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Review

Toxoplasmosis in sheep—The last 20 years

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ABSTRACT

Sheep are important to the economy of many countries because they are a source of food for humans Sheep are commonly infected with the protozoan parasite, *Toxoplasma gondii*. Infection with the parasite may cause early embryonic death and resorption, fetal death and mummification, abortion, stillbirth, and neonatal death. Severity of infection is associated with the stage of pregnancy at which the ewe becomes infected, the earlier in gestation, the more severe the consequences. Infected sheep meat is a source of *T. gondii* infection for humans and carnivorous animals. Most sheep acquire *T. gondii* infection after birth, and less than 4% of persistently infected sheep transmit the parasite vertically to the next generation. Recent studies by a group of researchers in England reported that repeat ovine transmission of *T. gondii* may be more common than previously believed but these findings are soley based on PCR data and require additional data using other techniques to verify the findings. Following infection with *T. gondii*, sheep develop humoral and cell-mediated immune responses against the parasite that provides effective protection against disease in subsequent pregnancies. A commercial vaccine is available, comprising a live, incomplete strain of *T. gondii*. The vaccine is administered to sheep prior to mating to protect against lamb losses due to toxoplasmosis. In the present paper, information on the prevalence, transmission, and control of ovine toxoplasmosis in the last 20 years is reviewed.

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1. Introduction

Toxoplasma gondii infection is widely prevalent in humans and animals worldwide (Dubey and Beattie, 1988). Humans become infected post-natally by ingesting tissue cysts from undercooked meat, consuming food or drink contaminated with oocysts, or by accidentally ingesting oocysts from the environment. The ingestion of undercooked infected lamb is considered an important source of infection for humans. Cook et al. (2000) identified eating uncooked lamb as a risk factor for *T. gondii* infection in pregnant women in Europe. In a retrospective study of 131 mothers in the USA who had given birth to children infected with *T. gondii*, 50% recalled having eaten uncooked meat (Boyer et al., 2005). Toxoplasmosis also causes heavy economic losses to sheep industry worldwide (Dubey and Beattie, 1988; Buxton et al., 2007). We previously reviewed worldwide information on ovine toxoplasmosis up to 1988 (Dubey and Towle, 1986; Dubey and Beattie, 1988). In the present paper, information on prevalence, transmission, epidemiology, immunology and control of ovine toxoplasmosis since 1988 is reviewed.

2. Natural infections

2.1. Serologic prevalence

Antibodies to *T. gondii* have been found in sheep worldwide (Table 1). The prevalence of antibodies in ewes was more than twice that in lambs, but results were dependent on the age of lambs sampled (Lundén et al., 1994; Gorman et al., 1999; Figliuolo et al., 2004; Rozette et al., 2005; Dumètre et al., 2006; Ragozo et al., 2008). Seroprevalence was shown to increase with age, reaching 95% in 6-year-old ewes in some flocks (Dubey and Kirkbride, 1989a), suggesting that most animals acquire infection post-natally. In general, most sheep acquired infection before 4 years of age, but one-third of old ewes were still seronegative in highly endemic flocks (Dubey and Kirkbride, 1989a). Prevalence was also higher in ewes on farms where epizootics of abortions were reported (Dubey and Kirkbride, 1989a). Seroprevalence in intensively managed sheep was lower than in semi-intensive management (Savio and Nieto, 1995; Abu Samra et al., 2007; Romanelli et al., 2007; Ragozo et al., 2008).

There is little information on the rate of seroconversion in sheep under different management systems, except in a study reported by Lundén et al. (1994), in which a flock of sheep in Sweden was followed serologically for 6 years. Most sheep became infected in autumn. An interesting finding was that 39% of 136 ewes became seropositive while only 6% of lambs seroconverted.

A few studies analyzed risk factors associated with *T. gondii* seropositivity in sheep (Skjerve et al., 1998; Klun et al., 2006; Abu Samra et al., 2007; Vesco et al., 2007; Ragozo et al., 2008). In a report from Serbia, ewes from State-owned flocks had a higher seropositivity than ewes from privately-owned flocks (Klun et al., 2006). Vesco et al. (2007) reported that in a survey of 1961 sheep from 62 farms in southern Italy, the presence of cats on the farm, using surface water for drinking water, and farm size were factors associated with seropositivity. A study from Spain found the presence of cats and abortion history as risk factors associated with *T. gondii* infection in sheep and goats (Mainar et al., 1996); however, this study did not separate data for goats and sheep. Caballero-Ortega et al. (2008b) in Mexico found that altitude and farm size affected infection rates; prevalence was higher at low altitudes and on large farms.

Fusco et al. (2007) conducted a thorough cross-sectional serological survey of sheep in Italy using 10 adult sheep from each of 117 farms. Additionally, they tested milk samples (all 10 samples were pooled for each farm) for anti-*Toxoplasma* antibodies and for *T. gondii* DNA. *T. gondii* DNA was found in only 4 milk samples and antibodies were found in 91 of 117 milk samples. Measuring specific antibody levels in milk samples is an economical method of conducting epidemiological surveys.

The data presented in Table 1 are not comparable among different locations because of the use of different serological tests and different cut-off values used to determine seropositivity. For example, most authors used 1:16 as a cut-off in immunofluorescent assays (IFA), but O'Brien and Geraghty (1990) and Masala et al. (2003) used 1:256, and 1:200, respectively as cut-off values. The year of sampling might also influence the results. For example, seroprevalence (IFAT, 1:16) had declined by more than 50% in samples collected a decade later than other samples (Shkap et al., 1992).

2.2. Comparison of different serological tests for detecting *T. gondii* antibodies in persistently infected sheep

To this author's knowledge, there is no validation of any serological test for the detection of *T. gondii* infection in sheep using isolation of the parasite as a gold standard to verify positivity. Available data on different serological tests in naturally-infected sheep are summarized in Table 2. Some data are available comparing parasite isolation results with seropositivity using the modified agglutination test (MAT). *T. gondii* was isolated from eight out of 30 (26.6%) ewes from France with MAT titers of 1:20 or higher; from one of eight (12.5%) ewes with titers of 1:80, and from seven of 11 ewes (64%) with titers of 1:160

Table 1
Seroprevalence of *T. gondii* in naturally exposed sheep.

Location	Reference	Test	No. exam	% Pos.	Cut-off titer	Notes*
Argentina	West et al. (1998)	IFA	29	41.4	64	a
Austria	Edelhofer and Aspöck (1996)	IFA	4079	66.4	40	a
Bangladesh	Samad et al. (1993b)	LAT	56	64	8	a
	Samad et al. (1993a)	LAT	17	17.6	64	b
Brazil						
Bahia	Gondim et al. (1999)	LAT	240	18.8	64	a
Paraná	Zonta et al. (1987–1988)	IHA	662	18.2	64	a
	Freire et al. (1995)	IFA	370	47.8	64	a,e,f
	Garcia et al. (1999)	IFA	228	51.8	64	a,c,e,f
	Romanelli et al. (2007)	IFA	305	51.5	64	a,c,e,f
	de Moura et al. (2007)	IFA	157	7	64	
Pernambuco	da Silva et al. (2003)	IFA	173	35.3	16	
Rio grande do Norte	Clementino et al. (2007)	ELISA	102	29.4	NS	a,e,f
	Soares et al. (2009)	IFA	409	20.7	64	a,d.f
Rio grande do Sul	da Silva and Langoni (2001)	IFA	522	7.7	16	b,e,h
		IFA	173	35.3	16	
	Silva and de la Rue (2006)	IFA	123	39	20	a,g
		IHA	123	21.1	16	a,e
Rondônia	Cavalcante et al. (2004)	IFA	141	46.8	64	a
São Paulo	Oliveira-Sequeira et al. (1993)	IFA	177	22.5	16	a,g
	da Silva et al. (2002)	IFA	100	23	16	g
	Meireles et al. (2003)	ELISA	200	31	NS	b
	Figliuolo et al. (2004)	IFA	597	34.7	64	a,e
Federal District	Ueno et al. (2009)	IFA	1028	38.2	64	a,c
Bulgaria	Ragozo et al. (2008)	MAT	495	24.2	25	b,e,f,h
	Prelezov et al. (2008)	IHA	380	48.2	80	a
Burkina Faso	Deconinck et al. (1996)	IHA	65	23	64	
Cameroon	Achu-Kwi and Ekue (1994)	LAT	211	31.8	64	a,e,f
Canada	Waltner-Toews et al. (1991)	ELISA	3872	57.6	NS	a,c,d
Chile	Gorman et al. (1999)	IFA	408	28	16	a,g
Côte d'Ivoire -Ivory (coast)	Deconinck et al. (1996)	IHA	62	68	64	
Czech Republic	Hejlíček and Literák (1994)	DT	886	55	4	
	Sedlák and Bártová (2007)	IFA	24	4	40	
	Bártová et al. (in press)	ELISA	547	59	NS	a
Djibouti	Deconinck et al. (1996)	IHA	183	13	64	
Egypt	El Ridi et al. (1990)	IHA	17	29	NS	
	El-Ghaysh and Mansour (1994)	MAT	102	49	49	a,d,g
	Shaapan et al. (2008)	MAT	300	43.7	25	b,d,g
Ethiopia	Bekele and Kasali (1989)	IHA	899	22.9	64	b
	Negash et al. (2004)	MAT	116	52.6	NS	a,g
	Deconinck et al. (1996)	IHA	94	26	NS	
France	Dumètre et al. (2006)	MAT	93 ewes	65.6	20	b,h
			164 lambs	22	20	b
Haute-Vienne	Rozette et al. (2005)	MAT	141 lambs	20.5	20	
			65 ewes	59.1	20	
Germany	Seineke (1996)	ELISA	1122	33	NS	
Ghana	van der Puije et al. (2000)	ELISA	732	33	NS	
India	Verma et al. (1988)	IHA	164	8	16	d
	Dubey et al. (1993)	MAT	88	22.7	25	a
	Mirdha et al. (1999)	IHA	40	25	18	a
Punjab	Sharma et al. (2008)	ELISA	186	3.8	NS	a
Iran						
Ahvaz	Hamidinejat et al. (2008)	ELISA	150	72.6	NS	a,g
Khoozestan	Hoghooghi-Rad and Afraa (1993)	LAT	138	13.8	8	b
	Hashemi-Fesharki (1996)	LAT	2209	24.3	8	a,g
		IHA	1102	24.6	64	a,g
Ardabil	Ghazaei (2005)	ELISA	200	30	NS	b
Mazandaran	Sharif et al. (2007)	IFA	588	35	16	b

Table 1 (*Continued*)

Location	Reference	Test	No. exam	% Pos.	Cut-off titer	Notes*
	Zia-Ali et al. (2007)	LAT	105	20.9	8	b,h
Ireland	O'Brien and Geraghty (1990)	IHA	837	55.6	256	a
Israel	Shkap et al. (1992)	IFA	259 (1979–1981)	72	16	
		IFA	372 (1985–1990)	30	16	
Italy	Sindoni et al. (1989)	MAT	169	13.61	16	a
	Balbo et al. (1991)	ELISA	1531	13	NS	a
	Masala et al. (2003)	IFA	7194	28.4	200	a,d
	Fusco et al. (2007)	IFA	1170	28.5	100	a,c
Sicily	Vesco et al. (2007)	ELISA	1876	49.9	NS	a,d,e
Central Alps	Gaffuri et al. (2006)	LAT	1056	78	32	
Jordan	Harps (1993)	LAT	559	20.6	16	a
Lithuania	Stimbirys et al. (2007)	ELISA	354	42.1	NS	a,e
Malaysia	Rajamanickam et al. (1990)	IHA	106	22.6	64	a
Mexico	García-Vázquez et al. (1990)	IFA	495	30	16	a
	Cruz-Vazquez et al. (1992)	IFA	702	37.9	16	a,c
	Caballero-Ortega et al. (2008b)	ELISA	351	29.1	NS	a,c,e,f
Morocco	Benkirane et al., 1989	LAT	304	29	64	a,d
	Sawadogo et al. (2005)	ELISA	261	27.6	NS	b
Netherlands	Cremers et al. (1991)	ELISA	40	65	NS	
Niger	Weitzman et al. (1991)	LAT	70	14	NS	a
	Deconinck et al. (1996)	IHA	77	19.5	64	
Norway	Skjerve et al. (1998)	ELISA	1940	16.2	NS	a,c
Pakistan	Zaki (1995)	LAT	40	3	64	
Peru	Reif et al. (1989)	IHA	211	27	64	a,d
Poland	Górecki et al. (2008)	IFA	41	53.6	8	a
	Michalski and Platt-Samoraj (2004)	MAT	20	55	40	
Saudi Arabia	Hossain et al. (1987)	IHA	210	11	64	b
	Amin and Morsy (1997)	IHA	100	39	64	b
	Sanad and Al-Ghabban (2007)	IFA	397	52.2	NS	b,g
	El-Metenawy (2000)	IHA	150	3.3	128	d
Senegal	Pangui et al. (1993)	IFA	190	46.3	16	a,e,g
	Deconinck et al. (1996)	IHA	52	11.5	64	
Serbia	Klun et al. (2006)	MAT	511	84.5	25	c,e,f
Slovakia	Kováčová (1993)	DT	1939	10	4	
South Africa	Abu Samra et al. (2007)	IFA	600	5.6	64	a,b,c,g
Spain	Moreno et al. (1991)	IFA	550	34.9	40	a,g
	Marca et al. (1996)	IFA	2306	42.7	40	a,g
	Mainar-Jaime and Barberán (2007)	MAT	203	40.4	40	a,d,g
Sweden	Lundén et al. (1992)	ELISA	704	19	NS	a,d
Turkey	Zeybek et al. (1995)	LAT	1050	14.6	64	a
	Oz et al. (1995)	IHA	42	9.5	NS	
	Mor and Arslan (2007)	ELISA	460	95.7	NS	
	Acici et al. (2008)	DT	95	49.5	16	a,e,f
Samsun	Babür et al. (1997)	DT	62	88.7	16	
	Altintaş (1996)	DT	603	31.1	16	a
Ka Yseri	Inci et al. (1999)	DT	154	33.8	16	a
Afyon	Çiçek et al. (2004)	DT	172	54.6	16	a
Nigde	Karatepe et al. (2004)	DT	110	50.9	16	a,d
Istanbul	Oncel and Vural (2006)	ELISA	181	31	NS	a,c,e,f
Kars	Mor and Arslan (2007)	ELISA	460	95.7	NS	a
	Dumanli et al. (1991)	IHA	295	27.7	32	a
	Oz et al. (1995)	IHA	259 (Aborted)	25.5	64	
			42 (Normal)	9.5	64	
Yozgat	Babur et al. (2001)	DT	152	45.4	16	
Yalova	Öncel et al. (2005)	DT	63	66.6	16	
UK-Scotland	Johnston (1988)	LAT	909	26.2	512	a
Uruguay	Savio and Nieto (1995)	IFA	526	32.5	8	a,d,g
	Freyre et al. (1999)	MAT	1613	28.7	64	a

Table 1 (*Continued*)

Location	Reference	Test	No. exam	% Pos.	Cut-off titer	Notes*
USA						
New York	Dubey and Welcome (1988)	MAT	592	73.8	50	a,d,e
North central	Dubey and Kirkbride (1989a)	MAT	1564	65.5	64	a,d
Northeastern	Malik et al. (1990)	ELISA	654	58.6	NS	b,g
Michigan	Underwood and Rook (1992)	NS	63	60	NS	a,d
Maryland, Virginia	Dubey et al. (2008)	MAT	Lambs 383	27.1	25	b,h
Zimbabwe	Pandey and van Knapen (1992)	ELISA	216	6	NS	b,e,g
	Hove et al. (2005)	IFA	23	47.8	50	a

DT = Dye test. ELISA = enzyme linked immuno-sorbent assay. IFA = Indirect fluorescent antibody. IHA = Indirect hemagglutination antibody. LAT = Latex agglutination test. MAT = Modified agglutination test. PCR = Polymerase chain reaction. WB = Western blot. NS = Not stated.

* a = farm, b = abattoir, c = risk factors, d = abortion, e = age, f = sex, g = serological test compared, h = isolation of *T. gondii*.

or higher (Dumètre et al., 2006). In our studies, *T. gondii* was isolated from 53 of 68 (77.9%) seropositive lambs; from six of 13 (46.1%) lambs with MAT titers of 1:50 and 1:100, and from 47 of 54 (87%) lambs with titers of 1:200 or higher (Dubey et al., 2008). The parasite was not isolated from one lamb with an MAT titer of 1:25 or from 44 lambs with a MAT titer of <1:25. These data taken collectively suggest that a MAT titer of 1:100 or above would be indicative of a persistently infected animal.

Marca et al. (1996) tested 2306 sera by MAT and IFA and found excellent correlation between tests at a serum dilution of 1:80; 42.7, 33.7, 29.3 and 21.2% were positive at dilutions of 40, 80, 160 and 320, respectively by IFA and 38.1, 35.2, 34.8 and 33.7% were positive at the same dilutions, by MAT.

Various ELISA methods using crude, fractionated, or recombinant antigens have been used to detect *T. gondii* antibodies in ovine sera. Tenter et al. (1992) compared ELISA based on crude and recombinant antigens and found varying degrees of specificity in naturally and experimentally-infected sheep. Caballero-Ortega et al. (2008a) reported a good correlation between their in-house crude *T. gondii* antigen ELISA and Western blots in 103 naturally-exposed sheep; 91% of the samples showed the same result in both tests. The highest sensitivity was with immunoblots, while specificity was higher with ELISA. They also reported on the value of an avidity ELISA to investigate the duration of infection in sheep.

Standardization of ELISA is a major problem because only a few kits are commercially available for use in animals. One ELISA kit (CHECKIT, Table 2) has been tested against MAT (Klun et al., 2007; Mainar-Jaime and Barberán, 2007). Klun et al. (2007) compared the sensitivity and specificity of the CHECKIT ELISA using two cut-off values (low-30% and high-

Table 2
Comparisons of different serological tests for detection of *T. gondii* antibodies in naturally-exposed sheep.

Country	No. of sera	% positive (cut-off value)					Reference
		MAT	IFA	IHA	LAT	ELISA	
Brazil	177	ND	17.5 (1:16)	22.5 (1:16)	ND	ND	Oliveira-Sequeira et al. (1993)
	100	27 (1:16)	23 (1:16)	ND	ND	ND	Silva et al. (2002)
	123	ND	39 (1:20)	20.3 (1:16)	ND	ND	Silva and de la Rue (2006)
Chile	408	ND	28 (1:16)	12 (1:16)	ND	ND	Gorman et al. (1999)
Egypt	102	49 (1:40)	50 (1:40)	ND	ND	46	El-Ghaysh and Manssur (1994)
	300	43.7 (1:25)	37(1:64)	ND	ND	41.7	Shaapen et al. (2008)
Ethiopia	116	52.6	ND	ND	ND	56	Negash et al. (2004)
Iran	150	69.3 (1:80)	ND	ND	ND	72.6	Hamidinejat et al. (2008)
Senegal	190	ND	46.3	ND	ND	55.2	Pangui et al. (1993)
Serbia	180	90.6 (1:25) 75.6 (1:50) 57.2(1:100)	ND	ND	ND	68.3[b] 35[c]	Klun et al. (2007)
Saudi Arabia	397	ND	ND	41.8	23.4	ND	Sanad and Al-Ghabban (2007)
South Africa	600	ND	5.6 (1:64)	ND	ND	4.3[a,b]	Abu Samra et al. (2007)
Spain	550	35.1(1:40)	34.9(1:40)	ND	ND	ND	Moreno et al. (1991)
	203	40.4 (1:40)	ND	ND	ND	36.9[a]	Mainar-Jaime and Barberán (2007)
	2306	35.2 (1:40)	33.7 (1:40)	ND	ND	ND	Marca et al. (1996)
Sweden	58	36.2 (1:40)	36.2 (1:40)	ND	ND	ND	Ljungström et al. (1994)
Uruguay	526	ND	33.6 (1:16)	34.2 (1:16)	ND	ND	Savio and Nieto (1995)
Zimbabwe	107	ND	ND	5.6 (1:64)	ND	2.8	Pandey and van Knapen (1992)

[a] CHECKIT-Toxo test, IDEXX test kit (Dr BommeliAG, Switzerland). ND = No data.
[b] (low,30% cut-off).
[c] (high,100% cut-off).

Table 3
Isolation of viable *T. gondii* from tissues of naturally-infected sheep.

Country	Type	No exam.	No. positive (%)	Tissues bioassayed	Isolate designation	Genetic data	Reference
Brazil	NS	136	5 (3.6) [b]	Brain	No	No	Spósito Filha et al. (1992)
	NS	40[a]	10 (25)	Brain, diaphragm	None	No	da Silva and Langoni (2001)
	Lambs, adults	82[a]	16 (19.5)	Brain, heart, diaphragm	TgShBr1-16	No	Ragozo et al. (2008)
France	Adults	30	8 (26.6)	Heart	Fr2-2005-*Ovi ari*1-6, Fr2-2006-*Ovi ari*1-2	Yes	Dumètre et al. (2006)
Iran	Adults	105	4 (3.8)	Brain	No	Yes	Zia-Ali et al. (2007)
USA	Lambs	68	53 (77.9)	Heart	TgShUs1-53	Yes	Dubey et al. (2008)

NS = not stated.
[a] Seropositive.
[b] Authors state that they found additional tissue cysts in H and E stained sections of mice inoculated with brains of 15 sheep.

100%) with the MAT at 1:25, 1:50, and 1:100 dilutions using sera from 180 sheep; these sera were selected from 511 sera tested in a general survey (see Table 1). Overall agreement was best (sensitivity of 58.3%, and specificity of 96.1%) with the MAT at a 1:100 dilution and the high ELISA cut-off. They concluded that that ELISA and MAT compared favorably under specific conditions. Mainar-Jaime and Barberán (2007) also found good correlation between MAT (at a 1:40 dilution) and ELISA optical density values of 30% or higher. Using another commercial ELISA kit (Institute of Pourquier, France) a good correlation was found between MAT (69.3%, 1:80) and ELISA (72.6%) with 150 sheep sera from Iran (Hamidinejat et al., 2008).

2.3. Isolation and genetic characterization of *T. gondii* from tissues of naturally-exposed sheep

2.3.1. Isolation of *T. gondii*

Viable *T. gondii* have been recovered from tissues of persistently infected sheep (Table 3). In addition, attempts were made to isolate *T. gondii* from sheep using a variety of techniques. Elmassry et al. (1990) isolated *T. gondii* from sheep using a bioassay in cats. In this assay, cats fed minced mutton from samples pooled from 40 sheep in Egypt and were found to shed *T. gondii* oocysts. Cremers et al. (1991) did not find bradyzoites by microscopic examination of digests of 5 g muscle samples from 40 sheep although 65% of these sheep were seropositive. Jungersen et al. (2002) isolated viable *T. gondii* from five slaughtered sheep and seven aborted lambs in Denmark but did not provide additional details. Owen and Trees (1999) isolated *T. gondii* from the hearts of two healthy lambs in England; no details were given. Belbacha et al. (2004) found tissue cysts by direct microscopic examination in 30% of 50 brains taken from sheep in Morocco.

2.3.2. Genetic characterization

Little genetic typing has been performed on *T. gondii* isolates from sheep. Owen and Trees (1999) found that DNA amplified directly from the placentas of 13 aborted sheep from 10 widely separated farms in the United Kingdom and two isolates from the hearts of lambs from an undefined location were all Type II, based on the SAG2 locus. Jungersen et al. (2002) reported that 11 isolates of *T. gondii* from Denmark (six from aborted lambs, five from healthy sheep) were also Type II. The results from the United Kingdom and Denmark are of interest because there was no difference in genotype based on health (abortion) status of the animals. Dumètre et al. (2006) found that all eight *T. gondii* isolates from adult sheep, persistently infected with *T. gondii*, from France were clonal Type II, using the SAG2 locus and five satellite markers (TUB2, TgM-A,W35, B17, and B18). Using the same markers as Dumètre et al. (2006), Zia-Ali et al. (2007) found that of four isolates of *T. gondii* from adult sheep in Iran, two isolates were Type II and two were Type III.

Dubey et al. (2008) found 15 genotypes among 57 *T. gondii* isolates using 10 PCR-RFLP markers, suggesting high genetic diversity of the parasite in lambs from Maryland, Virginia and West Virginia. Phylogenetic analysis indicated that the clonal Type II lineage and its closely related genotypes accounted for 68% (39 of 57) of the isolates. The Type III lineage accounted for 14% (8 of 57) of the strains and was the second most prevalent genotype. The identification of unique alleles in several loci including SAG1 in 2 isolates, c22-8 in 1 isolate, and PK1 in 1 isolate, indicates that the genetic makeup of the non-clonal genotypes are quite diverse. Most of the non-clonal genotypes have a combination of alleles of types I, II and III from different loci. It is not clear if those are simply recombinants from genetic crosses of clonal Type I, II and III strains, or are diverged lineages. In summary, published data indicated that Type II was the predominant lineage of the strains isolated from sheep. Interestingly, no Type I isolate of *T. gondii* has been found in sheep to date.

2.4. Abortion and lamb losses due to toxoplasmosis

T. gondii has been recognized as one of the main causes of infective ovine abortion in New Zealand, Australia, UK, Norway, and the USA (Dubey and Beattie, 1988). Actual losses in lambs due to toxoplasmosis are difficult to estimate because (1) the disease is usually sporadic, (2) only a small number of aborted lambs are submitted for diagnosis, (3) those submitted may be inadequately examined, (4) unsuitable material may be sent for diagnosis, (5) the serologic test may not be specific, and (6) toxoplasmosis does not produce clinical disease in the ewe, so this disease does not alarm the farmer as much as other bacterial and viral infections.

Table 4 summarizes reports of abortion in sheep as a result of *T. gondii* infection in different countries in the last

Table 4
Reports of *T. gondii* induced abortion in sheep.

Country	No. of fetuses exam.	% positive	Diagnostic methods[a]				Reference
			Other causes investigated	IHC	PCR	Fetal serology	
Germany	47	10.6	Yes	Yes	Yes	No	Steuber et al. (1995)
Italy	582	11.1	No	No	Yes	No	Masala et al. (2003)
	366	18.1	Yes	No	Yes	No	Masala et al. (2007)
Spain	53	16.9	Yes	No	Yes	Yes	Hurtado et al. (2001)
	173	23.1	No	Yes	Yes	Yes	Pereira-Bueno et al. (2004)
USA	556	13.7	Yes	Yes	No	Yes	Dubey et al. (1990)
	1201	17.5	Yes	Yes	No	Yes	Dubey and Kirkbride (1990)

PCR = polymerase chain reaction.
[a] IHC = immunohistochemistry.

20 years. *T. gondii* or *T. gondii* DNA was detected in up to 23% of aborted fetuses. Pereira-Bueno et al. (2004) made an extensive investigation on aborted fetuses in Spain. They detected *T. gondii* DNA in 23.1% of 173 fetuses submitted to one diagnostic facility. Of these, 106 fetuses were examined by 3 techniques: histology, fetal serology, and PCR. *T. gondii* was detected in 37 (34.9%) of 106 fetuses by one or more of the techniques: 4 by all three tests, 7 by PCR and serology, 2 by histology and serology, and 24 by only 1 test (6 by histology, 1 by PCR, and 17 by serology). Of the 2 serologic tests used, there was a 100% agreement between IFA (titer 1:32) and ELISA (50 IU). *T. gondii* aborted fetuses submitted for examination, were in the mid (60%) or last (40%) term of gestation (Pereira-Bueno et al., 2004).

Masala et al. (2003, 2007) reported *T. gondii*, detected by PCR, in the tissues of aborted sheep in Sardina, Italy. In the first survey (1999–2002), *T. gondii* DNA was found in 271 of 2471 (11.1%) fetuses; in 42 of 133 (31.5%) placentas, 76 of 496 (15.3%) muscle samples, 63 of 500 (12.6%) brains, 32 of 420 (7.6%) abomasi, 34 of 479 (7%) livers, and 24 of 394 (6%) spleens. In the second survey of sheep from 98 farms (2003–2005), they found *T. gondii* DNA in 53 of 292 (18.1%) fetuses; in 10 of 76 (13.1%) placentas, 34 of 274 (12.4%) muscle samples, 33 of 275 (12.0%) brains, 21 of 250 (8.4%) abomasi, 22 of 268 (8.2%) livers, and 18 of 230 (7.8%) spleens. In both surveys, DNA detection was the highest using muscle and brain samples of the fetuses. They also reported prevalence of DNA of other abortifacients, including *Neospora caninum*. However, histological examination was not done. Several researchers from the UK (Wheeler et al., 1990; Turner et al., 1991; Greig et al., 1993) reported on the value of detection of *T. gondii* DNA in aborted specimens, especially from autolyzed fetuses that are unsuitable for histologic examination.

In addition to the information presented in Table 4, there have been other reports of abortion in sheep linked to *T. gondii* infection. Thamsborg et al. (1994) reported toxoplasmic abortion in a small flock in Denmark; 9 of 15 ewes aborted and the diagnosis was confirmed in 5 fetuses using histological techniques or by bioassay in mice. Verma et al. (1989a) isolated *T. gondii* from 3 of 77 aborted fetuses in India. Szeredi and Bacsadi (2002) identified *T. gondii* immunohistochemically in 5 ovine fetuses from 5 flocks in Hungary; in 4 of these fetuses there was also concurrent *Chlamydophila abortus* infection. Weissmann (2003) reported presumptive *T. gondii* abortion in a Canadian flock based on history, and serology of the dam, and the finding of placentitis in a fetus. Underwood and Rook (1992) diagnosed toxoplasmosis in a flock of sheep in the USA that had a 27.2% lamb mortality; placental lesions characteristic of toxoplasmosis and *T. gondii* antibodies were found in all 11 aborted fetuses examined.

Johnston (1988) suggested that *T. gondii* may cause barrenness in sheep. Although fetal serology and DNA detection are useful aids, histopathology is essential to establish a cause–effect association, because *T. gondii* can be passively transmitted transplacentally and fetuses can die of other causes.

Detection of *T. gondii* antibodies in fetal fluids or serum is useful in the diagnosis of ovine abortion. Arthur and Blewett (1988) examined fetal fluids from 171 aborted fetuses from 55 flocks in Scotland and found that an IFA titer of 1:256 was diagnostic of exposure to *T. gondii*. Trees et al. (1988) examined 478 aborted ovine fetuses in England and found *T. gondii* antibodies in 40.4% by the MAT, in 40% by the dye test, in 37.4% by IFA, and in 29% by the latex agglutionation test (LAT), using a dilution of 1:16 in all tests.

It has been reported that 7–32% of abortion outbreaks in New Zealand sheep from 1973–1989 were caused by *T. gondii* with higher rates in the 1980s (Gumbrell, 1990; Orr, 1989). *T. gondii* has been suspected as a cause of ovine abortion in Morocco (Benkirane et al., 1990), Egypt (Hassanain et al., 1992), Turkey (Öncel et al., 2005), and Uruguay (Savio and Nieto, 1995; Freyre et al., 1997) based on dam serology.

2.5. Sub-clinical congenital transmission

The rate of sub-clinical congenital transmission has not been documented because only a few animals are tested once a diagnosis has been established. An example where sub-clinical congenital transmission of *T. gondii* was documented in sheep is provided in the paper of Dubey and Kirkbride (1989b). In this study, a flock of 80 Hampshire ewes were pastured in 1987 on a farm in South Dakota, USA. The 80 ewes produced 144 lambs of which 30 were stillborn. Toxoplasmosis was confirmed in 11 of 30 aborted lambs based on fetal serology and

immunohistochemical examination. *T. gondii* antibodies (MAT) were found in 68 of 114 (40.3%) surviving lambs 3-4 months after birth; 2 had titers of 1:64, 1 of 1:256, 12 of 1:1024, and 53 had titers of 1:4096. These high titers are indicative of an immune response initiated by an active infection and not due to passive immunity acquired through suckling of colostrum. Eight lambs, with MAT titers of 1:1024 or higher, were slaughtered when they were seven month old. *T. gondii* was isolated from the hearts of three lambs, the tongues of seven lambs, the leg muscles of eight lambs, and from the chops of seven lambs using a bioassay in mice with 100 g samples of each tissue. (Dubey and Kirkbride, 1989b). Serological examination of the ewes 2-3 weeks after lambing revealed that 56 of 80 ewes had MAT titers of 1:64 or more, indicating a high rate of infection in the ewes.

2.6. Prenatal, post-natal, and repeat transmission of T. gondii

Until recently, the prevailing view was that most sheep acquire *T. gondii* infection after birth. Although exact data are not available it is thought that <2% of sheep become congenitally-infected with *T. gondii*, and less than 4% of persistently infected sheep transmit it to the next generation (Dubey and Beattie, 1988; Buxton et al., 2006, 2007). Evidence for these conclusions is based on three older studies (Hartley, 1961; Watson and Beverley, 1971; Munday, 1972) and one recent study (Rodger et al., 2006). Studies reported by Hartley (1961) and Watson and Beverley (1971) studied experimentally infected ewes. Of 38 ewes infected with *T. gondii* during a previous pregnancy, all but one gave birth to uninfected lambs; *T. gondii* was isolated from only one placenta (Hartley, 1961). Of 26 ewes inoculated with *T. gondii* during a previous pregnancy, 24 had uninfected, live lambs; one aborted twins and one was barren. *T. gondii* was isolated from the brain of the aborted lamb (Watson and Beverley, 1971). Of 178 lambs born to 135 persistently naturally infected ewes, none had precolostral *T. gondii* antibodies; the placenta of one was infected with *T. gondii* (Munday, 1972). Recent studies by a group of researchers from Scotland (Buxton et al., 2006; Rodger et al., 2006) supported these findings that congenital transmission of *T. gondii* from ewes persistently infected with the parasites is infrequent. Their observations were based on a flock of 46 Scottish black ewes; 31 of these were seropositive and 15 were seronegative for *T. gondii* (Buxton et al., 2006; Rodger et al., 2006). Progeny of these ewes and placental tissue were tested using histopathology, PCR, pre-colostral lamb serology, along with clinical outcome to determine the presence of *T. gondii*. The seropositive ewes delivered 43 live and six dead lambs, but none of the lambs were infected with *T. gondii* based on histopathology, DNA analysis, or the presence of *T. gondii* antigen, and/or intact tachyzoites in immunohistochemical analysis of tissues. Antibodies were not found by IFA in fetal fluids from the dead lambs or in precolostral sera from all but two live twin lambs using western blot. Thus at the most, only one of the 31 (3.2%) naturally infected ewes had transmitted the infection transplacentally. The seronegative ewes produced 24 live uninfected lambs. In conclusion, all 4 studies discussed above reached the same conclusion that congenital transmission of *T. gondii* from ewes persistently infected with the parasite, may occur, but is very infrequent.

Recently, a series of papers was published from a group of researchers in England (Duncanson et al., 2001; Morley et al., 2005; Williams et al., 2005; Morley et al., 2008). These authors proposed that repeat transplacental transmission of *T. gondii* in sheep maybe more common than previously believed. However, all the evidence they presented was based on the detection of *T. gondii* DNA by PCR. In the first paper, placental and fetal tissues from a flock of 88 Suffolk cross sheep in Worcestershire were tested for *T. gondii* DNA. They detected DNA in the placenta of 37 of 70 sheep birthing live lambs, indicating a 42% congenital transmission. *T. gondii* DNA was detected in 17 of 18 placental and fetal tissues; from the brains of 15, and hearts of 14 (Duncanson et al., 2001). Similar findings were reported in a study of two pedigree Charollais sheep flocks in Cheshire and a Suffolk flock Worcestershire (presumably the same flock studied by Duncanson et al., 2001). In these three flocks 4.5–18.9% of lambs aborted with a 91% *T. gondii* infectivity rate based on PCR (Williams et al., 2005). More interestingly, 65% of live lambs had evidence of *T. gondii* DNA based on placental or cord samples. These three flocks were geographically separated. The higher abortion rate observed in the Charollais as compared with the Suffolk flock raises the important question of breed susceptibility (Williams et al., 2005), although abortion may be caused by several other agents eg: *chlamydophila abortus*, so it is important to do a differential diagnosis on abortion material submitted for examination. In the third paper, abortion and *T. gondii* infection were associated with different families of Charollais sheep within a flock (Morley et al., 2005). In this study, abortion data in 765 ewes from 27 families in one flock were analyzed. The abortion rate varied from 0-100%. In total, tissues of 155 aborted lambs were tested for *T. gondii* DNA. The frequency of *T. gondii* positive lambs also varied from 0 to 100% (Morley et al., 2005), however again the group did not look for the presence of other abortifacient agents in this study. In a fourth paper (Morley et al., 2008), further observations were conducted on the Charollais sheep flock reported on by Morley et al. (2005). In this study, 29 ewes were selected based on whether they had two or more lambings during the 2000–2003 seasons. Of these 29 ewes, nine (31%) produced *T. gondii*-positive progeny over two successive lambings. Of the 35 lambs from these nine ewes, 12 were born alive and 22 were aborted or mummified; more importantly 33 of the 35 lambs were PCR-positive (Morley et al., 2008). A major shortcoming of these studies is that their conclusions are based solely on *T. gondii* DNA detection; they have not demonstrated *T. gondii*-associated lesions using conventional histopathology which would have helped to establish a cause-effect relationship nor do they look for the presence of other abortifacient agents.

3. Experimental toxoplasmosis in sheep

In the last 20 years, several authors reported on experimental studies of ovine toxoplasmosis using differ-

Table 5
Experimental toxoplasmosis in sheep orally inoculated with oocysts.

T. gondii isolate	Dose	No. other details	Remarks	Reference
M1	2000, 12000	20 (10 + 10), 91–94 gestation	Fever, abortion, dead lambs. Antibody titers compared in IFA, IHA, and LAT	Buxton et al. (1988), Trees et al. (1989)
TS1, 2	10000	9 (3 per strain) pregnant ewes, 3 rams	Fever, diarrhea, abortion, dead lambs	Aganga et al. (1988)
Nigerian isolates			*T. gondii found* in semen and milk	
M1	2000, 200, 20	59 ewes	All 19 ewes fed 2000 oocysts became feverile, and became immune, only 3 of 10 ewes fed 200 oocysts seroconverted. Ewes seroconverted starting 14 days p.i. 20 oocysts were not infective	McColgan et al. (1988)
M1	2000	13 ewes at 91 day gestation	Fever, abortion, dead lambs. Ewes seroconverted starting 14 days p.i.	Buxton et al. (1989)
M3	**A**. 20, 70, 200, 700, 2000 **B**. 2000	14, mo-old lambs fed graded doses 20, 80–90 day gestation ewes	All lambs developed fever and antibodies to *T. gondii*. Fever, abortion, dead lambs. Ewes seroconverted starting 14 days p.i.	Buxton et al. (1991)
TS1	100	17, 105-days to late gestation	Fever, dead congenitally-infected lambs	Kirkbride et al. (1992)
M3	2000	34, 90-day gestation	Fever, abortion, dead lambs. Ewes seroconverted satating 14 days p.i.	Buxton et al. (1993a)
Not stated	2000	4 pregnant ewes	Fever, abortion	Samad and Clarkson (1995)
M1		21, 90-day gestation	Fever, abortion, mummification, dead lambs, live lambs. Seropositive at 21 days p.i. Sera tested using recombinant ELISA.	Coughlan et al. (1995), Owen et al. (1998a)
	1500 (in 3 doses of 500)	ewes	Direct PCR-found sensitive for diagnosis of congenital toxoplasmosis	
M1	2000	18, 80–90 day gestation	Fever, abortion, resorption, live congenitally-infected lambs. PCR found sensitive for diagnosis	Owen et al. (1998b)
M3	1000–100,000	12, 9-mo-old Grey face	Fever, parasitemia between 3–10 days p.i., seroconversion starting 12 days in ELISA and IFA. Tissue cysts not found by histology. DNA found in tissues.	Esteban-Redondo and Innes (1998)
M3	2000	10, adult sheep	Fever, parasitemia, viable *T. gondii* found in ovine tissues by bioassays in mice.	Esteban-Redondo et al. (1999)
M3	2000	30, 89-day gestation	Fever, dead congenitally-infected lambs	Buxton et al. (1993b)
M3	200	30, 89-day gestation	Fever, dead congenitally-infected lambs	Buxton et al. (1996)

ent strains of *T. gondii*. Results from these studies are summarized below.

3.1. Clinical disease in sheep orally inoculated with oocysts

Sheep can be easily infected by feeding them sporulated *T. gondii* oocysts. Most of the experimental studies performed were in the UK, using the M1 or M3 isolates of *T. gondii* originally isolated from sheep. The data from all of these studies are summarized in Table 5 in order to facilitate future studies with respect to experimental doses used to infect sheep. In this author's opinion, even a few live oocysts can infect sheep; pigs fed as few as one oocyst developed patent *T. gondii* infection (Dubey et al., 1996). Lambs (14 month-old) fed 20 oocysts each became infected (Buxton et al., 1991), and ewes given 100 oocysts produced congenitally infected lambs (Kirkbride et al., 1992). Before dosing sheep it is advisable to verify the infectivity of oocysts by bioassay in mice because some oocysts may not be viable. This may be the reason that in the study reported by McColgan et al. (1988), only 3 of 10 sheep fed 200 oocysts became infected.

Sheep given an oral dose of viable oocysts developed fever and occasionally showed some respiratory distress, but recovered by 14 days p.i. (Table 5). None of the sheep died as a result of administration of an oral dose of *T. gondii* oocysts. Some ewes aborted during the acute phase (second week) of infection, probably due to pyrexia and hormonal regulation (Trees et al., 1989; Owen et al., 1998b; Aiumlamai et al., 1990; Fredrickson et al., 1990). Ewes fed 100 or more oocysts at mid gestation aborted or produced dead lambs, usually 4 weeks p.i. In one study even ewes infected in the last trimester produced dead lambs (Kirkbride et al., 1992).

3.2. Immunological responses

Sheep can develop very high levels of *T. gondii* antibodies during acute infection and high IgG antibodies can persist for months or years (Dubey and Beattie, 1988). Trees et al. (1989) followed the kinetics of IgG and IgM antibodies in 20 sheep fed oocysts using three tests (IFA, LAT, indirect heamagglutination assay (IHA) and drew the following conclusions. A titer of 1:16 in the LAT was

considered non-specific because this titer was found in pre-inoculation samples and titers increased 8-fold after feeding of oocysts to sheep. In general, antibodies were detected later by IHA as compared with IFA. *T. gondii* specific IgM antibodies peaked at 3 weeks p.i. and preceded an IgG response. Similar kinetics for a *T. gondii* specific IgG response, in sheep orally infected with oocysts, were found using an ELISA (McColgan et al., 1988; Coughlan et al., 1995; Esteban-Redondo and Innes, 1998; Esteban-Redondo et al., 1999) and also in sheep parenterally inoculated with tachyzoites (Payne et al., 1988). In sheep inoculated intravenously with the RH strain tachyzoites, IgM was detected by one month p.i., persisted for three month p.i. and re-infection did not increase the antibody titer (McColgan et al., 1988). Tenter et al. (1992) reported that two sheep fed 5000 or 50,000 oocysts developed antibodies detectable by ELISA by 14 day p.i. Sheep inoculated intraperitoneally with RH strain tachyzoites developed antibodies detectable by immunoprecitation by 24 days p.i. (Verma et al., 1989b).

As *T. gondii* is an obligate intracellular parasite, protective immunity in sheep, similar to the situation in other host species, involves cellular responses and most of the information in this regard is derived from studies by a group of researchers from Scotland using sheep infected with the S48 (vaccine) strain (Wastling et al., 1993, 1994, 1995; Buxton and Innes, 1995; Innes et al., 1995a). Using a technique involving chronic cannulation of the efferent duct of a lymph node draining the site of infection, this group was able to monitor the development of immune responses *in vivo* over the course of an acute infection with *T. gondii*. Immune cells from the infected animals were also used in functional studies to examine their anti-parasite activity. They concluded that T-cells (CD4+, CD8+) and IFN-γ were critical in recovery from a primary infection with *T. gondii* and were an important component of the protective immune response (Innes et al., 1995b).

3.3. Detection of T. gondii in tissues of sheep fed oocysts

Earlier studies in this author's laboratory using an oral dose of 10,000 oocysts demonstrated that *T. gondii* can persist for more than five month in tissues of asymptomatic sheep (see Dubey and Beattie, 1988). Esteban-Redondo and Innes (1998), and Esteban-Redondo et al. (1999) compared several methods to detect *T. gondii* in ovine tissues. Sheep (4 for each dose) were fed 1000, 10,000, or 100,000 oocysts and killed 6 weeks p.i. (Esteban-Redondo and Innes, 1998). Samples of all the major organs were collected to allow analysis for the presence of *T. gondii* using a range of different techniques. Samples of all tissues were fixed in formalin and studied histologically, in addition approximately 2 g samples of brain, heart, skeletal muscle, and peripheral blood monocytes from 1 to 14 days p.i. were tested for the presence of *T. gondii* DNA at 6 weeks p.i., *T. gondii* DNA was detected in 8 of 12 sheep. It was more readily detected in the samples from the group of sheep receiving the highest dose of oocysts and the parasite was more frequently detected in the brain, and heart than in muscle samples. *T. gondii* was not detected using histological techniques (Esteban-Redondo and Innes, 1998). Similar results were found in a second study by these authors (Esteban-Redondo et al., 1999). In this study, sheep were examined at six weeks and six months after feeding 1000 or 100,000 oocysts. *T. gondii* DNA was detected more frequently from tissues of sheep fed the higher dose, and more readily from the brain and heart than from muscle tissue. As a comparison, the authors performed a similar experiment in cattle and did not detect *T. gondii* DNA from any of the tissues examined from cattle fed a similar number of *T. gondii* oocysts as those used to dose sheep (Esteban-Redondo et al., 1999).

4. Reducing losses in sheep due to toxoplasmosis through prophylaxis

4.1. Vaccine

A live vaccine (Toxovax®) is commercially marketed in the UK, France and New Zealand for reducing losses to the sheep industry from congenital toxoplasmosis (Buxton and Innes, 1995). This vaccine was initially developed in New Zealand (O'Connell et al., 1988; Wilkins et al., 1988; Wilkins and O'Connell, 1992), and further efficacy studies were conducted by Buxton and his colleagues in Scotland. The vaccine consists of a modified strain (S48) of *T. gondii*, originally isolated from an aborted lamb in New Zealand. By repeated passage in mice for many years, the strain lost the capacity to form tissue cysts and oocysts. The commercial vaccine consists of live cell culture-grown tachyzoites that have a shelf life of 10 days. It is recommended to be given 3 weeks before mating. One subcutaneous injection of this 2 ml suspension induces protective immunity for at least 18 months (Buxton and Innes, 1995). After subcutaneous inoculation, S48 tachyzoites multiply locally, producing parasitemia and fever. Tachyzoites are controlled by the host immune response as soon as 10 days p.i. and are not demonstrable by bioassay at 6 month p.i. (Wilkins and O'Connell, 1992, Buxton et al., 1991, 1993b, 1994). Vaccinated sheep develop humoral and cellular immunity involving CD4, CD8 T cells, and IFN-*y* (Wastling et al., 1993, 1994, 1995; Innes et al., 1995b). The mechanism of this persistent immunity in the absence of demonstrable live *T. gondii* is most intriguing and needs further research.

The search for a non-infectious vaccine should continue because of the existing shortcomings of the live vaccine—a short shelf life and safety concerns for those handling it. Identifying new methods of antigen delivery to induce appropriate immune responses and characterization of the protective antigens should continue (Buxton et al., 1989; Lundén, 1995; Stanley et al., 2004).

4.2. Prophylactic treatment

Prophylactic treatment of ewes with monensin has been reported to reduce fetal mortality due to toxoplasmosis (Buxton et al., 1988). Ewes given daily doses of 16.8 or 27.9 mg of monensin had fewer abortions than unmedicated ewes. Both groups were challenged with 2000 or 12,000 *T. gondii* oocysts at 91–94 days of pregnancy; lamb mortality was 16.7% in ewes fed monensin versus 55.2% mortality in lambs from ewes

not fed monensin. Similar results were obtained by feeding sulfamezathine and pyrimethamine (Buxton et al., 1993b) or decoquinate (Samad and Clarkson, 1995; Buxton et al., 1996). However, feeding another ionophore, lasalocid, did not prevent fetal loss due to toxoplasmosis (Kirkbride et al., 1992).

5. Concluding remarks

Despite the advances in our understanding of ovine toxoplasmosis, several aspects of the disease in sheep require further research effort. These include understanding whether there may be breed differences or genetic differences relating to disease susceptibility in sheep In addition, the extent to which *T. gondii*-associated repeat abortion may occur in flocks, and the need for a non-viable vaccine to prevent infection and abortion.

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References

Abu Samra, N., McCrindle, C.M.E., Penzhorn, B.L., Cenci-Goga, B., 2007. Seroprevalence of toxolasmosis in sheep in South Africa. J. S. Afr. Vet. Assoc. 78, 116–120.

Achu-Kwi, M.D., Ekue, N.F., 1994. Prevalence of *Toxoplasma gondii* antibodies in Djallonke sheep flocks in the Vina division, Cameroon. Bull. Anim. Health Prod. Afr. 42, 89–92.

Acici, M., Babur, C., Kilic, S., Hokelek, M., Kurt, M., 2008. Prevalece of antibodies to *Toxoplasma gondii* infection in humans and domestic animals in Samsun province, Turkey. Trop. Anim. Health Prod. 40, 311–315.

Aganga, A.O., Umoh, J.U., Kyewalabye, E.K., Ekwempu, C.C., 1988. Comparative experimental trasmission studies with Nigerian isolates and TS-1 strain of *Toxoplasma gondii* in sheep. J. Anim. Prod. Res. 8, 104–120.

Aiumlamai, S., Fredriksson, G., Uggla, A., Kindahl, H., Edqvist, L.E., 1990. The effect of *Toxoplasma gondii* infection in flunixin meglumine treated pregnant ewes as monitored by plasma levels of 15- ketodihydroprostaglandin F_{2a}, progesterone, oestrone sulphate and ultrasound scanning. J. Vet. Med. A 37, 23–34.

Altintaş, K., 1996. *Toxoplasma gondii* infections of animals in Turkey. Türkiye Parazitoloji Dergisi. 20, 479–487.

Amin, A.M., Morsy, T.A., 1997. Anti-*Toxoplasma* antibodies in butchers and slaughtered sheep and goats in Jeddah Municipal abattoir, Saudi Arabia. J. Egypt Soc. Parasitol. 27, 913–918.

Arthur, M.J., Blewett, D.A., 1988. IFAT detection of IgG specific to *Toxoplasma* in thoractic fluids from aborted lambs: evluation on routine diagnostic submissions. Vet. Rec. 122, 29–31.

Babür, C., Inci, A., Karaer, Z., 1997. Detection on seropositivity of *Toxoplasma gondii* in sheep and goats in around of Çankiri using Sabin-Feldman dye test. Türkiye Parazitoloji Dergisi. 21, 409–412 [in Turkish].

Balbo, S.M., Di Bella, C., Piraino, C., Vesco, G., 1991. Contributo alla diagnostica della toxoplasmosi ovina. Atti Fe. Me. S. P. Rum. 1, 281–284.

Bártová, E., Sedlák, K., Literák, I., in press. *Toxoplasma gondii* and *Neospora caninum* antibodies in sheep in the Czech Republic. Vet. Parasitol.

Bekele, T., Kasali, O.B., 1989. Toxoplasmosis in sheep, goats and cattle in central Ethiopia. Vet. Res. Commun. 13, 371–375.

Belbacha, I., Hafid, J., Sung, T.M.R., Flori, P., Raberin, H., Aboufatima, R., Regragui, A., Dalal, A., Chait, A., 2004. *Toxoplasma gondii*: taux de portage chez les ovins de la région de Marrakech (Mnabha). Schweiz. Arch. Tierheilkd. 146, 561–564.

Benkirane, A., Jabli, N., Rodolakis, A., 1990. Fréquence d'avortement et séroprévalence des principales maladies infectieuses abortives ovines dans la région de Rabat (Maroc). Ann. Rech. Vet. 21, 267–273.

Boyer, K.M., Holfels, E., Roizen, N., Swisher, C., Mack, D., Remington, J., Withers, S., Meier, P., McLeod, R., 2005. The toxoplasmosis study group, 2005. Risk factors for *Toxoplasma gondii* infection in mothers of infants with congenital toxoplasmosis: implications for prenatal management and screening. Am. J. Obstet. Gynecol. 192, 564–571.

Buxton, D., Blewett, D.A., Trees, A.J., McColgan, C., Finlayson, J., 1988. Further studies in the use of monensin in the control of experimental ovine toxoplasmosis. J. Comp. Pathol. 98, 225–236.

Buxton, D., Uggla, A., Lövgren, K., Thomson, K., Lundén, A., Morein, B., Blewett, D.A., 1989. Trial of a novel experimental *Toxoplasma* iscom vaccine in pregnant sheep. Brit. Vet. J. 145, 451–457.

Buxton, D., Thomson, K., Maley, S., Wright, S., Bos, H.J., 1991. Vaccination of sheep with a live incomplete strain (S48) of *Toxoplasma gondii* and their immunity to challenge when pregnant. Vet. Rec. 129, 89–93.

Buxton, D., Thomson, K.M., Maley, S., Wright, S., Bos, H.J., 1993a. Experimental challenge of sheep 18 months after vaccination with a live (S48) *Toxoplasma gondii* vaccine. Vet. Rec. 133, 310–312.

Buxton, D., Thomson, K.M., Maley, S., 1993b. Treatment of ovine toxoplasmosis with a combination of sulphamezathine and pyrimethamine. Vet. Rec. 132, 409–411.

Buxton, D., Thomson, K.M., Maley, S., Wastling, J.M., Innes, E.A., Panton, W.R.M., Nicoll, S., 1994. Primary and secondary responses of the ovine lymph node to *Toxoplasma gondii*: cell output in efferent lymph and parasite detection. J. Comp. Pathol. 111, 231–241.

Buxton, D., Innes, E.A., 1995. A commercial vaccine for ovine toxoplasmosis. Parasitology 110, S11–S16.

Buxton, D., Brebner, J., Wright, S., Maley, S.W., Thomson, K.M., Millard, K., 1996. Decoquinate and the control of experimental ovine toxoplasmosis. Vet. Rec. 138, 434–436.

Buxton, D., Rodger, S.M., Maley, S.W., Wright, S.E., 2006. Toxopplasmosis: the possibility of vertical transmission. Small Rumin. Res. 62, 43–46.

Buxton, D., Maley, S.W., Wright, S.E., Rodger, S., Bartley, P., Innes, E.A., 2007. *Toxoplasma gondii* and ovine toxoplasmosis: new aspects of an old story. Vet. Parasitol. 149, 25–28.

Caballero-Ortega, H., Quiroz-Romero, H., Olazarán-Jenkins, S., Correa, D., 2008a. Frequency of *Toxoplasma gondii* infection in sheep from a tropical zone of Mexico and temporal analysis of the humoral response changes. Parasitology 135, 897–902.

Caballero-Ortega, H., Palma, J.M., García Márquez, L.J., Gildo-Cárdenas, A., Correa, D., 2008b. Frequency and risk factors for toxoplasmosis in ovines of various regions of the State of Colima, Mexico. Parasitology 135, 1385–1389.

Cavalcante, G.T., Aguiar, D.M., Chiebao, D.P., Meireles, L.R., Andrade, H.F., Camargo, L.M.A., Labruna, M.B., Ruiz, V.L.A., Gennari, S.M., 2004. Ocorrência de anticorpos anti-*Toxoplasma gondii* em humanos e animais domésticos da zona Monte Negro, Rondônia. Thirteenth Congresso Brasileiro de Parasitologia Veterinária, Ouro Preto, Minas Gerais, vol. 13 (Suplemento), Brasil, 20-24 de setembro de 2006, Rev. Brasil. Parasitol. Vet. 217.

Cicek, H., Babür, C., Karaer, Z., 2004. Seroprevalence of *Toxoplasma gondii* in sheep using Sabin-Feldman (SF) dye test in Afyon province. Ankara Üniv. Vet. Fak. Derg. 51, 229–231 [in Turkish].

Clementino, M.M., Souza, M.F., Andrade Neto, V.F., 2007. Seroprevalence and *Toxoplasma gondii*-IgG avidity in sheep from Lajes. Brazil. Vet. Parasitol. 146, 199–203.

Cook, A.J.C., Gilbert, R.E., Buffolano, W., Zufferey, J., Petersen, E., Jenum, P.A., Foulon, W., Semprini, A.E., Dunn, D.T., 2000. Sources of toxoplasma infection in pregnant women: European multicentre case-control study. Br. Med. J. 321, 142–147.

Coughlan, S.N., Saman, E., Jacobs, D., Mercier, C., Cesbron-Delauw, M.F., Trees, A.J., 1995. Cellular and humoral immune responses to recombinant antigens in sheep infected with *Toxoplasma gondii*. Parasite Immunol. 17, 465–468.

Cremers, H.J.W.M., van Knapen, F., Panggabean, S.O., den Hartog, J.M., 1991. Problemen bij het aantonen van *Toxoplasma gondii* in spierweefsel van schapen. Tijdschr. Diergeneesk. 116, 3–6.

Cruz-Vazquez, C., Garcia-Vazquez, Z., Rosario-Cruz, R., Solorzano-Salgado, M., 1992. Ovine toxoplasmosis in Huitzilac, Morelos, Mexico. Prev. Vet. Med. 12, 27–33.

da Silva, A.V., Langoni, H., 2001. The detection of *Toxoplasma gondii* by comparing cytology, histopathology, bioassay in mice, and the polymerase chain reaction (PCR). Vet. Parasitol. 97, 191–198.

da Silva, A.V., Cutolo, A.A., Langoni, H., 2002. Comparação da reação de imunofluorescência indireta e do método de aglutinação direta na detecção de anticorpos anti-toxoplasma em soros de ovinos, caprinos, caninos e felinos. Arq. Inst. Biol. (São Paulo) 69, 7–11.

da Silva, A.V., Cunha, E.L.P., Meireles, L.R., Gottschalk, S., Mota, R.A., Langoni, H., 2003. Toxoplasmose em ovinos e caprinos: estudo soro-epidemiológico em duas regiões dp Estado de Pernambuco, Brasil. Ciência Rural, Santa Maria 33, 115–119.

de Moura, A.B., Osaki, S.C., Zulpo, D.L., Marana, E.R.M., 2007. Ocorrência de anticorpos contra *Toxoplasma gondii* em suínos e ovinos abatidos no

município de Guarapuava, PR, Brasil. Rev. Brasil Parasitol. Vet. 16, 54–56.
Deconinck, P., Pangui, L.J., Akakpo, J., Garrouste, A., Ouattara, L., Roger, F., Tibayrenc, R., Dorchies, P., 1996. Prévalence de la toxoplasmose chez les petits ruminants en Afrique tropicale: résultats d'une enquête séro-épidémiologique sur 1042 animaux. Rev. Méd. Vét. 147, 377–378.
Dubey, J.P., Towle, A., 1986. Toxoplasmosis in Sheep: A Review and Annotated Bibliography. Commonwealth Institute of Parasitology, St. Alberts, Herts, UK, pp. 1–152.
Dubey, J.P., Beattie, C.P., 1988. Toxoplasmosis of Animals and Man. CRC Press, Boca Raton, Florida, pp. 1–220.
Dubey, J.P., Welcome, F.L., 1988. *Toxoplasma gondii* induced abortion in sheep. J. Am. Vet. Med. Assoc. 193, 697–700.
Dubey, J.P., Kirkbride, C.A., 1989a. Enzootic toxoplasmosis in sheep in North-Central United-States. J. Parasitol. 75, 673–676.
Dubey, J.P., Kirkbride, C.A., 1989b. Economic and public health considerations of congenital toxoplasmosis in lambs. J. Am. Vet. Med. Assoc. 195, 1715–1716.
Dubey, J.P., Kirkbride, C.A., 1990. Toxoplasmosis and other causes of abortions in sheep from the north central United States. J. Am. Vet. Med. Assoc. 196, 287–290.
Dubey, J.P., Sonn, R.J., Hedstrom, O., Snyder, S.P., Lassen, E.D., 1990. Serologic and histologic diagnosis of toxoplasmic abortions in sheep in Oregon. J. Am. Vet. Med. Assoc. 196, 291–294.
Dubey, J.P., Somvanshi, R., Jithendran, K.P., Rao, J.R., 1993. High seroprevalence of *Toxoplasma gondii* in goats from Kumaon region of India. J. Vet. Parasitol. 7, 17–21.
Dubey, J.P., Lunney, J.K., Shen, S.K., Kwok, O.C.H., Ashford, D.A., Thulliez, P., 1996. Infectivity of low numbers of *Toxoplasma gondii* oocysts to pigs. J. Parasitol. 82, 438–443.
Dubey, J.P., Sundar, N., Hill, D., Velmurugan, G.V., Bandini, L.A., Kwok, O.C.H., Majumdar, D., Su, C., 2008. High prevalence and abundant atypical genotypes of *Toxoplasma gondii* isolated from lambs destined for human consumption in the USA. Int. J. Parasitol. 38, 999–1006.
Dumanli, N., Güler, S., Köroglu, E., Orak, S., 1991. The incidence of *Toxoplasma gondíí* in sheep in the Elazig region. Tr. J. Vet. Anim. Sci. 16, 10–18 [in Turkish].
Dumètre, A., Ajzenberg, D., Rozette, L., Mercier, A., Dardé, M.L., 2006. *Toxoplasma gondii* infection in sheep from Haute-Vienne, France: seroprevalence and isolate genotyping by microsatellite analysis. Vet. Parasitol. 142, 376–379.
Duncanson, P., Terry, R.S., Smith, J.E., Hide, G., 2001. High levels of congenital transmission of *Toxoplasma gondii* in a commercial sheep flock. Int. J. Parasitol. 31, 1699–1703.
Edelhofer, R., Aspöck, H., 1996. Infektionsquellen und Infektionswege aus der Sicht des Toxoplasmose-Screenings der Schwangeren in Österreich. Mitt. Österr. Ges. Tropenmed. Parasitol. 18, 59–70.
El-Ghaysh, A.A., Mansour, M.M., 1994. Detection of antibodies to *Toxoplasma gondii* in an Egyptian sheep-herd using modern serological techniques. J. Egypt. Assoc. Immunol. 1, 117–121.
El-Metenawy, T.M., 2000. Seroprevalence of *Toxoplasma gondii* antibodies among domesticated ruminants at Al-Qassim Region, Saudi Arabia. Dtsch. Tierärztl. Wschr. 107, 32–33.
El Ridi, A.M., Nada, S.M., Aly, A.S., Habeeb, S.M., Aboul-Fattah, M.M., 1990. Serological studies on toxoplasmosis in Zagazig slaughterhouse. J. Egypt. Soc. Parasitol. 20, 677–681.
Elmassry, A.A., Abdel-Gawad, A.M., Nassar, A.M., 1990. Isolation of *Toxoplasma gondii*, *Isospora felis*, and *Isospora revolta* from (sheep, goats, chicken) in Egypt. J. Egypt. Vet. Med. Assoc. 50, 275–284.
Esteban-Redondo, I., Innes, E.A., 1998. Detection of *Toxoplasma gondii* in tissues of sheep orally challenged with different doses of oocysts. Int. J. Parasitol. 28, 1459–1466.
Esteban-Redondo, I., Maley, S.W., Thomson, K., Nicoll, S., Wright, S., Buxton, D., Innes, E.A., 1999. Detection of *T. gondii* in tissues of sheep and cattle following oral infection. Vet. Parasitol. 86, 155–171.
Figliuolo, L.P.C., Kasai, N., Ragozo, A.M.A., de Paula, V.S.O., Dias, R.A., Souza, S.L.P., Gennari, S.M., 2004. Prevalence of anti-*Toxoplasma gondii* and anti-*Neospora caninum* antibodies in ovine from São Paulo State, Brazil. Vet. Parasitol. 123, 161–166.
Fredrickson, G., Buxton, D., Uggla, A., Kindahl, H., Edqvist, L.E., 1990. The effect of *Toxoplasma gondii* infection in unvaccinated and iscom-vaccinated pregnant ewes as monitored by plasma levels of 15-ketodihydroprostaglandin-F_{2a}, progesterone, and estrone sulfate. J. Vet. Med. A 37, 113–122.
Freire, R.L., Giraldi, N., Vidotto, O., Navarro, I.T., 1995. Levantamento soroepidemiológico da toxoplasmose em ovinos na rgião de Londrina, Paraná. Arquivos Brasileiro de Medicina Veterinária e Zootecnia 47, 609–612.
Freyre, A., Bonino, J., Falcón, J., Castells, D., Correa, O., Casaretto, A., 1997. The incidence and economic significance of ovine toxoplasmosis in Uruguay. Vet. Parasitol. 73, 13–15.
Freyre, A., Bonino, J., Falcón, J., Castells, D., Correa, O., Casaretto, A., 1999. The incidence and economic significance of ovine toxoplasmosis in Uruguay. Vet. Parasitol. 81, 85–88.
Fusco, G., Rinaldi, L., Guarino, A., Proroga, Y.T.R., Pesce, A., Giuseppina, D.M., Cringoli, G., 2007. *Toxoplasma gondii* in sheep from the Campania region (Italy). Vet. Parasitol. 149, 271–274.
Gaffuri, A., Giacometti, M., Tranquillo, V.M., Magnino, S., Cordioli, P., Lanfranchi, P., 2006. Serosurvey of roe deer, chamois, and domestic sheep in the central Italian Alps. J. Wildlife Dis. 42, 685–690.
Garcia, J.L., Navarro, I.T., Ogawa, L., de Oliveira, R.C., 1999. Soroprevalência do *Toxoplasma gondii*, em suínos, bovinos, ovinos e eqüinos, e sua correlação com humanos, felinos e caninos, oriundos, de propriedades rurais do norte do Paraná-Brasil. Ciência Rural. 29, 91–97.
García-Vázquez, Z., Rosario-Cruz, R., Solorzano-Salgado, M., 1990. Prevalence of antibodies against *Toxoplasma gondii* in sheep and goats in three states of Mexico. Prev. Vet. Med. 10, 25–29.
Ghazaei, C., 2005. Serological survey of antibodies to *Toxoplasma gondii*. Afr. J. Health Sci. 12, 114–117.
Gondim, L.F.P., Barbosa, H.V., Ribiero Filho, C.H.A., Saeki, H., 1999. Serological survey of antibodies to *Toxoplasma gondii* in goats, sheep, cattle, and water buffaloes in Bahia State, Brazil. Vet. Parasitol. 82, 273–276.
Gorman, T., Pablo Arancibia, J., Lorca, M., Hird, D., Alcaino, H., 1999. Seroprevalence of *Toxoplasma gondii* infection in sheep and alpacas (*Llama pacos*) in Chile. Prev. Vet. Med. 40, 143–149.
Górecki, M.T., Andrzejewska, I., Steppa, R., 2008. Is ordeer of voluntarily entrance to milking parlour related to *Toxoplasma gondii* infection in sheep—a brief note. Appl. Anim. Behav. Sci. 110, 392–396.
Greig, A., Buxton, D., Savva, D., 1993. Diagnosis of *Toxoplasma* abortion in sheep. Vet. Rec. 132, 226–227.
Gumbrell, R.C., 1990. Sheep abortion in New Zealand: 1989. Surveillance 17, 22–23.
Hamidinejat, H., Goraninejad, S., Ghorbanpoor, M., Nabavi, L., Akbarnejad, F., 2008. Role of *Toxoplasma gondii* in abortion of ewes in Ahvaz (South-West Iran). Bull. Vet. Inst. Puawy 52, 369–371.
Harps, O., 1993. Untersuchungen über die Seroprävalenz von *Toxoplasma*-Infektionen bei kleinen Wiederkäueren in Jordanien. D.V.M.Thesis. Hannover, Germany, pp. 1–137.
Hartley, W.J., 1961. Experimental transmission of toxoplasmosis in sheep. N. Z. Vet. J. 9, 1–6.
Hashemi-Fesharki, R., 1996. Seroprevalence of *Toxoplasma gondii* in cattle, sheep and goats in Iran. Vet. Parasitol. 61, 1–3.
Hassanain, M.A., Ezzo, O.H., Deghidy, B.S., 1992. Some biochemical and hormonal changes in *Toxoplasma*-infected and aborted ewes. Egypt. J. Comp. Pathol. Clin. Pathol. 5, 221–227.
Hejlíček, K., Literák, I., 1994. Toxoplazmóza jatecnych zvirat. Hygienicka problematika Veterinarstvi. 44, 576–581.
Hoghooghi-Rad, N., Afraa, M., 1993. Prevalence of toxoplasmosis in humans and domestic animals in Ahwaz, Capital of Khoozestan Province, South-West Iran. J. Trop. Med. Hyg. 96 (3), 163–168.
Hossain, A., Bolbol, A.S., Bakir, T.M.F., Bashandi, A.M., 1987. A serological survey of the prevalence of *Toxoplasma gondii* in slaughtered animals in Saudi-Arabia. Ann. Trop. Med. Parasitol. 81, 69–70.
Hove, T., Lind, P., Mukaratirwa, S., 2005. Seroprevalence of *Toxoplasma gondii* infection in goats and sheep in Zimbabwe. Onderstepoort J. Vet. Res. 72, 267–272.
Hurtado, A., Aduriz, G., Moreno, B., Barandika, J., García-Pérez, A.L., 2001. Single tube nested PCR for the detection of *Toxoplasma gondii* in fetal tissues from naturally aborted ewes. Vet. Parasitol. 102, 17–27.
Inci, A., Aydin, N., Babür, C., Çam, Y., Akdongan, C., Kuzan, S., 1999. Seroepidemiological studies on toxoplasmosis and brucellosis in cattle and sheep around Kayseri. Pendik Vet. Microbiyol. Derg. 30, 41–46 [in Turkish].
Innes, E.A., Panton, W.R.M., Thomson, K.M., Maley, S., Buxton, D., 1995a. Kinetics of interferon gamma production in vivo during infection with the S48 vaccine strain of *Toxoplasma gondii*. J. Comp. Pathol. 113, 89–94.
Innes, E.A., Panton, W.R.M., Sanderson, A., Thomson, K.M., Wastling, J.M., Maley, S., Buxton, D., 1995b. Induction of $CD4^+$ and $CD8^+$ T cell responses in efferent lymph responding to *Toxoplasma gondii* infection: analysis of phenotpye and function. Parasite Immunol. 17, 151–160.
Johnston, W.S., 1988. An investigation into toxoplasmosis as a cause of barrenness in ewes. Vet. Rec. 122, 283–284.
Jungersen, G., Jensen, L., Rask, M.R., Lind, P., 2002. Non-lethal infection parameters in mice separate sheep type II *Toxoplasma gondii* isolates by virulence. Comp. Immunol. Microbiol. Infect. Dis. 25, 187–195.

Karatepe, B., Babur, C., Karatepe, M., Cakmak, A., Nalbantoglu, S., 2004. Seroprevalance of toxoplasmosis in sheep and goats in the Nigde Province of Turkey. Indian Vet. J. 81, 974–976.
Kirkbride, C.A., Dubey, J.P., Libal, M.C., 1992. Effect of feeding lasalocid to pregnant ewes experimentally infected with *Toxoplasma gondii*. Vet. Parasitol. 44, 299–303.
Klun, I., Djurkovic-Djakovic, O., Katic-Radivojevic, S., Nikolic, A., 2006. Cross-sectional survey on *Toxoplasma gondii* infection in cattle, sheep and pigs in Serbia: seroprevalence and risk factors. Vet. Parasitol. 135, 121–131.
Klun, I., Djurkovic-Djakovic, O., Thulliez, P., 2007. Comparison of a commercial ELISA with the modified agglutination test for the detection of *Toxoplasma gondii* infection in naturally exposed sheep. Zoonoses Public Health. 54, 165–168.
Kovâčovâ, D., 1993. Sérologická prevelencia *Toxoplasma gondii* u oviec. Veterinarstvi 43, 51–54.
Ljungström, B.L., Lundén, A., Höglund, J., Zakrisson, G., 1994. Evaluation of a direct agglutination test for detection of antibodies against *Toxoplasma gondii* in cat, pig and sheep sera. Acta Vet. Scand. 35, 213–216.
Lundén, A., Carlsson, U., Näslund, K., 1992. Toxoplasmosis and border disease in 54 swedish sheep flocks - Seroprevalence and incidence during one gestation period. Acta Vet. Scand. 33, 175–184.
Lundén, A., Näsholm, A., Uggla, A., 1994. Long-term study of *Toxoplasma gondii* infection in a Swedish sheep flock. Acta Vet. Scand. 35, 273–281.
Lundén, A., 1995. Immune responses in sheep after immunization with *Toxoplasma gondii* antigens incorporated into iscoms. Vet. Parasitol. 56, 23–35.
Mainar-Jaime, R.C., Barberán, M., 2007. Evaluation of the diagnostic accuracy of the modified agglutination test (MAT) and an indirect ELISA for the detection of serum antibodies against *Toxoplasma gondii* in sheep through Bayesian approaches. Vet. Parasitol. 148, 122–129.
Mainar, R.C., de la Cruz, C., Asensio, A., Domínguez, L., Vázquez-Boland, J.A., 1996. Prevalence of agglutinating antibodies to *Toxoplasma gondii* in small ruminants of the Madrid region, Spain, and identification of factors influencing seropositivity by multivariate analysis. Vet. Res. Commun. 20, 153–159.
Malik, M.A., Dreesen, D.W., de la Cruz, A., 1990. Toxoplasmosis in sheep in northeastern United States. J. Am. Vet. Med. Assoc. 196, 263–265.
Marca, M.C., Ramos, J.J., Loste, A., Sáez, T., Sanz, M.C., 1996. Comparison of indirect immunofluorescent antibody test and modified direct agglutination test methods for detection of *Toxoplasma gondii* antibodies in adult sheep in Spain. Vet. Parasitol. 67, 99–103.
Masala, G., Porcu, R., Madau, L., Tanda, A., Ibba, B., Satta, G., Tola, S., 2003. Survey of ovine and caprine toxoplasmosis by IFAT and PCR assays in Sardinia, Italy. Vet. Parasitol. 117, 15–21.
Masala, G., Porcu, R., Daga, C., Denti, S., Canu, G., Patta, C., Tola, S., 2007. Detection of pathogens in ovine and caprine abortion samples from Sardinia, Italy, by PCR. J. Vet. Diagn. Invest. 19, 96–98.
McColgan, C., Buxton, D., Blewett, D.A., 1988. Titration of *Toxoplasma gondii* oocysts in non-pregnant sheep and the effects of subsequent challenge during pregnancy. Vet. Rec. 123, 467–470.
Meireles, L.R., Galisteo, A.J., Andrade, H.F., 2003. Serological survey of antibodies to *Toxoplasma gondii* in food animals from São Paulo state, Brazil. Braz. J. Vet. Res. Anim. Sci. 40, 267–271.
Michalski, M., Platt-Samoraj, A., 2004. Extent of *Toxoplasma gondii* invasion in goat and sheep from the Olsztyn region. Medycyna Weterynaryjna 60, 70–71.
Mirdha, B.R., Samantaray, J.C., Pandey, A., 1999. Seropositivity of *Toxoplasma gondii* in domestic animals. Indian J. Public Health. 43, 91–92.
Mor, N., Arslan, M.O., 2007. Kars yöresindeki koyunlarda *Toxoplasma gondii* nin seroprevalansi. Kafkas Univ. Vet. Fak Derg. 13, 165–170.
Moreno, T., Martinez-Gomez, F., Hernandez-Rodriguez, S., de Setefilla Martinez-Cruz, M., Martinez-Moreno, A., 1991. The seroprevalence of ovine toxoplasmosis in Cordoba, Spain. Ann. Trop. Med. Parasitol. 85, 287–288.
Morley, E.K., Williams, R.H., Hughes, J.M., Terry, R.S., Duncanson, P., 2005. Significant familial differences in the frequency of abortion and *Toxoplasma gondii* infection within a flock of Charollais sheep. Parasitology 131, 181–185.
Morley, E.K., Williams, R.H., Hughes, J.M., Thomasson, D., Terry, R.S., Duncanson, P., Smith, J.E., Hide, G., 2008. Evidence that primary infection of Charollais sheep with *Toxoplasma gondii* may not prevent foetal infection and abortion in subsequent lambings. Parasitology 135, 169–173.
Munday, B.L., 1972. Transmission of *Toxoplasma* infection from chronically infected ewes to their lambs. Br. Vet. J. 128, 71–72.
Negash, T., Tilahun, G., Patton, S., Prévot, F., Dorchies, P., 2004. Serological survey on toxoplasmosis in sheep and goats in Nazareth, Ethiopia. Rev. Méd. Vét. 155, 486–487.
O'Brien, D., Geraghty, V., 1990. A serological survey for toxoplasmosis in sheep in Ireland. Irish Vet. J. 43, 76–176.
O'Connell, E., Wilkins, M.F., Te Punga, W.A., 1988. Toxoplasmosis in sheep. II. The ability of a live vacine to prevent lamb losses after an intravenous challenge with Toxoplasma gondii. N. Z. Vet. J. 36, 1–4.
Oliveira-Sequeira, T.C.G., Aamarante, A.F.T., Salata, E., Sogayar, R., 1993. Serological survey for *Toxoplasma* infection in sheep in São Paulo State, Brazil. Vet. Zootec. 5, 121–125.
Oncel, T., Vural, G., 2006. Occurrence of *Toxxoplasma gondii* antibodies in sheep in Istanbul, Turkey. Veterinarski Arhiv. 76, 547–553.
Öncel, T., Vural, G., Babur, C., Kiliç, S., 2005. Detection of toxoplasmosis *gondii* seropositivity in sheep in Yalova by Sabin Feldman dye test and latex agglutination test. Turkiye Parazitol. Derg. 29, 10–12.
Orr, M.B., 1989. Sheep abortions—invermay 1988. Surveillance 16, 24–25.
Owen, M.R., Clarkson, M.J., Trees, A.J., 1998a. Diagnosis of toxoplasma abortion in ewes by polymerase chain reaction. Vet. Rec. 142, 445–448.
Owen, M.R., Clarkson, M.J., Trees, A.J., 1998b. Acute phase toxoplasma abortions in sheep. Vet. Rec. 142, 480–482.
Owen, M.R., Trees, A.J., 1999. Genotyping of *Toxoplasma gondii* associated with abortion in sheep. J. Parasitol. 85, 382–384.
Oz, I., Ozyer, M., Corak, R., 1995. Adana yoresi sigir koyun ve kecilerinde ELISA ve IHA testleri ile toxoplasmosis'in yayginliginin arastirilmasi. J. Etlik Vet. Microbiol. 8, 87–89.
Pandey, V.S., van Knapen, F., 1992. The seroprevalence of toxoplasmosis in sheep, goats and pigs in Zimbabwe. Ann. Trop. Med. Parasitol. 86, 313–315.
Pangui, L.J., Lahamdi, A., Samb, F., 1993. Utilisation de l'IFI et de l'ELISA dans une enquête sérologique de la toxoplasmose chez le mouton «de case» à Dakar-Sénégal. Rec. Méd. Vét. 169, 45–46.
Payne, R.A., Joynson, D.H.M., Wilsmore, A.J., 1988. Enzyme linked immunosorbent assays for the measurement of specific antibodies in experimentally induced ovine toxoplasmosis. Epidemiol. Infect. 100, 205–212.
Pereira-Bueno, J., Quintanilla-Gozalo, A., Pérez-Pérez, V., Álvarez-García, G., Collantes-Fernández, E., Ortega-Mora, L.M., 2004. Evaluation of ovine abortion associated with *Toxoplasma gondii* in Spain by different diagnostic techniques. Vet. Parasitol. 121, 33–43.
Prelezov, P., Koinarski, V., Georgieva, D., 2008. Seroprevalence of *Toxoplasma gondii* infection among sheep and goats in the Stara Zagora region. Bulgarian J. Vet. Med. 11, 113–119.
Ragozo, A.M.A., Yai, L.E.O., Oliveira, L.N., Dias, R.A., Dubey, J.P., Gennari, S.M., 2008. Seroprevalence and isolation of *Toxoplasma gondii* from sheep from São Paulo State. Brazil. J. Parasitol. 94, 1259–1263.
Rajamanickam, C., Cheah, T.S., Paramasvan, S., 1990. Antibodies to *Toxoplasma gondii* from domestic animals in Malaysia. Trop. Anim. Health Prod. 22, 61–62.
Reif, J.S., Samame, H.A., Ameghino, E., Lopez-Nieto, E., Demartini, J.C., 1989. Adverse reproductive outcome and antibody to *Toxoplasma gondii* in a cohort of Peruvian sheep. Prev. Vet. Med. 7, 225–228.
Rodger, S.M., Maley, S.W., Wright, S.E., Mackellar, A., Wesley, F., Sales, J., Buxton, D., 2006. Role of endogenous transplacental transmission in toxopasmosis in sheep. Vet. Rec. 159, 768–772.
Romanelli, P.R., Freire, R.L., Vidotto, O., Marana, E.R.M., Ogawa, L., de Paula, V.S.O., Garcia, J.L., Navarro, I.T., 2007. Prevalence of *Neospora caninum* and *Toxoplasma gondii* in sheep and dogs from Guarapuava farms, Paraná State, Brazil. Res. Vet. Sci. 82, 202–207.
Rozette, L., Dumètre, A., Couquet, C.Y., Dardé, M.L., 2005. Seroprevalence de la toxoplasmose chez des ovins et des bovins en Haute-Vienne. Épidémiologie et Santé Animale 48, 97–99.
Samad, M.A., Rahman, K.B., Halder, A.K., 1993a. Seroprevalence of *Toxoplasma gondii* in domestic ruminants in Bangladesh. Vet. Parasitol. 47, 157–159.
Samad, M.A., Rahman, K.M.B., Basher, S.A., 1993b. Serological status to natural *Toxoplasma gondii* infection in mixed flocks of sheep and goats in Bangladesh. J. Protozool. Res. 3, 25–28.
Samad, M.A., Clarkson, M.J., 1995. Prophylactic efficacy of decoquinate in the control of experimentally induced toxoplasmosis in pregnant ewes. Bangladesh Vet. J. 29, 43–50.
Sanad, M.M., Al-Ghabban, A.J., 2007. Serological survey on toxoplasmosis among slaughtered sheep and goats in Tabouk, Saudi Arabia. J. Egypt. Soc. Parasitol. 37, 329–340.
Savio, E., Nieto, A., 1995. Ovine toxoplasmosis: seroconversion during pregnancy and lamb birth rate in Uruguayan sheep flocks. Vet. Parasitol. 60, 241–247.
Sawadogo, P., Hafid, J., Bellete, B., Sung, R.T.M., Chakdi, M., Flori, P., Raberin, H., Hamouni, I.B., Chait, A., Dalal, A., 2005. Seroprevalence of *T. gondii* in sheep from Marrakech, Morocco. Vet. Parasitol. 130, 89–92.
Sedlák, K., Bártová, E., 2007. Toxoplasmoza zvirat a jeji laboratorni diagnostika v Ceske republice. Veterinarstvi 57, 562–567.

Seineke, P., 1996. Seroprävalenz von Antikörpern gegen *Toxoplasma gondii* bei Schafen, Zeigen. Schweinen in Niedersachsen Dr. Med. Vet. Thesis, Hannover, 171.

Shaapan, R.M., El-Nawawi, F.A., Tawfik, M.A.A., 2008. Sensitivity and specificity of various serological tests for the detection of *Toxoplasma gondii* infection in naturally infected sheep. Vet. Parasitol. 153, 359–362.

Sharif, M., Gholami, S., Ziaei, H., Daryani, A., Laktarashi, B., Ziapour, S.P., Rafiei, A., Vahedi, M., 2007. Seroprevalence of Toxoplasma gondii in cattle, sheep and goats slaughtered for food in Mazandaran province, Iran, during 2005. Vet. J. 174, 422–424.

Sharma, S., Sandhu, K.S., Bal, M.S., Kumar, H., Verma, S., Dubey, J.P., 2008. Serological survey of antibodies to *Toxoplasma gondii* in sheep, cattle, and buffaloes in Punjab, India. J. Parasitol. 94, 1174–1175.

Shkap, V., Pipano, E., Marcus, S., Rapoport, E., 1992. The prevalence of *Toxoplasma gondii* antibodies in sheep and cattle in Israel. Israel J. Med. Sci. 47, 100–102.

Silva, K.L.M.V., de la Rue, M.L., 2006. Possibilidade da transmissão congênita de *Toxoplasma gondii* em ovinos através de seguimento sorológico no município de Rosário do Sul, RS, Brasil. Ciência Rural, Santa Maria 36, 892–897.

Sindoni, L., Cananzi, F., Ciano, V., 1989. Indagine sieroepidemiologica sulla prevalenza di anticorpi antitoxoplasma in ovini, caprini e bovini stanzianti in alcune zone della Sicilia: valutazione dei tests utilizzati. Arch. Vet. Ital. 40, 118–127.

Skjerve, E., Waldeland, H., Nesbakken, T., Kapperud, G., 1998. Risk factors for the presence of antibodies to *Toxoplasma gondii* in Norwegian slaughter lambs. Prev. Vet. Med. 35, 219–227.

Soares, H.S., Ahid, S.M.M., Bezerra, A.C.D.S., Pena, H.F.J., Dias, R.A., Gennari, S.M., 2009. Prevalence of anti-*Toxoplasma gondii* and anti-Neospora caninum antibodies in sheep from Mossoró, Rio Grande do Norte. Brazil. Vet. Parasitol. 160, 211–214.

Spósito Filha, E., do Amaral, V., Macruz, R., Rebouças, M.M., Santos, S.M., Drumond, L.S., 1992. *Toxoplasma gondii* em ovinos: isolamento do parasita a partir de diafragmas de animais procedentes do estado do Rio Grande do Sul abatidos em matadouros de São Paulo, para consumo humano. Rev. Brasil. Parasitol. Vet. 1, 117–119.

Stanley, A.C., Buxton, D., Innes, E.A., Huntley, J.F., 2004. Intranasal imunisation with *Toxoplasma gondii* tachyzoite antigen encapsulated into PLG microspheres induces humoral and cell-mediated immunity in sheep. Vaccine 22, 3929–3941.

Steuber, S., Niu, A., Bauer, C., Reetz, J., Roth, A., Janitschke, K., 1995. Der Nachweis von *Toxoplasma gondii* in Abortgeweben vom Schaf mittels der Polymerase-Kettenreaktion. Dtsch. Tierärztl. Wschr. 102, 91–93.

Stimbirys, A., Bagdonas, J., Gerulis, G., Russo, P., 2007. A serological study on the prevalence of *Toxoplasma gondii* in sheep of Lithuania. Pol. J. Vet. Sci. 10, 83–87.

Szeredi, L., Bacsadi, A., 2002. Detection of *Chlamydophila* (*Chlamydia*) *abortus* and *Toxoplasma gondii* in smears from cases of ovine and caprine abortion by the streptavidin-biotin method. J. Comp. Pathol. 127, 257–263.

Tenter, A.M., Vietmeyer, C., Johnson, A.M., 1992. Development of ELISAs based on recombinant antigens for the detection of *Toxoplasma gondii*-specific antibodies in sheep and cats. Vet. Parasitol. 43, 189–201.

Thamsborg, S.M., Ilsoe, B., Henriksen, S.A., Lind, P., 1994. *Toxoplasma*-abort hos får. Dansk Veterinaertidsskrift 77, 925–930.

Trees, A.J., Al-Atiya, S.A., Balfour, A.H., 1988. Diagnosis of ovine toxoplasmosis. Vet. Rec. 124, 554.

Trees, A.J., Crozier, S.J., Buxton, D., Blewett, D.A., 1989. Serodiagnosis of ovine toxoplasmosis: an assessment of the latex agglutination-test and the value of IgM specific titres after experimental oocyst-induced infections. Res. Vet. Sci. 46, 67–72.

Turner, C.B., Mohammed, S., Savva, D., 1991. Detection of toxoplasma DNA in ovine samples. Vet. Rec. 129, 436–1436.

Ueno, T.E.H., Gonçalves, V.S.P., Heinemann, M.B., Dilli, T.L.B., Akimoto, B.M., de Souza, S.L.P., Gennari, S.M., Soares, R.M., 2009. Prevalence of *Toxoplasma gondii* and *Neospora caninum* infections in sheep from Federasl District, central region of Brazil. Trop. Anim. Health Prod. 41, 547–552.

Underwood, W.J., Rook, J.S., 1992. Toxoplasmosis infection in sheep. Comp. Cont. Edu. Pract. Vet. 14, 1543–1549.

van der Puije, W.N.A., Bosompem, K.M., Canacoo, E.A., Wastling, J.M., Akanmori, B.D., 2000. The prevalence of anti-*Toxoplasma gondii* antibodies in Ghanaian sheep and goats. Acta Tropica 76, 21–26.

Verma, S.P., Bhardwaj, R.M., Gautam, O.P., 1988. Seroprevalene of *Toxoplasma* antibodies in aborted ewes. Indian J. Vet. Med. 8, 132–133.

Verma, S.P., Bhardwaj, R.M., Gautam, O.P., 1989a. Isolation of *Toxoplasma gondii* from the foetal brain of aborted ewes. Indian J. Vet. Med. 9, 40–41.

Verma, S.P., Bhardwaj, R.M., Gautam, O.P., 1989b. Application of counter-current immunoelectrophoresis (CIEP) in the sero-diagnosis of *Toxoplasma* antibodies in sheep. J. Vet. Parasitol. 3, 61–62.

Vesco, G., Buffolano, W., La Chiusa, S., Mancuso, G., Caracappa, S., Chianca, A., Villari, S., Currò, V., Liga, F., Petersen, E., 2007. *Toxoplasma gondii* infections in sheep in Sicily, southern Italy. Vet. Parasitol. 146, 3–8.

Waltner-Toews, D., Mondesire, R., Menzies, P., 1991. The seroprevalence of *Toxoplasma gondii* in Ontario sheep flocks. Can. Vet. J. 32, 734–737.

Wastling, J.M., Nicoll, S., Buxton, D., 1993. Comparison of two gene amplification methods for the detection of *Toxoplasma gondii* in experimentally infected sheep. J. Med. Microbiol. 38, 360–365.

Wastling, J.M., Harkins, D., Buxton, D., 1994. Western blot analysis of the IgG response of sheep vaccinated with S48 *Toxoplasma gondii* (Toxovax). Res. Vet. Sci. 57, 384–386.

Wastling, J.M., Harkins, D., Maley, S., Innes, E., Panton, W., Thomson, K., Buxton, D., 1995. Kinetics of the local and systemic antibody response to primary and secondary infection with S48 *Toxoplasma gondii* in sheep. J. Comp. Pathol. 112, 53–62.

Watson, W.A., Beverley, J.K.A., 1971. Ovine abortion due to experimental toxoplasmosis. Vet. Rec. 88, 42–45.

Weissmann, J., 2003. Presumptive *Toxoplasma gondii* abortion in a sheep. Can. Vet. J. 44, 322–324.

Weitzman, G.L., Stem, E., Gilfillan, R.S., Lindenmayer, J.M., 1991. Preliminary serological survey for bluetongue and toxoplasmosis in sheep in Niger. Trop. Anim. Health Prod. 23, 258–1258.

West, M., Venturini, C., Passucci, J., González, C., Bacigalupe, D., 1998. Seroconversion de titulos anti-*Toxoplasma gondii* en hembras ovinas lecheras de 3 a 4 meses de edad. Vet. Arg. 15, 176–179.

Wheeler, R., Wilmore, H., Savva, D., Turner, C.B., 1990. Diagnosis of ovine toxoplasmosis using PCR. Vet. Rec. 126, 249–1249.

Wilkins, M., O'Connell, E., 1992. Vaccination of sheep against *Toxoplasma* abortion. Surveillance 19, 20–24.

Wilkins, M.F., O'Connell, E., Te Punga, W.A., 1988. Toxoplasmosis in sheep III. Further evaluation of the ability of a live Toxoplasma gondii vaccine to prevent lamb losses and reduce congenital infection following experimental oral challenge. N. Z. Vet. J. 36, 86–89.

Williams, R.H., Morley, E.K., Hughes, J.M., Duncanson, P., Terry, R.S., Smith, J.E., Hide, G., 2005. High levels of congenital transmission of *Toxoplasma gondii* in longitudinal and cross-sectional studies on sheep farms provides evidence of vertical transmission in ovine hosts. Parasitology 130, 301–307.

Zaki, M., 1995. Seroprevalence of *Toxoplasma gondii* in domestic animals in Pakistan. J. Pak. Med. Assoc. 45, 4–5.

Zeybek, H., Yarali, C., Nishikawa, H., Nishikawa, F., Dündar, B., 1995. The prevalence of toxoplasmosis in sheep of Ankara region. Etlik Vet. Mikrob. Derg. 8, 80–86 [in Turkish].

Zia-Ali, N., Fazaeli, A., Khoramizadeh, M., Ajzenberg, D., Dardé, M., Keshavarz-Valian, H., 2007. Isolation and molecular characterization of *Toxoplasma gondii* strains from different hosts in Iran. Parasitol. Res. 101, 111–115.

Zonta, J.C., Araujo, F.A.P., Stobbe, N.S., Chaplin, E.L., Santos da Silva, N.R., 1987–1988. Prevalência de anticorpos toxoplásmicos em ovinos de Marau e de Uruguaiana. RS. Arq. Fac. Vet. UFRGS 15/16, 59–61.