Romania. *Scientia Parasitologica* **13**, 163–168.
- \*Barabás-Hajdu, E., Miklós, A., Miklós, T. and Simó, A. (2007). Serodiagnostics of toxoplasmosis in Mures County Hospital's Central Laboratory in 2000–2005. *Revista Romana de Parazitologie* **17**, 33–36.
- \*Boer, C. L., Iacobiciu, I., Olariu, R. T., Marțincu, C., Gheorghiu, E. and Macovievici, G. (2002). Considerations on the immunodiagnostic value of the AxSYM Toxo IgM and IgG assay for the detection of specific anti-*Toxoplasma gondii* antibodies. *Revista Romana de Parazitologie* **12**, 53–55.
- \*Bondoc, I., Asiminei, S. and Antoci, R. (2007). Immunoenzymatic study regarding the incidence of toxoplasmosis at some species of animals. *Revista Romana de Parazitologie* **17**, 19–22.
- \*Cambrea, S. C., Ilie, M., Cambrea, M., Ionescu, V. and Rugină, S. (2007). Cerebral toxoplasmosis in seropositive teenagers. *Revista Romana de Parazitologie* **17**, 89–93.
- \*Chitjmia, L., Nălbaru, A. M., Banu, T., Apostu, C. and Constantinoiu, C. (2011). Seroprevalence of ovine toxoplasmosis by indirect immunofluorescence test. *Revista Romana de Medicina Veterinara* **21**, 136–141.
- \*Codarcea, M., Gorun, E., Rugină, S., Muja, E., Ilie, M. and Neagu, M. (2000). Toxoplasmic encephalitis in the settings of AIDS. *Revista Romana de Parazitologie* **10**, 51–53.
- \*Codarcea, M., Blebea, E., Basca, E., Dumea, E., Cernat, R. and Rugină, C. (2007). Cerebral toxoplasmosis in HIV/AIDS patients; two case presentations. *Revista Romana de Parazitologie* **17**, 95–97.
- Codreanu, R. R. and Rădulescu, S. (2007). Clinical and therapeutical aspects in ganglionic toxoplasmosis. *Revista Romana de Parazitologie* **17**, 59–66.
- \*Colțan, G., Marin, C. and Rebedea, I. (2000). Cerebral toxoplasmosis – difficulties of diagnosis and treatment (case presented). *Revista Romana de Parazitologie* **10**, 48–50.
- Coroiu, Z., Radu, R., Molnar, A. and Bele, J. (2009). Seroprevalence of anti *Toxoplasma gondii* antibodies in the healthy population from north-western and central Romania. *Scientia Parasitologica* **1**, 37–42.
- Costache, C., Junie, M. and Coroiu, Z. (2004). Toxoplasmosis in Cluj county. *Scientia Parasitologica* **5**, 50–53.
- Costache, C., Colosi, I. and Junie, M. (2008a). The importance of anti-*Toxoplasma* Ig A detection in newborns. *Congress: European Multicolloquium of Parasitology, France* **10**, 43–47.
- Costache, C. A., Tigan, S. I., Colosi, I. and Coroiu, Z. (2008b). Toxoplasmic infection in pregnant women from Cluj County and neighbouring area. *Applications in Medical Informatics* **23**, 31–36.
- Costache, C. A., Colosi, I. and Junie, M. (2010). Testing for toxoplasmic immunity in women of fertile age – are we prepared? *Scientia Parasitologica* **11**, 44–46.
- Costache, C. A., Colosi, H. A., Blaga, L., Györke, A., Pastiu, A. I., Colosi, I. A. and Ajzenberg, D. (2013). First isolation and genetic characterization of a *Toxoplasma gondii* strain from a symptomatic human case of congenital toxoplasmosis in Romania. *Parasite* **20**, 11.
- \*Cozma, V., Șuteu, O., Titilincu, A. and Osztian, R. M. (2007). Seroprevalence of *Toxoplasma gondii* antibodies in dogs from Cluj-Napoca. *Revista Romana de Parazitologie* **17**, 23–26.
- Crețu, C., Cilievici, S., Neagu, R., Voinea, L. and Corbu, C. (2007). Is ocular toxoplasmosis an important health problem in Romania? *Revista Romana de Parazitologie* **17**, 81–84.
- Crețu, C. M., Rădulescu, S., Ristea, L. F., Pop de Popa, D., Tacorian, D., Voinea, L., Mihăilescu, P. and Popă, L. (2000). Ocular toxoplasmosis: clinical, diagnosis and therapeutics aspects. *Revista Romana de Parazitologie* **10**, 50–51.
- \*Crucerescu, E. (1998). Epidemiological data on toxoplasmosis. The aspects of congenital toxoplasmosis. *Bacteriology, Virology, Parasitology and Epidemiology* **43**, 147–155.
- Crucerescu, E. and Lovin, D. (2001). Toxoplasmosis risk in eastern Romania. *Jurnal de Medicina Preventiva* **9**, 54–60.
- Crucerescu, E. and Lovin, D. (2002). Study on specific IgG avidity as a tool for recent primary *Toxoplasma gondii* infection diagnosis. *Jurnal de Medicina Preventiva* **10**, 56–62.
- Csep, A. (2010a). Epidemiological and clinico-biological correlations in the diagnosis of achieved toxoplasmosis. *Analele Universitatii din Oradea* **10**, 247–254.

- Csep, A. (2010b). Aspects of toxoplasmosis diagnosis in women at the procreation age and pregnant women. *Analele Universitatii din Oradea* **10**, 261–268.
- Csep, A. (2010c). Clinico-biological aspects in congenital toxoplasmosis. *Analele Universitatii din Oradea* **10**, 255–260.
- Dărăbuș, Gh., Aftenie, M., Olariu, R. T., Ilie, M. S., Balint, A. and Hotea, I. (2011a). Epidemiological remarks on *Toxoplasma gondii* infection in Timisoara Zoo. *Scientia Parasitologica* **12**, 33–37.
- Dărăbuș, Gh., Hotea, I., Oprescu, I., Morariu, S., Brudiu, I. and Olariu, R. T. (2011b). Toxoplasmosis seroprevalence in cats and sheep from Western Romania. *Revue de Médecine Veterinaire* **162**, 316–320.
- \*Dogan, D. and Farah, C. (2004). Clinical and serologic research in toxoplasmic uveitis in children. *Ophthalmologia* **48**, 55–63.
- \*Dragomir, C. (1956). Isolation on the animal (white mice) of a strain of *Toxoplasma* from a case of human toxoplasmosis. *Microbiology, Parasitology and Epidemiology* **4**, 18–26.
- Dubey, J. P. (2010). *Toxoplasmosis of Animals and Humans*, 2nd Edn. CRC Press, Boca Raton, FL, USA.
- Dubey, J. P. and Beattie, C. P. (1988). *Toxoplasmosis of Animals and Man*. CRC Press, Boca Raton, FL, USA.
- \*Dumitru, I., Rugină, S. and Rugină, C. (2007). Toxoplasmosis with hepatitis and lymphadenopathy – case presentation. *Revista Romana de Parazitologie* **17**, 67–69.
- Elias, M. I. (1966). Beitrage zur Epidemiologie der Toxoplasmosis. *Zeitschrift fur Tropenmedizin und Parazitologie* **17**, 87–99.
- \*Elias, M. I. and Budiu, T. (1973). *Human and Animal Toxoplasmosis*. Eatura Facla, Timisoara.
- \*Elias, M. I. and Porsche, T. (1961). Serological research on the incidence of toxoplasmosis in apparently healthy people. *Microbiology (Bucharest)* **6**, 155–159.
- \*Elias, M. I. and Pucă-Ciudin, M. (1967). Research on the effectiveness of diagnostic methods in toxoplasmosis. *Microbiologia, Parazitologia, Epidemiologia* **12**, 67–72.
- \*Elias, M. I., Gluhovschi, N., Pucă-Ciudin, M. and Costin, P. (1963a). Observations on a focus of ovine toxoplasmosis. *Microbiologia, Parazitologia, Epidemiologia* **8**, 133–137.
- \*Elias, M. I., Pucă-Ciudin, M., Costin, P., Porshe, T., Borbil, I. and Bogdan, F. (1963b). Clinical and epidemiological aspects of congenital toxoplasmosis. *Microbiologia, Parazitologia, Epidemiologia* **8**, 127–131.
- Erscoiu, S., Ungureanu, E., Alecu, D., Ionescu, C. and Mihăilă, L. (2007). Clinical and therapeutical aspects of cerebral toxoplasmosis in Romanian AIDS patients. *Revista Romana de Parazitologie* **17**, 108–109.
- Georgescu, G., Tudor, P., Tudor, N., Grosu, F. and Ionescu, A. (2009). A clinical case of toxoplasmic encephalitis in a cat. *Scientific Works C series Veterinary Medicine, Bucuresti* **55**, 222–225.
- \*Georgescu, M. (1976). Structural aspects at the individual level in the epidemiological process in human toxoplasmosis. *Bacteriology, Virology, Parasitology, Epidemiology and Pneumophysiology* **21**, 105–110.
- Gheoca, D., Hărănglăvean, A. and Gheoca, V. (2009). Aspects of *Toxoplasma*. *Toxoplasma*-like parasitism in small mammal species in Transylvania and their role in *Toxoplasma* dissemination. *Studia Universitatis Vasile Goldis Arad* **19**, 69–73.
- \*Ghinea, M., Niculescu, Z. and Nicoară, A. (2007). On inflammatory adenopathies diagnosis. *Revista Romana de Parazitologie* **17**, 55–58.
- Györke, A., Opsteegh, M., Mircean, V., Iovu, A. and Cozma, V. (2011). *Toxoplasma gondii* in Romanian household cats: evaluation of serological tests, epidemiology and risk factors. *Preventive Veterinary Medicine* **102**, 321–328.
- Hotea, I., Dărăbuș, Gh., Ilie, M. S., Imre, K., Ciocan, R., Balint, A., Indre, D. and Măndiță, D. (2009a). The prevalence of *Toxoplasma gondii* infection in cats from Hunedoara County. *Scientific Works C Series Veterinary Medicine Bucuresti* **55**, 312–319.
- \*Hotea, I., Dărăbuș, Gh., Mederle, N., Ilie, M. S., Imre, K., Oprescu, I., Morariu, S. and Mederle, N. (2009b). The identification of *Toxoplasma gondii* infection in sheep from Arad County. *Lucrări științifice Medicină Veterinară Timisoara* **42**, 16–21.
- Hotea, I., Dărăbuș, Gh., Mederle, N., Ilie, M. S., Imre, K., Balint, A. and Indre, D. (2009c). The prevalence of *Toxoplasma gondii* infection in cats in Arad County. *Lucrări științifice Medicină Veterinară, Iasi* **52**, 587–592.
- Hotea, I., Dărăbuș, Gh., Ilie, M. S., Imre, K., Balint, A., Indre, D., Imre, M., Sorescu, D., Ciocan, R., Costinar, L. and Pascu, C. (2010a). Seroprevalence of *Toxoplasma gondii* infection in pigs reared in intensive system from Timis County. *Lucrări științifice Medicină Veterinară Timisoara* **43**, 68–72.
- Hotea, I., Dărăbuș, Gh., Ilie, M. S., Imre, K., Balint, A., Indre, D., Imre, M., Sorescu, D. and Ciocan, R. (2010b). Preliminary study of toxoplasmic infection in domestic pigs from Timis County. *Scientific Works C Series Veterinary Medicine Bucuresti* **56**, 53–58.
- Hotea, I., Dărăbuș, Gh., Păcurar, C., Rugea, T., Muntean, P., Ilie, M. S., Imre, K., Imre, M., Sorescu, D., Balint, A. and Indre, D. (2010c). Preliminary study on the prevalence of *Toxoplasma gondii* infection in wild boars from Timis County. *Lucrări științifice Medicină Veterinară, Iasi* **53**, 70–73.
- Hotea, I., Ilie, M. S., Imre, M., Sorescu, D. and Dărăbuș, Gh. (2011a). Determining of toxoplasmosis seroprevalence, by ELISA, in lambs in Timis County. *Lucrări științifice Medicină Veterinară, Iasi* **54**, 161–164.
- Hotea, I., Oprescu, I., Ilie, M. S., Imre, K. and Dărăbuș, Gh. (2011b). Seroprevalence of *Toxoplasma gondii* infection, by ELISA, in rams in Timis County. *Lucrări științifice Medicină Veterinară, Iasi* **54**, 325–328.
- Hotea, I., Oprescu, I., Sorescu, D., Ciocan, R. and Dărăbuș, Gh. (2011c). Toxoplasmosis seroprevalence in pigs from Arad County. *Lucrări științifice Medicină Veterinară Timisoara* **44**, 58–62.
- Hotea, I., Ilie, M. S., Imre, M., Sorescu, D., Indre, D., Brudiu, I., Colibar, O. and Dărăbuș, Gh. (2012). *Toxoplasma gondii* seroprevalence in cats and sheep from Caras-Severin County, Romania. *Lucrări științifice Medicină Veterinară, Iasi* **55**, 104–109.
- Iovu, A., Titilincu, A., Mircean, V., Blaga, R., Bejan, A. and Cozma, V. (2008a). Serosurvey of *Toxoplasma gondii* in sheep for human consumption in two slaughter-houses. *Bulletin UASVM Veterinary Medicine* **65**, 40–43.
- \*Iovu, A., Titilincu, A., Mircean, V., Blaga, R. and Cozma, V. (2008b). Preliminary results regarding the seroprevalence of *Toxoplasma gondii* infection in pigs from intensive farming. *Scientia Parasitologica* **2**, 21–25.
- \*Iovu, A., Traversa, D., Milillo, M., Titilincu, A. and Cozma, V. (2008c). A quantitative PCR method to determine the *Toxoplasma gondii* infection in the tissues of abortion sheep. *Revista Romana de Parazitologie* **18**, 111.
- Iovu, A., Titilincu, A., Mircean, V., Blaga, R., Voinescu, V. and Cozma, V. (2009). Seroprevalence of *Toxoplasma gondii* infection in indoor pigs from Center and North-Western of Romania. *Bulletin UASVM Veterinary Medicine* **66**, 103–107.
- Iovu, A. I., Titilincu, A., Voinescu, V. and Cozma, V. (2010). Molecular investigation of sow abortions for the detection of *Toxoplasma gondii* and *Hammondia hammondi*. *Scientia Parasitologica* **11**, 101–104.
- Iovu, A., Györke, A., Mircean, V., Gavrea, R. and Cozma, V. (2012). Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in dairy goats from Romania. *Veterinary Parasitology* **186**, 470–474.
- \*Junie, M. and Coroiu, Z. (1995). Incidence and consequences of acute *Toxoplasma gondii* infection in pregnancy evolution. *Obstetrica si Ginecologia* **43**, 45–48.
- Junie, M., Coroiu, Z., Costache, C. and Stranț, M. (2000). Toxoplasmic congenital malformations. *Revista Romana de Parazitologie* **10**, 44–47.
- Junie, M., Coroiu, Z., Mihalache, M. and Costache, C. (2002). The medical human importances of the toxoplasmic infection acquired during pregnancies. *Scientia Parasitologica* **2**, 62–66.
- \*Jurja, S. (2007). Toxoplasmic necrotizing retinitis – clinical case. *Revista Romana de Parazitologie* **17**, 85–87.
- Lazăr, L. and Barbu, I. (2007). Fetal and placental abnormalities with neurological and ocular sequelae during congenital toxoplasmosis. *Revista Romana de Parazitologie* **17**, 106–107.
- Lazăr, L., Radu-Niculescu, M., Ștefănescu, A. and Agârbiceanu, I. (2002). Profile of ocular toxoplasmosis within retinoveal pathology: clinical and evolutive post chemotherapy outcome. *Revista Romana de Parazitologie* **12**, 51–52.
- \*Lupașcu, G., Bossie-Agavriloaei, A., Atanasiu, M., Dahnovici, V., Burnuz, M., Elias, M. I. and Pucă-Ciudin, M. (1963). Contribution to the study of human toxoplasmosis. Investigation of several different groups of the population with the toxoplasmin intradermoreaction. *Microbiology, Parasitology and Epidemiology* **9**, 119–126.
- \*Marcaș, C., Cambrea, C., Ilie, M. and Rugină, S. (2007). Cerebral toxoplasmosis evolution in HIV infected adolescent – case presentation. *Revista Romana de Parazitologie* **17**, 99–102.
- Mării, C., Iancu, M., Miu, N., Zaharie, G., Samașcă, G. and Idrizi, E. (2008). Primary and secondary care of congenital infection with *Toxoplasma gondii*. *Applications in Medical Informatics* **22**, 21–26.
- Mederle, N., Dărăbuș, Gh., Oprescu, I., Morariu, S., Ilie, M. S., Imre, K., Hotea, I. and Mederle, O. (2008). Correlation between histological, serological and epidemiological investigations in human toxoplasmosis. *Lucrări Științifice Medicina Veterinara Timisoara* **41**, 355–360.
- Medrea, V. and Constantinescu, V. (1991). Untersuchungen zur Diagnose der *Toxoplasma gondii*-Infektion beim Wiederkäuer. *Monatshefte für Veterinärmedizin* **46**, 467–468.
- Mello, U. (1910). Un cas de toxoplasmosis du chien observé à Turin (2). *Bulletin de la Société de Pathologie Exotique* **3**, 359–363.
- \*Mihai, M., Marinov, B., Munteanu, L. and Condruz, E. (1976). Evolution of immunity to *Toxoplasma gondii* in an apparently healthy

- human population. *Bacteriology, Virology, Parasitology, Epidemiology and Pneumophysiology* **21**, 155–158.
- \*Mihai, M., Marinov, R., Baci, G., Renner, L., Vinătoru, E., Blum, L., Chirculescu, O., Tîrziman, I., Pricop, M., Gilcă, M. and Gilcă, D. (1978). Prospective evaluation of the risk of congenital toxoplasmosis. II. Further data on the immune response during pregnancy. *Bacteriology, Virology, Parasitology and Epidemiology* **23**, 147–151.
- Militaru, D., Ştirbu-Teofănescu, B., Militaru, M. and Ciobotaru, E. (2008). Serological detection of anti-*Toxoplasma gondii* antibodies in abattoir slaughtered pigs and sheep. *Revista Romana de Parazitologie* **18**, 145.
- Mircean, V., Titilincu, A. and Cozma, V. (2010). Prevalence of endoparasites in household cat (*Felis catus*) populations from Transylvania (Romania) and association with risk factors. *Veterinary Parasitology* **171**, 163–166.
- Navolan, D.B., Ciohat, I.M., Tigla, A.E., Vasies, D. and Dumitrascu, V. (2012). Risk assessment for TORCH complex infection agents during pregnancy – preliminary study. *Timisoara Medical Journal* **62**, 15–19.
- Neagoe, I., Gherman, R., Rădulescu, A., Pop, M., Bucurenci, N., Dumitrache, A. and Steriu, D. (2007). Determination of a possible congenital toxoplasmosis by detection specific antigens (NTP-hydrolase) with monoclonal antibodies in pregnant women with seroconversion. *Revista Romana de Parazitologie* **17**, 43–47.
- Nicolle, C. and Manceaux, L. (1908). Sur une infection à corps de Leishman (ou organismes voisins) du gondi. *Comptes Rendus des Séances de l'Académie des Sciences* **147**, 763–766.
- Nicolle, C. and Manceaux, L. (1909). Sur un protozoaire nouveau du gondi. *Comptes Rendus des Séances de l'Académie des Sciences* **148**, 369–372.
- Olariu, T.R., Dărăbuş, Gh., Creţu, O., Jurovits, O., Giura, E., Erdelean, V., Marinu, I., Iacobiciu, I., Petrescu, C. and Koreck, A. (2008). Prevalence of *Toxoplasma gondii* antibodies among women of childbearing age in Timis County. *Lucrări Stiintifice Medicină Veterinară Timisoara* **41**, 367–371.
- Oprea, C., Ungureanu, E., Rădoi, R., Ene, L., Tardei, G., Tetrarov, S., Gigoianu, R. and Duiculescu, D. (2007). Atovaquone, an alternative therapeutic regimen in cerebral toxoplasmosis in HIV-1 infected children. *Revista Romana de Parazitologie* **17**, 114–115.
- Opsteegh, M., Haveman, R., Swart, A. N., Mensink-Beerepoot, M. E., Hofhuis, A., Langelaar, M. F. M. and van der Giessen, J. W. B. (2012). Seroprevalence and risk factors for *Toxoplasma gondii* infection in domestic cats in the Netherlands. *Preventive Veterinary Medicine* **104**, 317–326.
- Panaitecu, D., Steriu, D., Proca-Cioban, M., Ştefănoiu, V., Silard, R. and Potcoavă, R. (1978). Studies concerning the assessment of the incidence of human toxoplasmosis. *Archives Roumaine of Pathology and Experimental Microbiology* **37**, 399–405.
- Panaitecu, D., Căpraru, T. and Bugarin, V. (1995a). Study of the incidence of intestinal and systemic parasitoses in a group of children with handicaps. *Roumaine Archive of Microbiology and Immunology* **54**, 65–74.
- Panaitecu, D., Steriu, D., Ştefănoiu, V. and Pop, M. (1995b). Research on congenital toxoplasmosis. *Roumaine Archive of Microbiology and Immunology* **54**, 313–324.
- Păstărnac, N. (2009). Toxoplasmosis in mink (*Mustela vison*). *Revista Romana de Medicină Veterinară* **19**, 82–95.
- Paştiu, A. I., Györke, A., Blaga, R., Mircean, V., Rosenthal, B. M. and Cozma, V. (2013). In Romania, exposure to *Toxoplasma gondii* occurs twice as often in swine raised for familial consumption as in hunted wild boar, but occurs rarely if ever among fattening pigs raised in confinement. *Parasitology Research* **112**, 2403–2407.
- Petriceanu, G., Guţu, E., Rădulescu, R. A., Ragalie, A. and Tănăsuiică, R. (2007). The prevalence of serological diagnosed toxoplasmosis cases in animals from Romania between 2006 and 2007 years. *Institutul de Diagnostic si Sanatate Animala Bucuresti* **1**, 1–14.
- Pop, A., Cerbu, A., Pop, A. and Andreescu, N. (1986). The seasonal prevalence of *Toxoplasma gondii* infections in stray cats from urban areas, studied by parasitological methods. *Archives Roumaine of Pathology and Experimental Microbiology* **45**, 57–63.
- Pop, A., Opreşan, A., Pop, A., Cerbu, A., Stavarache, M. and Niţu, R. (1989). Toxoplasmosis prevalence parasitologically evaluated in meat animals. *Archives Roumaine of Pathology and Experimental Microbiology* **48**, 373–378.
- Proca-Cioban, M., Ştefănoiu, V., Potcoavă, R., Panaitecu, D. and Steriu, D. (1981). Incidence of anti-*Toxoplasma* antibodies in children with neurological manifestations and ocular diseases. *Archives Roumaine of Pathology and Experimental Microbiology* **40**, 91–99.
- Radacovici, E. and Atanasiu, M. (1959). Contributions to the study of human toxoplasmosis in our country. *Microbiology, Parasitology and Epidemiology* **4**, 329–335.
- Radbea, N., Mederle, O., Barbu, D. and Izvernariu, D. (2006). Human corioretinitis with *Toxoplasma gondii* – a case report. *Lucrari Stiintifice Medicina Veterinara Timisoara* **39**, 19–21.
- Rugină, S., Dumitru, I. and Gorun, E. (2007). Toxoplasmosis in pregnant women. *Revista Romana de Parazitologie* **17**, 71–75.
- Sabin, A. B. and Feldman, H. A. (1948). Dyes as microchemical indicators of a new immunity phenomenon affecting a protozoan parasite (*Toxoplasma*). *Science* **108**, 660–663.
- Sharma, S. P. (1980). Prevalence of *Toxoplasma* infection in sheep in Romania. *Veterinary Parasitology* **7**, 19–23.
- \*Siloşi, I., Rogoz, S., Siloşi, C., Rogoz, I., Avramescu, C. and Badea, P. (2006). Immune serological evaluation in toxoplasmosis. *Revista Romana de Parazitologie* **16**, 23–26.
- \*Siloşi, I., Ungureanu, A., Rogoz, S., Siloşi, C., Avramescu, C., Muşetescu, A., Drackoulagona, O. and Neamţu, C. (2007). Immunoserological investigations in toxoplasmosis. *Revista Romana de Parazitologie* **17**, 49–53.
- Splendore, A. (1908). Un nuovo protozoo parassita de conigli incontrato nelle lesioni anatomiche d'una malattia che ricorda in molti punti il Kala-azar dell' uomo. Nota preliminare. *Revista da Sociedade Scientifica de São Paulo* **3**, 109–112.
- \*Ştefănoiu, V., Panaitecu, D., Steriu, D. and Proca Ciobanu, M. (1983). Incidence of anti-*Toxoplasma* antibodies in patients with lymph node reactions. *Bacteriology, Virology, Parasitology and Epidemiology* **28**, 371–376.
- \*Ştirbu-Teofănescu, B., Amzuţa, A., Comârzan, A. M. and Militaru, D. (2005). Researches concerning the ovine toxoplasmosis serodiagnosis by ELISA. *Lucrari Stiintifice Medicina Veterinara Timisoara* **38**, 647–652.
- \*Ştirbu-Teofănescu, B., Militaru, D., Militaru, M. and Diaconu, I. (2008). Surveillance of porcine toxoplasmosis through IgG type antibodies detection by immunoenzymatic test. *Lucrari Stiintifice Medicina Veterinara, Iasi* **51**, 950–952.
- \*Stroia, V. and Ungureanu, A. (2007). Congenital toxoplasmosis. *Revista Romana de Parazitologie* **17**, 77–80.
- Teodorescu, C., Steriu, D., Teodorescu, I., Miclos, M. and Pop, M. (2006). Cases of cerebral hydatidosis, cysticercosis and toxoplasmosis in Romania. *Revista Romana de Parazitologie* **16**, 27–32.
- \*Teodorescu, C., Manea, V. and Teodorescu, I. (2007). *Toxoplasma gondii* diagnosis in pregnant women by PCR and ELISA technics. *Revista Romana de Parazitologie* **17**, 37–41.
- Teodorescu, C., Teodorescu, I., Raneti, C., Dumitrica, D. M. and Ştefan, C. (2008). The anti-*Toxoplasma gondii* antibodies incidence to the different ocular diseases patients cases of human ocular toxoplasmosis infections in Romania. *Oftalmologia* **52**, 88–94.
- \*Titilincu, A., Blaga, R., Halos, L., Mircean, V., Boireau, P. and Cozma, V. (2008a). Diagnosis of *Toxoplasma gondii* infection in sheep by ELISA and MAT. *Scientia Parasitologica* **2**, 15–20.
- \*Titilincu, A., Mircean, V., Blaga, R., Chiţimia, L., Cernea, M., Mirescu, F. and Cozma, V. (2008b). Research regarding the prevalence of *Toxoplasma gondii* infection in cats. *Revista Romana de Medicină Veterinara* **18**, 108–122.
- Titilincu, A., Mircean, V. and Cozma, V. (2008c). Seroprevalence of *Toxoplasma gondii* infection in goats from centre and north-west of Romania. *Lucrări ştiinţifice Medicină Veterinară, Iaşi* **51**, 544–547.
- Titilincu, A., Mircean, V., Iovu, A. and Cozma, V. (2009). Development of an indirect ELISA test using tachyzoite crude antigen for sero-diagnosis of sheep *Toxoplasma gondii* infection. *Bulletin UASVM Veterinary Medicine* **66**, 137–141.
- Toma, R., Guguianu, E., Busuioc, C., Stoicescu, A., Tisu, A. and Toma, V. (1967). Epidemiological aspects of toxoplasmosis in the city of Bucharest. I. Brief investigations on the incidence of toxoplasmosis among certain categories of suspected and exposed populations. *Microbiology, Parasitology and Epidemiology* **12**, 59–66.
- Turcitu, M. A., Pricop, L., Cioranu, R., Bărbuceanu, F., Diaconu, C., Banu, T., Chiţimia, L., Codreanu, M. D., Codreanu, I. and Antoniu, S. (2012). Incidence of *Toxoplasma gondii* in sheep in Romania. Preliminary results. *Revista Romana de Medicină Veterinara* **22**, 112–117.
- Wolf, A., Cowen, D. and Paige, B. (1939). Human toxoplasmosis: occurrence in infants as an encephalomyelitis verification by transmission to animals. *Science* **89**, 226–227.