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Research paper

Maternal immune response in the placenta of sheep during recrudescence of natural congenital infection of *Neospora caninum*

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ABSTRACT

In order to gain further insight into the pathogenesis and transmission of ovine neosporosis, the serological response of 13 naturally infected pregnant sheep was monitored. All sheep were euthanized upon the detection of a sharp increase in the level of specific antibodies against *N. caninum* in order to study the maternal immune response after the recrudescence of a chronic infection. Ten sheep were euthanized between 84 and 118 days of gestation, whereas the three remaining and three control not infected, pregnant sheep were euthanized at 135 days of gestation after no sharp increase in antibodies was detected. Vertical transmission was confirmed in 11 sheep by detection of *N. caninum*-DNA in at least one fetus, confirming recrudescence. Not all of fetuses showed pathologic microscopic lesions, however, multifocal non-purulent encephalitis was the main finding. Furthermore, nine out of the 11 vertical transmission positive sheep had lesions in placentomes (mainly multifocal necrotic foci), and the parasite was detected in eight out of 11 placentas by PCR and/or immunohistochemestry. The placentomes from sheep that suffered recrudescence showed an increased number of T lymphocytes CD3+ (CD4/CD8 < 1) and macrophages (MHC-II+), assessed by immunohistochemestry, together with an up-regulation of IFN-γ, IL-10, IL-4, TNFα, IL-2 and IL-18. IL-17 was only upregulated in the three infected sheep that did not have a sharp increase in antibody levels. In the sheep that showed fetal death at the time of euthanasia ($n = 3$) the placental microscopic lesions were more severe, the inflammatory infiltrate was higher, and the upregulation of cytokines was greater than in those sheep carrying viable fetuses. This study suggests that, similarly to bovine neosporosis, the time of gestation when recrudescence occurs determines the viability of the fetuses and, thus, seems to be related to the severity of lesions and immune response in the placenta. These results suggest that there might be a correlation, either as cause or as a consequence, between protection against vertical transmission of the parasite and a milder maternal serological response together with a high level of transcription of IL-17 in the placenta.

1. Introduction

Neospora caninum is a protozoan parasite considered as one of the main causes of abortion in cattle worldwide (Dubey et al., 2007). Since Dubey et al. (1990) described *N. caninum* in a naturally infected lamb, the number of reports of ovine neosporosis has notably increased worldwide (Bártová et al., 2009; González-Warleta et al., 2014; Díaz et al., 2016; Amdouni et al., 2018; Hecker et al., 2019; Villagra-Blanco et al., 2019). In fact, recent studies consider neosporosis an important abortifacient disease in sheep with a still unknown economic impact (Moreno et al., 2012; Romo-Gallegos et al., 2019). Cattle, and presumably sheep, become infected by the ingestion of food and/or water contaminated with *N. caninum*-oocysts shed by canids (exogenous transmission), or by transplacental transmission during gestation (endogenous or vertical transmission). Vertical transmission is considered the most efficient and the most common infection route (Trees and

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