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Toxoplasma gondii: Prototype immunization of lambs against formation of muscle and brain cysts

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ABSTRACT

Nine lambs were immunized with 10^6 oocysts of the relatively avirulent ME-49 strain of *Toxoplasma gondii*, and were challenged 45 days later with 4×10^6 oocysts of the M3 strain of this parasite. Less than 5 tissue cysts of the M3 strain formed per 8 g of muscle – if any – judging from a bioassay in mice in which all mice survived. In contrast, mortality occurred in mice fed muscle specimens from 4 lambs that had not been immunized with ME-49 prior to challenge with the M3 strain. This finding suggests the possibility of creating a vaccine for lambs that could diminish or abolish lamb and mutton as sources of human infection with *T. gondii*, and which could also prevent toxoplasmosis abortion in sheep.

1. Introduction

Toxoplasmosis can cause fetal damage in humans and abortion in sheep, goats, pigs, and rabbits if first contracted during pregnancy (Dubey and Beattie, 1988; Remington and Desmonts, 1990). The incidence of human congenital toxoplasmosis has been shown to be 1–6/1000 births. Although most infected newborns are asymptomatic at birth, adverse sequelae may develop later in life in a large proportion of affected patients (Remington and Desmonts, 1990). In addition, reactivation of latent *Toxoplasma gondii* infection is often fatal in patients who are immunosuppressed in the course of the acquired immunodeficiency syndrome (AIDS), during cancer therapy, or after organ transplantation (Luft and Hafner, 1990).

The importance of consumption of insufficiently cooked meat as a source of human infection, is stressed in two recent reviews (Kijlstra and Jongert, 2008, 2009). Three large case–control studies have pinpointed uncooked meat as the most important risk factor for pregnant women in Europe (Kapperud et al., 1996; Baril et al., 1999; Cook et al., 2000).

Insufficiently cooked lamb or mutton is a potential source of *Toxoplasma* infection for people. Oral inoculation of sheep with *Toxoplasma* oocysts results in muscle encystment that endures at least 4 months (Dubey and Sharma, 1980), thereby creating a food safety risk for people who may consume rare or raw meat from sheep. *Toxoplasma* was isolated from muscles of naturally infected sheep that had specific antibody titers (Jacobs et al., 1963), and since that time many other investigations have isolated *Toxoplasma* from mutton (reviewed by Freyre and Falcon, 1989; and by Dubey and Beattie, 1988). Table 1 summarizes studies from several countries in which *Toxoplasma* was isolated from 3 to 68% of mutton specimens, collectively demonstrating the high prevalence of infection in sheep meat. It is generally concluded that *Toxoplasma* does not survive after smoking, curing or deep freezing of mutton, but can remain infective after microwaving, possibly due to uneven heating (Lunden and Uggla, 1992). However, *Toxoplasma* cysts have been recovered from frozen meat (Dubey and Kirkbride, 1989), viable *Toxoplasma* has been discovered in cured meat (Warnekulasuriya et al., 1998), and consumption of cured

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Table 1
Isolation of *Toxoplasma gondii* from mutton in several countries.

Country	No. sheep examined	% isolation	Reference
Australia	32	25	Munday (1970)
Brazil	495	4.7	Ragozo et al. (2008)
Denmark	31	22	Work (1967)
France	50	16	Dumètre et al. (2006)
Germany	50	12	Janitschke et al. (1967)
Germany	461	4.8	Boch et al. (1979)
India	67	2.9	Gupta et al. (1979)
Iran	40	3.5–50[a]	Zia-Ali et al. (2007)
Japan	26	11.5	Maitani (1970)
Japan	17	29.4	Hagiwara et al. (1970)
Japan	26	26.9	Hagiwara et al. (1978)
New Zealand	34	67.6	Jacobs et al. (1963)
Norway[b]	186	37	Waldeland (1976)
Rumania	1340	9	Pop et al. (1989)
UK	9	67[c]	Aspinall et al. (2002)
Uruguay	1613	28.7[d]	Freyre et al. (1999)
USA	50	4	Remington (1968)
USA	383	21	Dubey et al. (2008)

[a] Depending on the antibody titers.
[b] Waldeland (1976) calculated that *T. gondii* was present in 25–37% of the carcasses from mature sheep and in 10–15% of the lambs carcasses in Norway.
[c] *Toxoplasma* identified by PCR.
[d] Seroimmune prevalence.

meat is a risk factor for congenital toxoplasmosis (Cook et al., 2000).

The use of a vaccine to prevent the infection of meat animals with *Toxoplasma* is desirable, so as to reduce the rate of human *Toxoplasma* infection from the ingestion of undercooked infected meat. A vaccine against toxoplasmosis abortion in sheep is commercially available in New Zealand and the UK (Buxton et al., 1991), but it has not been demonstrated to prevent formation of cysts of *Toxoplasma* in the ewe or in its offspring. It has been shown that 52% of 18 pigs vaccinated with a cyst-deficient *Toxoplasma* strain, had no demonstrable tissue cysts after the challenge infection (Kringel et al., 2004) and that a DNA vaccine primes anti-*Toxoplasma* immune responses in pigs, but protection studies are pending (Jongert et al., 2008).

In previous experiments of premunition against acquired toxoplasmosis in a rat model (Freyre et al., 2006), we tested protection conferred by two strains of *Toxoplasma* against brain colonization, and obtained high rates of protection with one of them (Freyre et al., 2006). Evidence has been advanced that rats immunized with the ME-49 strain of *Toxoplasma* do not form muscle cysts when later challenged with the M-7741 strain of this parasite (Freyre et al., 2008). The purpose of the present investigation was to determine if the findings with the rat model could be extended to sheep for the prevention of *Toxoplasma* encystment in meat.

2. Materials and methods

2.1. Experimental design

Prototype immunization of lambs was performed by inoculating them with a strain of *Toxoplasma* of very low pathogenicity for mice (ME-49), and then challenging the lambs with a highly pathogenic strain of *Toxoplasma* (M3). Next, mice consumed muscles from the lambs, to determine the rate of protection achieved in the lambs—if M3 cysts developed in the lambs' muscles, then mice would become infected with this pathogenic strain and high mortality would result; however, if vaccination prevented formation of M3 cysts in lambs, then mice would not be exposed to the pathogenic M3 strain and little or no mortality would occur. It was previously tested if mice survive after eating muscle samples of lambs that had received known amounts of oocysts of strain ME-49. Previous experiments have also shown that ingestion of 5 or more M3 tissue cysts causes mortality in CF-1 mice (Freyre et al., 2008). The present investigation required us to first determine a dose of M3 oocysts to administer to lambs that would subsequently cause high mortality in mice that consumed muscle and brain from the infected lambs.

The experiments performed comply with current laws of Uruguay.

2.2. Animals

Sera from 3-month old, 20 kg Corriedale lambs were screened for *Toxoplasma* antibodies with the direct agglutination (DA) test of Desmonts and Remington (1980) at a final dilution of 1:64. Twenty-nine seronegative lambs were selected for the study. CF-1 mice were *Toxoplasma* seronegative (using DA reaction) and weighed 20 g. Recently weaned cats with a negative DA test (1:64 dilution) were used to produce oocysts. They were from the cat colony of the Laboratory for Toxoplasmosis of the College of Veterinary Sciences of Montevideo.

2.3. Strains of Toxoplasma

Toxoplasma gondii strains ME-49 and M3 were used in the present study (Freyre et al., 2004). They are of low and high pathogenicity for mice, respectively, and belong to genotype II (Howe and Sibley, 1995; M.L. Dardé personal communication). Strain Me-49 was isolated from sheep muscle in California in 1960 (Dubey et al., 1995). Strain M3 was isolated from sheep abortion in Scotland (Buxton et al., 1991).

2.4. Toxoplasma gondii oocysts

Each cat consumed the brain and the carcass of a mouse with a chronic *Toxoplasma* infection. Fecal specimens were collected 4–7 days after inoculation and oocysts were concentrated with the method of Sheather (Frenkel, 1977). They were incubated at room temperature with agitation in 2% sulfuric acid for 4 days. After sporulation, they were stored in the refrigerator until used. Oocysts suspensions were neutralized with a solution of NaOH, counted in a hemocytometer and diluted in water if necessary. The lambs were fed the stated number of oocysts in a volume of 20 ml (Table 2).