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Interferon-gamma expression and infectivity of *Toxoplasma* infected tissues in experimentally infected sheep in comparison with pigs

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ABSTRACT

Livestock animals are a potential risk for transmission of toxoplasmosis to humans. Sheep and pigs still remain an important source because their meat is often eaten undercooked which has been regarded as a major route of infection in many countries. Moreover, porcine tissues are processed in many food products.

In the current study, the IFN-gamma (T-helper 1 cells), IL-4 (Th2 cells) and IL-10 mRNA (Treg cells) expression by blood mononuclear cells, and the serum antibody response against *Toxoplasma gondii* total lysate antigen, recombinant *T. gondii* GRA1, rGRA7, rMIC3 and rEC2, a chimeric antigen composed of MIC2, MIC3 and SAG1, was studied in sheep the first two months after a *T. gondii* infection and compared with these responses in pigs. At the end of this period, the parasite distribution in heart, brain and two skeletal muscles in sheep was compared with this in pigs.

Whereas the parasite distribution was similar in sheep and pigs, the antibody response differed considerably. In sheep, antibodies appeared against all tested *T. gondii* antigens, but mainly against rGRA7, $rMIC3_{234307}$ and TLA whereas in pigs only rGRA7-specific antibodies could be demonstrated. Also, the cytokine response differed. Both in sheep and pigs an IFN-gamma response occurred which seemed to be a slightly more pronounced in sheep. In sheep, also IL-10 and IL-4 mRNA expression showed an increase, but later than IFN-gamma and with more variation. However, in pigs no such increase was seen.

As concerning diagnosis, results indicate that serum antibodies against GRA7 in live sheep and pigs and heart tissue for bioassay and qPCR in slaughtered animals are the best targets to demonstrate presence of *T. gondii* infection.

1. Introduction

Toxoplasma gondii, an obligate intracellular protozoan parasite, is one of the most common parasitic zoonosis worldwide (Tenter et al., 2000). The parasite can establish a chronic infection in animals and humans. By

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estimation, 5×10^8 people worldwide or one third of the world population has been infected (Denkers and Gazzinelli, 1998; Montoya and Liesenfeld, 2004). Livestock animals are a potential risk for transmission of toxoplasmosis to humans. Consumption of raw or undercooked meat has been regarded as a major route of infection in many countries (Cook et al., 2000; Kijlstra and Jongert, 2008). Seroprevalence studies showed that in certain countries 90% of the sheep are infected (Dubey, 2009). In contrast the seroprevalence in pigs is very low, almost zero (Kijlstra et al., 2004). Nevertheless sheep and pigs still remain an important source because their meat is often eaten undercooked and porcine tissues are incorporated into many food.

Following oral ingestion of oocysts/tissue cysts by sheep, the parasites transform into tachyzoites, which invade the small intestine, where they rapidly replicate in the epithelial cells. Simultaneously, they spread to other gut associated tissues. This is accompanied with the induction of cellular and humoral immune responses. Cellular responses occur first with interferon gamma (IFN-gamma) responses peaking in mesenteric lymph nodes and splenocytes 4 days post infection and IL-12 responses in mesenteric lymph nodes most-likely as a result of innate immune responses (Verhelst et al., 2014a). A second peak occurs 1–3 weeks later and is most-likely due to T-cell responses (Verhelst et al., 2014a). These T-cell responses are necessary for the survival of the host and as a result the tachyzoite stage of the parasite transforms into the bradyzoite stage, which escapes the immune system and persist in a cyst form in the host tissues (Denkers and Gazzinelli, 1998). Since the parasite becomes undetectable in most intestinal and systemic lymphoid tissues around 3 weeks post infection (Verhelst et al., 2014a), we postulated that this is the result of clearance of the parasite from these tissues by an immune response. Tissue cysts appear 7–10 days post infection in visceral organs such as lungs, liver and kidneys but predominantly in the central nervous system and in muscle tissue, where they stay for the entire host's life (Black and Boothroyd, 2000).

The objectives of the present study were to determine the distribution of the parasite and to assess the status of the adaptive immune response in sheep 4–8 weeks post infection, which is shortly after the parasite left the intestinal tissues. Furthermore we wanted to compare the immune response in sheep with this in pigs. Differences in antibody and cytokine responses against different antigens could reflect differences in host pathogen interaction. Furthermore we aimed to obtain information on the optimal antigen(s) target for sero-diagnosing infection in both species.

2. Materials and methods

2.1. Parasites

T. gondii Prugniaud (PRU) for infecting the sheep (Martrou et al., 1965) and IPB-G strain for the pig study (Vercammen et al., 1998) are routinely maintained by mouse passage. Both type II strains were previously used in sheep (Isabelle Moiré, personal communication) and pigs (Vercammen et al., 1998), and were harvested from the brains of chronically infected Swiss mice. Mice were euthanized by cervical dislocation, and *T. gondii* brain cysts were counted under a microscope. For experimental infection, the mouse brain homogenates were diluted in PBS at a concentration of 300 cysts/ml.

2.2. Animals

Four adult Belgian cross-breed sheep and six indoor-born Belgian Landrace pigs were weaned at an age of 4 weeks and housed in isolation units. The animals were *T. gondii* seronegative as determined by indirect immunofluorescence assay (IIFA) (described in Section 2.4). Three sheep were orally infected with 3000 tissue cysts of the *T. gondii* PRU strain and five pigs with 3000 tissue cysts of the *T. gondii* IPB-G. The remaining sheep and pig served as negative control and were each given orally half a brain of a non-infected mouse.

All pigs were bled weekly from infection till euthanasia, 6 weeks post infection (PI). Sheep were bled before infection and weekly from 4 weeks PI till euthanasia, 8 weeks PI. In a previous study on sheep the antibody response had been determined on sera sampled daily from infection till 3 weeks PI (Verhelst et al., 2014a). Euthanasia was performed by intravenous injection of an overdose natriumpentobarbital 20% (Kela, Hoogstraten, Belgium).

Animal experimentation was performed with the prior approval of the animal ethics committee of the faculties of Bioscience Engineering and Veterinary Medicine (EC 2007/103).

2.3. Detection of parasites by bio-assay and real-time quantitative PCR

In order to determine whether parasite load was different between tissues, the numbers of bradyzoites were determined by qPCR in brain, heart, mgastr and mlongd samples for sheep and pigs. The infectivity of the tissues was further evaluated by bioassay. After euthanasia of the animals, 100 g of brain, heart, musculus gastrocnemius (mgastr) and musculus longissimus dorsi (mlongd) were collected from each animal for detection of parasites by bioassay and quantitative real-time PCR (qPCR). Hereto, the tissues were homogenized in 15 ml 0.9% NaCl, and the tissue suspensions were incubated 1–2 h in a 250 ml acidic pepsin solution (0.8 g l^{-1} pepsin and 7 ml l^{-1} HCl) at 37 °C. Thereafter the suspensions were filtered, centrifuged at 1180 × *g*, and the pellets were resuspended in 5 ml PBS with 0.04% gentamicin. Eighty-five μl of each suspension was used for qPCR quantification and the rest was used for the bioassay. For the latter one, 5 mice were intraperitoneally inoculated with 1 ml tissue suspension. Lungs and brains of mice that died from acute toxoplasmosis were examined for *T. gondii* cysts by phase-contrast microscopy and for *T. gondii* DNA by qPCR (Verhelst et al., 2011). From surviving mice, serum was sampled at day 45 PI for *T. gondii* antibody detection with the IIFA whereafter mice were euthanized. Their brain tissues were sampled and processed for