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DEVELOPMENT OF A METHOD FOR THE MEASURING CHLAMYDIAL
INFECTIOUS TITER AND ITS APPLICATION FOR
EXPERIMENTAL CHLAMYDIAL INFECTION OF QUAILS

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A focus-formation method using the peroxidase anti-peroxidase (PAP) staining technique was evaluated as a simple test for measuring chlamydial infectious titer.

In addition, the relationship between chlamydial propagation and antibody production was examined in a quail model of chlamydial infection.

The results are summarized as follows ;

1. Fixation with paraformaldehyde-lysine-periodate buffer (PLP) gave adequate preservation of the expressed antigens for PAP staining. Precise infectious titers were obtained by counting the dilution giving 20 to 100 foci per well. The appropriate incubation period for monolayer cells was at least 16 hours after the chlamydial infection. The required incubation times for focus formation were shorter in 4 chlamydial strains of 3 species than that required for the inclusion formation generally used for titration.

2. Using the focus-formation method, neutralization tests were performed for quail sera inoculated with *Chlamydia psittaci* strain Izawa-1. Anti-chlamydial neutralizing antibodies were complement-dependent and were detectable in the presence of fresh chicken serum.

3. In the sera of quails inoculated with *C. psittaci*, the IgM antibody titer had already reached the maximum level on the 8 days after infection, and then decreased rapidly. In contrast to the IgM antibody, the IgG antibody titer reached the maximum level at 14 days, and a high level was maintained until 42 days. The neutralizing antibody titer was negative at 8 days, but suddenly reached a high level at 14 days. Thereafter, however, it decreased rapidly like the IgM antibody, and became negative at 42 days.

4. The nested polymerase chain reaction (PCR) was applied to detect *Chlamydiae* in spleens and feces from inoculated quails. The detection rate of the chlamydial genome in spleens was clearly higher than that in feces. It was suggested that some quails did not excrete *Chlamydiae* in feces but had it in the spleen. In addition, attempts were made to detect infectious *Chlamydiae*. As a result, a high detection rate was recognized from the spleen and the infectious titer was at high levels at 8 and 14 days after inoculation. However, the infectious titer decreased rapidly like the

decreasing pattern of the neutralizing antibody titer. From feces, only 1 sample was positive for infectious *Chlamydiae*. Therefore, nested PCR is expected to be more practical than the focus-formation method for detection of *Chlamydiae* from feces.