WIADOMOŚCI PARAZYTOLOGICZNE 2005 • tom 51 • suplement • s. 61–62

Bovine neosporosis; pathogenesis of abortion and immunity

David Buxton[1], Willem Wouda[2] and J. P Dubey[3]

[1]Moredun Research Institute, Edinburgh, Scotland
[2]Animal Health Service Ltd, Deventer, The Netherlands
[3]Animal Parasitic Diseases Laboratory, USDA, Beltsville, USA

Abstract

Bovine neosporosis is a disease of cattle caused by infection with the protozoan parasite *Neospora caninum*, which may lead to fetal death and abortion. Infection of the placenta and fetus follows a maternal parasitaemia, triggered either as the result of a primary (exogenous) maternal infection or following recrudescence of a persistent (endogenous) infection during pregnancy. In the latter, which is the more common of the two, disease follows reactivation of a persistent infection, while a primary infection occurs after the ingestion of sporulated *N. caninum* oocysts. These then release sporozoites into the gut lumen to parasitise the intestinal epithelium, transform into tachyzoites and undergo a phase of multiplication, possibly in the mesenteric lymph nodes. From here they are released into the blood (parasitaemia). In non-pregnant cattle infection is rapidly controlled, largely by cell mediated immune (CMI) mechanisms, with cytotoxic T lymphocytes likely to have a significant protective role. This degree of protection is carried into the early stage of pregnancy but around midgestation immunity appears to be modified, with *in-vitro* tests showing a down regulation of cellular responses to mitogen, a reduction in cell proliferation in response to specific *N. caninum* antigen and a corresponding reduction in IFNγ production. These effects then gradually return to pre-pregnancy values through the rest of pregnancy.

A parasitaemia in pregnant cattle, triggered either by a primary infection or the recrudescence of a persistent infection, will allow the parasite to invade the gravid uterus where it can establish itself in the maternal caruncular septa before crossing to the fetal placental villi. Thereafter the development and progression of lesions is influenced by the stage of gestation.

The gestation period in cattle is around 280 days, and the fetal immune system develops progressively throughout. In the first trimester, the fetus is exceptionally vulnerable to a *N. caninum* infection, and is unlikely to survive. In the middle third of pregnancy fetuses may be able to mount an immune response to the parasite but this may or may not be sufficient to save it. In the third trimester the fetus is capable of an increasingly competent defence against the pathogen, aiding survival. Endogenous transplacental transmission is more likely to occur later in gestation with, in the majority of cases, the birth of clinically normal, infected calves.

In mammals, complex immunological mechanisms have evolved to allow the dam to nurture a fetus that is genetically a "foreign body" (allograft) rather than to reject it, and as such a very precise maternal and fetal immunological balance pertains in the placenta. Central to this process are cytokines, soluble mediators secreted locally that allow the producing cell to exert a powerful local effect on certain other cells of lymphoid and non-lymphoid origin. During pregnancy maternal immune responses in the placenta are modified to favour a micro-environment dominated by "beneficial" cytokines such as the haemopoietic cytokines (colony stimulating factor-1 [CSF-1] and granulocyte-macrophage CSF [GM-CSF]), the regulatory cytokines (transforming growth factor beta [TGF-ß] and interleukin-10 [IL-10]) and the Th2-type cytokines (interleukins 4 and 5). Intracellular pathogens, such as *N. caninum*, stimulate cell mediated immune responses which in turn invoke cytokines that may be harmful to pregnancy such as the Th1-type (inflammatory) cytokines, interferon

gamma (IFN-γ) and interleukin-2 (IL-2) and the proinflammatory cytokine tumour necrosis factor-alpha (TNF-α). If the stimulus from *N. caninum* infection is sufficient it is suggested that their production will not be adequately suppressed by the beneficial cytokines, the balance will tip in their favour, they will terminate pregnancy and trigger abortion. In some instances therefore, relatively small numbers of the parasite, causing relatively little local damage, may cause a very considerable effect by locally eliciting cytokines that endanger pregnancy. Similarly, placental infection and inflammation may trigger prostaglandin-induced luteolysis causing premature uterine contraction and fetal expulsion. To what extent these two mechanisms or other processes, including direct fetal damage, overlap or interact is not known.

In experimental infections the most severe lesions are normally found in the placenta and brain of the fetus. *N. caninum* causes focal destruction in both maternal and fetal tissue at the maternofetal interface and elicits a largely non-suppurative inflammatory response. The earliest lesions in cows injected with tachyzoites at 70 days gestation were seen 14 days later and consisted of necrosis of fetal placental villi and non-suppurative inflammation in the maternal septa. Preliminary analysis has shown that the influx of maternal inflammatory cells is composed in large part of $CD4^+$, $CD8^+$ and γδ T-cells and in situ hybridization has shown a proportion of the cells in the infiltrate to be capable of producing IFN-γ. This supports the hypothesis that in some cases, fetal death may be less a direct result of parasite replication and more due to the maternal immune response, triggered by the parasite. In pregnant cattle experimentally injected at 140 dg, lesions were shown to develop and then regress. In parallel studies, that used the same inoculum, cattle were allowed to go on and calve. These cows produced live calves that were congenitally infected with *N. caninum*. Descriptions of the natural disease in later pregnancy indicate that a nonsuppurative placentitis may extend out into the intercotyledonary chorioallantois.

Coincidental with the onset of placental infection the parasite enters the fetal bloodstream and invades further tissues, including the CNS where it initially locates in and around blood vessels. In the younger fetus, its uncontrolled multiplication can cause lethal widespread destruction of the neuropil but in older fetuses multiplication is more restricted, and necrosis is confined to small foci of damage surrounded by a relatively intense fetal inflammatory response involving microglia, reactive astrocytes and cells of the monocyte and lymphoid series. Mild meningitis may also be present. Lesions may also occur in the heart, skeletal muscle, lung and liver.

Thus *N. caninum* is a primary pathogen that is capable of causing abortion either through maternal placental inflammation, maternal and fetal placental necrosis, fetal damage or a combination of all three. The parasite is passed from mother to daughter with ease and while only in a minority of cases does it cause fetal death, these losses are of considerable economic significance to farmers.