



Title	EVALUATION OF THE POLYMERASE CHAIN REACTION to detect Chlamydia psittaci DNA
Author(s)	IMAI, Yumi
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INFORMATION

Hokkaido University granted the degree of the Doctor of Veterinary Medicine to the following 44 graduates of the Faculty of Veterinary Medicine on 25 March, 1993. The authors' summaries of their theses are as follows:

EVALUATION OF THE POLYMERASE CHAIN REACTION to detect *Chlamydia psittaci* DNA

Yumi IMAI

*Department of Veterinary Public Health,
Faculty of Veterinary Medicine,
Hokkaido University, Sapporo 060, Japan*

To establish a rapid and specific method for clinical diagnosis of psittacosis, we examined the application of the polymerase chain reaction (PCR).

We examined the sensitivity of PCR to detect chlamydiae using a primer pair which amplifies 1.2 kbp *omp1*: a major outer membrane protein (MOMP) gene in the chlamydial chromosome. We compared methods for extraction of template DNA and evaluated the effect of fecal constituents by PCR.

1. Stock solution of *Chlamydia psittaci* IZAWA-1 had an infectious titer of 4.8×10^7 IFU/ml and a particulate number of 8.4×10^8 cells/ml. PCR detected 8.4×10^2 chlamydial particles with an infectious titer of 4.8×10^1 IFU.

2. The infectious titer was little reduced by addition of 5% normal chicken feces to IZAWA-1 stock solution.

3. We compared PCR, Giemsa staining and the indirect immunofluorescent antibody technique (IFA) in BGM cell monolayers inoculated with 10-fold dilutions of IZAWA-1 stock solution. The sensitivity of PCR was 200 times higher than that of Giemsa staining and IFA.

4. Adding 5% fecal material to template DNA reduced the sensitivity of PCR by 10^{-3} . Various conditions for centrifugation to recover DNA from the template DNA with the feces were evaluated. PCR with centrifugation at 10,000 rpm for 5 min showed 10 times higher sensitivity.

5. Phenol: chloroform extraction and ethanol precipitation of a small amount of template DNA reduced the sensitivity of PCR.

6. Among 50 fecal samples from apparently healthy budgerigars, 6 were positive by yolk sac inoculation to chick embryos and 1 was positive by direct PCR from fecal materials. In addition, 5 were positive by PCR with template DNA from feces-inoculated monolayer cells and no samples were positive by Giemsa staining of inoculated monolayer cells.