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Congenital Toxoplasmosis in Wild Boar (*Sus scrofa*) and Identification of the *Toxoplasma gondii* Types Involved

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ABSTRACT: Congenital toxoplasmosis has been little described in wild animals. We report a case of vertical transmission in wild boar (*Sus scrofa*). Necropsy and histopathologic examination of a pregnant female and her three fetuses revealed all to have lesions compatible with acute toxoplasmosis. Nested polymerase chain reaction B1 gene detected *Toxoplasma gondii* in maternal (heart and diaphragm) and fetal (central nervous system, retina, optic nerve, heart, lung, tongue, and diaphragm) samples. The mother had a mixed infection of *T. gondii* types I and III. One fetus with type III infection developed no malformations, but the others—one with type I infection and one infected by types I and III—showed bilateral ocular agenesis, prognathism, and agenesis of the nasal cartilage. These results suggest the pathogenicity of the various *T. gondii* types may differ in wild boars.

Key words: Congenital, genetic characterisation, *Toxoplasma gondii*, vertical transmission, wild boar.

Toxoplasmosis is a worldwide zoonosis that affects many mammalian species. Its pathology depends on factors such as the susceptibility of the host species and the virulence of the infecting strain. The course of the disease is usually benign, but occasionally is associated with miscarriages, fetal malformation, and encephalitis. Few studies have been performed on the clinical manifestations and lesions caused by *Toxoplasma gondii* in wild animals. Episodes of clinical toxoplasmosis have been reported in macropods (Johnson et al., 1989; Bermúdez et al., 2009), wombats (*Vombatus ursinus*; Hartley, 2006), black-footed ferrets (*Mustela nigripes*; Burns et al., 2003), bobcats (*Lynx rufus*; Dubey et al., 1987; Smith et al., 1995), Pallas cats (*Otocolobus manul*; Basso et al., 2005), and white-tailed deer (*Odocoileus virginianus*; Dubey et al., 2008), but until now, no clinical cases have ever been reported in wild pigs (Dubey, 2009). We describe a case of natural congenital transmission of *T. gondii* in a gestating wild boar (*Sus scrofa*) that caused fetal malformations and identify the *T. gondii* types involved.

A 5-yr-old, 70-kg, female wild boar was killed by hunters on a fenced hunting estate in the Sierra de San Pedro mountain range (Province of Cáceres, southwestern Spain; 39°37′37″N, 7°11′30″W). Wildlife on the estate is free to feed on corn-based dietary supplements and is provided with watering holes. The hunted female was 2.5 mo into gestation (last trimester of pregnancy). Following a health inspection the animal was subjected to necropsy and histopathologic examination. Blood, diaphragm, and heart muscle samples were taken from the mother, and a wider range of samples (blood, brain, tongue, lung, eyes, heart, and diaphragm) was taken from the fetuses. Reproductive disease was suspected given the small number of fetuses carried—three instead of the normal four–eight—and the malformations shown by two of these (Fig. 1). Appropriate samples from the mother and fetuses were fixed, paraffined, stained with hematoxylin-eosin (H&E) (Isokit, Bio-Optica, Milano, Italy), and examined by microscopy for *T. gondii* cysts.

IgG anti-*Toxoplasma* antibodies were detected in maternal serum with the use of an indirect enzyme-linked immunosorbent assay (ID Screen® Toxoplasmosis

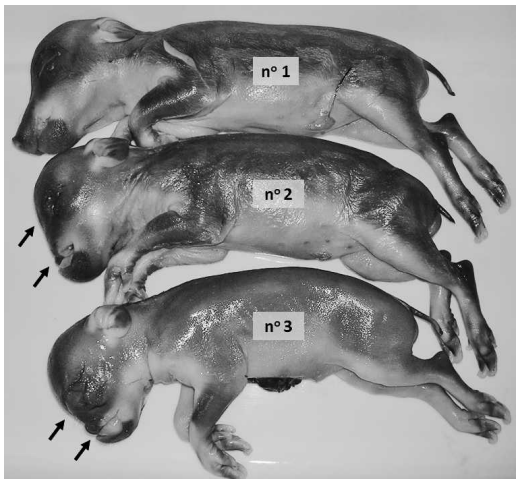

FIGURE 1. Fetuses removed from a female wild boar infected with *Toxoplasma gondii* in Spain. Fetus 1 (male; weight: 586.1 g), with no apparent macroscopic defects; fetus 2 (male; 504.4 g), and fetus 3 (male; 366.8 g) show significant abnormalities, including bilateral ocular agenesis, agenesis of the nasal cartilage, and prognathism.

Indirect, IDVET, Montpellier, France) according to the manufacturer's instructions (cutoff: 50% reactivity). Direct detection of the parasite was undertaken in fetal (central nervous system, retina, optic nerve, heart, lung, tongue, and diaphragm) and maternal (heart and diaphragm) tissues. All tissues (5 g) were digested with trypsin for 1 hr according to Erber (1977). DNA was extracted from 600 μL of the digest using the QIAamp Mini Kit (Qiagen, Cortaboeuf, France), and subjected to nested-polymerase chain reaction (PCR) (Fuentes et al., 1996). *Toxoplasma* were genotyped by nested-PCR-restriction fragment length polymorphism amplification of the SAG1, SAG3, GRA6 and BTUB genes as reported by Su et al. (2006) and Boughattas et al. (2010), and of the SAG2 gene as reported by Howe et al. (1997).

Necropsy revealed that the mother was in good body condition, although she carried fewer fetuses than normal. No gross abnormalities were recorded in fetus 1, which was of a normal size for the point in gestation reached when the mother was killed. The other two fetuses showed prognathism, agenesis of the nasal cartilage, oronasal communication, and bilateral ocular agenesis; they were also smaller and lighter than normal (Fig. 1). Histopathologic examination showed all three fetuses had marked interstitial pneumonia; in some cases the alveolar lumens were completely collapsed (Fig. 2A). The inflammatory component was mostly lymphocytes. Signs of pleurisy were evident, and all fetal lung samples showed clear interstitial edema. Bacteria were also detected in the blood vessels, which showed signs of necrosis (Fig. 2B).

The eyes, which had developed in only fetus 1, showed almost total loss of the cornea. Interstitial myositis was apparent, along with the possible remains of tachyzoites, in the ocular musculature (Fig. 2C); the inflammatory component was mainly lymphoplasmocellular. Similar inflammatory lesions were seen in the skeletal and cardiac muscles of the mother (Fig. 2D).

The mother had a high titer of anti-*Toxoplasma* IgG antibodies (percentage reactivity 260%). The PCR detected *T. gondii* DNA in all maternal and fetal samples except for central nervous system tissue samples of fetus 1. *Toxoplasma* genotyping showed the mother to have a mixed infection of *T. gondii* types I and III. Fetus 1 had a *T. gondii* type III infection, fetus 2 a mixed infection involving types I and III, and fetus 3 was infected with *T. gondii* type I (Table 1).

Our results confirm congenital transmission of *T. gondii* in wild boar in Spain. Experimental congenital transmission has been reported in domestic pigs and mice (Dubey and Urban, 1990; Rejmanek et al., 2010), as have descriptions of clinical toxoplasmosis acquired via natural infection in domestic pigs. However, this is the first gross and histopathologic description of the disease in wild boar; the absence of such a description was noted by Dubey (2009). Congenital transmission of *T. gondii* in domestic pigs is not common (Venturini et al., 1999).

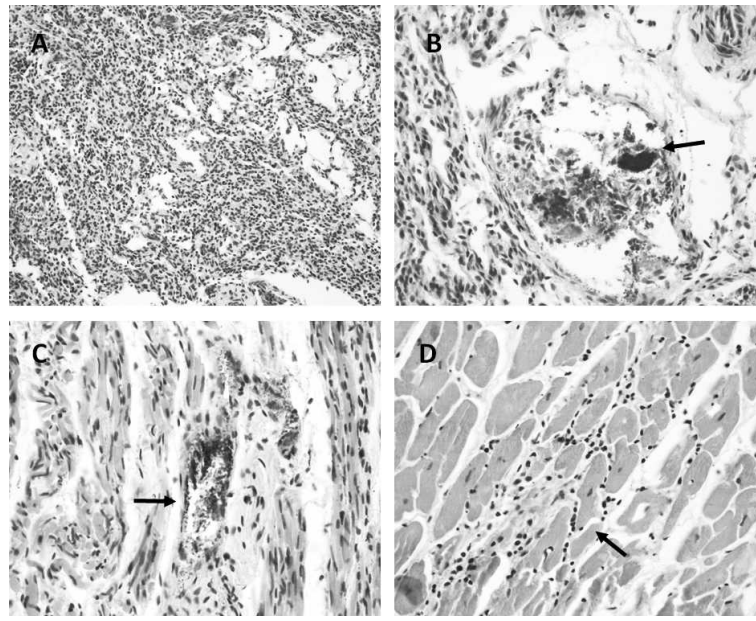

FIGURE 2. Histopathologic changes associated with *Toxoplasma gondii* infection in wild boar tissues in Spain. (A) Fetus 2. Lung. Interstitial pneumonia with a predominantly lymphocytic inflammatory component. H&E, ×200. (B) Fetus 3. Lung. Thrombus and bacterial elements in the lumen of a blood vessel. H&E, ×400. (C) Fetus 1. Eye. Focus of necrosis with possible traces of tachyzoites in the associated muscles. H&E, ×400. (D) Sow. Cardiac muscle. Note the interstitial lymphocytic infiltrate. H&E, ×400.

The litter carried by the sow was small, possibly due to fetal reabsorption. *Toxoplasma gondii* infection may therefore reduce the reproductive output of sows. Wild boar sows generally deliver four–eight piglets, a number expected from the mother given her good body condition and habitat (a fenced estate that provides supplementary feed for wildlife).

The histopathologic results suggest the sow and fetuses were suffering from acute *T. gondii* infection. This is indicated by signs of myositis, the necrosis seen in many samples, and the interstitial pneumonia in the fetuses. Similar signs have been reported in experimentally infected domestic pigs (Dubey et al., 1990). The apparent absence of tissue cysts that could

TABLE 1. Molecular diagnosis and identification of *Toxoplasma gondii* types infecting a wild boar (*Sus scrofa*) and her three fetuses in Spain. Dashes indicate no amplification.

Sample		Detection	Genotyping					
Individual	Tissue[a]	nPCR (B1 gene)	SAG1	(5′+3′) SAG2	SAG3	GRA6	BTUB	Type
Sow	Heart	+	I	I/III	—	I+III	I+III	I+III
Fetus 1	Heart	+	II/III	III	III	III	III	III
Fetus 2	Heart and CNS	+	—	I	—	I+III	—	I+III
Fetus 3	Heart and CNS	+	I	I/II	—	I	—	I
Reference RH	Mouse	+	I	I	I	I	I	Clonal I
Reference Me49	Mouse	+	II	II	II	II	II	Clonal II
Reference C56	Mouse	+	III	III	III	III	III	Clonal III

[a] CNS = central nervous system.

not be detected by histopathologic examination is consistent with that reported by García et al. (2006) in similar experiments, who demonstrated the infection by bioassay in mice. The malformations (prognathism, agenesis of the nasal cartilage and eyes) in two of the three fetuses would have compromised their chances of postnatal survival.

The parasite was detected by PCR in almost all the tissues of the fetuses and the sow. The mother showed a mixed infection involving *T. gondii* types I and III. In the only other study of *T. gondii* infection in wild boar, from France, a type II infection was detected, but no pathologic description was carried out (Richomme et al., 2009). Other authors have reported mixed infections in sheep (Berger-Schoch et al., 2011) and foxes (Herrmann et al., 2012). Fetus 1, which developed apparently normally, was infected with *T. gondii* type III, which appears to be less pathogenic than the others in several species (Dubey et al., 2004). Fetuses 2 and 3, however, which showed significant malformations, were both infected with *T. gondii* type I. This is thought to be a more virulent type in several species, although infection is believed to be rare in wild European animals (Aubert et al., 2010; De Craeye et al., 2011). Type I has also been commonly associated with congenital toxoplasmosis in humans in Spain (Fuentes et al., 2001). Like the sow, fetus 2 was also infected with type III. These findings suggest that the pathogenicity of toxoplasmosis in wild boar is related to the infecting *T. gondii* type.

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