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Immunodiagnosis of *Haemonchus contortus* infection in sheep by indirect enzyme linked immunosorbent assay (ELISA)

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Indirect plate enzyme-linked immunosorbent assay was standardized and evaluated for its effectiveness in immunodiagnosis of haemonchosis in experimental and clinical cases in sheep by using somatic whole adult antigen of *H. contortus*. Plate ELISA was standardized using 5 µg/well antigen concentration with 1:100 and 1:1000 of sera and conjugate dilution. Indirect plate ELISA was able to demonstrate the antibody titer at different weeks post infection in experimental sheep. A comparison of plate ELISA on suspected field sera and faecal sample examination by floatation method revealed that 74 samples were found to be positive by ELISA but only ten by faecal examination. Sensitivity of plate ELISA was found to be 80.0%, whereas specificity was 21.42% indicating that this test is quite sensitive for clinical cases; an early diagnosis, however, lacks specificity.

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Listeria moncytogenes induces inflammasome activation of macrophages regulating trophoblast invasion and immune function

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To investigate the molecular mechanism of inflammasome activation in Listeria monocytogenes (LM)-infected macrophages and the regulatory effects of secretory products of LM-infected macrophage on trophoblast invasion and immune functions, the mouse B6 macrophages and its derived NLRP3-/- B6 macrophages were infected with wild-type LM and Listeriolysin O-/- LM (LLO-/- LM), respectively. IL-1β concentration in the medium, caspase 1 and pro-IL-1β and TNFα mRNA in the B6 macrophages were detected by ELISA, western blot and RT-PCR, respectively. Apoptosis was detected by Annexin V/PI double staining. Then the mouse SM9-1 trophoblasts were treated by the secretory products of LM-infected macrophages. The trophoblast invasion and immune functions were detected in vitro by cell invasion assay and measurements of matrix Metalloproteinases (MMPs), monocyte chemotactic protein-1 (MCP-1) and TNFα and IL-6 by RT-PCR. Results showed that the wild-type, not LLO-deficient LM, could significantly induce the IL-1β release, caspase 1 maturation and pyroptosis of the B6 cells, but not NLRP3-/- B6 cells, indicating that LM, not LLO-/- LM, could activate NLRP3 inflammasome of mouse macrophages by its LLO. Regarding the regulation of LM-infected macrophage on trophoblasts, our results showed that LM-infected macrophage products, as well as recombinant IL-1β, could significantly facilitate trophoblast invasion, increase MMP-2, MMP-9 MMP-26 expressions, and up-regulate pro-inflammatory cytokine TNFα and IL-6 expressions and down-regulate MCP-1 expression of mouse trophoblasts in vitro, which indicate that the macrophage secretory products, mainly IL-1β can facilitate trophoblast invasion by increasing MMP expressions, and regulate trophoblast immune functions, including modulating monocyte/macrophage recruitments by inhibiting MCP-1 expression and shaping the inflammatory reaction by improving pro-inflammatory cytokine production. Above results indicate that LM can activate macrophage NLRP3 inflammasome and promote cell apoptosis by its LLO, the inflammasome activation pathway-mediated IL-1β release from LM-infected macrophages plays a crucial role in regulating trophoblast invasion and immune functions during LM infection.

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