



Title	Development of latex agglutination test for the detection of <i>Echinococcus multilocularis</i> coproantigens in the definitive hosts
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Citation	Japanese Journal of Veterinary Research, 48(1), 68-69
Issue Date	2000-05-31
Doc URL	http://hdl.handle.net/2115/2824
Type	bulletin (article)
File Information	KJ00003408158.pdf



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CD 4- and CD 8-positive cells were constantly lower in the peripheral blood of the susceptible line of chickens after 10 days pi, the severity of this continuous depression may also be a factor to determine susceptibility to MD.

The expression of cytokines, interleukin-2 (IL-2), interferon- γ (IFN- γ) and chicken myelomonocytic growth factor (cMGF) which is an avian homolog of mammalian interleukin-6, were also analyzed in both resistant and susceptible chickens by reverse-transcriptase polymerase chain reaction. The expression levels of IL-2 and IFN- γ appeared to be equal or suppressed compared to uninfected chickens followed by the enhancement

at 4 to 7 days pi., probably resulting from the immune suppression caused by MDV. Although the level of IFN- γ expression was higher in susceptible than in resistant chickens during MDV infection, the relationship between the level of IFN- γ expression and susceptibility to MD was not clarified in this study. No significant difference in the expression level of cMGF was observed between the two lines except for 4 days pi.

Thus, it would be necessary to study the differences between resistant and susceptible chickens for understanding the mechanism of lymphomagenesis.

Development of latex agglutination test
for the detection of *Echinococcus multilocularis*
coproantigens in the definitive hosts.

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In this study, the development of a simple and rapid latex agglutination test for the detection of *Echinococcus multilocularis* coproantigens in the definitive hosts was attempted. The optimal size of polybead carboxylated microspheres (latex beads), optimal monoclonal antibody and its reaction concentration for the sensitization of the latex beads were evaluated. It was determined that slide agglutination test was best performed using the latex beads (1.0 μ m in diameter) sensitized with 50 μ g/ml of EmA 9 monoclonal antibody raised against somatic antigen of adult *E. multilocularis*. Different kinds of dilution buffer for the fecal samples were also evalu-

ated, and 1% Tween 20 in PBS was selected. The latex agglutination test (Test 1) was performed on 35 non-heated and 82 heated feces of wild foxes, resulting in 47% and 61% in sensitivity, and 94% and 86% in specificity, respectively. By heating fecal samples to inactivate parasite eggs, higher sensitivity was obtained but specificity was reduced. To improve the sensitivity of the coproantigen detection, a small amount of excretory/secretory antigen of adult *E. multilocularis* (EmES antigen) was added to dilution buffer of feces (Test 2). By addition of EmES antigen, the sensitivity increased to 82% and 91%, but the specificity decreased to 44% and 61%, respec-

tively. Higher sensitivity and specificity were obtained by heating the fecal samples.

The positive predictive value of Test 1 and negative predictive value of Test 2 were 89% and 73% for the non-heated and 85% and 85% for the heated samples, respectively, thus the results can be accepted. All of the positive samples in Test 1 was also positive in Test 2. Considering the negative samples in Test 1, 56% and 47% of the samples turned to be positive, of which 43% and 61% were true worm positive for non-heated and heated samples, respectively. Therefore, those samples which were negative in Test 1 and positive in Test 2 were classified as suspicious and further tested by the more specific sandwich

ELISA developed in our laboratory. As a result, sensitivity and specificity of this combination judgment became 71% and 94% for the non-heated and 87% and 86% for the heated samples, respectively. If only the samples with ≥ 100 worm burden were taken, the sensitivity were 100% in either Test 2 or combination judgment for the heated samples.

Test 1 and Test 2 and their combination judgment revealed different spectra of sensitivity and specificity. More samples should be tested by each test for precise evaluation.

However, these tests could be applicable for field use and rapid diagnosis.

Humoral immune responses of hamsters infected
with adult *Echinococcus multilocularis*
in the course of reinfection and oral vaccination trial

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Humoral immune responses and acquired resistance against *Echinococcus multilocularis* were evaluated using alternative definitive host, golden hamsters, after immune

ing any antigens with CT. Time course of coproantigen excretion was examined by sandwich ELISA using monoclonal antibody EmA 9, raised against *E. multilocularis* adult

were found in the small intestine nor coproan-

tigen increased at the course of the infection.

Cloning and histological analysis of the mouse testis specific gene, Sperm tail associated protein (*Stap*)