Confirmation of *Chlamydophila abortus* in infected cell culture using Indirect Immunofluorescence technique

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Abstract

Chlamydophila abortus (C. abortus) is an important abortifacient agent in bovines and ovines. Clinical diagnosis of the disease is often difficult. An early diagnosis can be achieved based on direct demonstration of the organism in clinical material and through the cultural recovery of the organism in embryonated chicken egg. For confirmatory diagnosis antigen detection methods or serological techniques can be adopted. The present study is aimed at the confirmatory diagnosis of *C. abortus* infection by indirect immunofluorescence technique following the isolation of the organism in cell culture. Specific apple green fluorescing inclusions of *C. abortus* in McCoy cell lines was detected from 72 h to 96 h post infection employing anti-chlamydial group specific monoclonal antibodies. Thus, a confirmatory diagnosis of the infection was possible with this study.
Keywords: *Chlamydophila abortus,* Indirect immunofluorescence, McCoy cell lines, Monoclonal antibodies.

Introduction

Abortion is causing heavy economic loss in livestock industry and is recognized as a world wide problem. A variety of bacterial, viral, parasitic and nutritional factors cause abortion in domestic ruminants. *Chlamydophila abortus (C. abortus)* is an important one among the abortifacients in bovines and ovines (Jee *et al.* 2004).

Chlamydophila abortus is an obligate intracellular bacterium that was formerly known as *Chlamydia psittaci*. The family *Chlamydiaceae* which previously contained the single genus *Chlamydia* has been reclassified into two genera, *Chlamydia* and *Chlamydophila*, and nine species (Everett *et al.* 1999). Of these, the most economically important animal pathogen is *C. abortus*. In animals abortion in late pregnancy is the most common pathological effect caused by these bacteria. The organism also has affinity for human placenta and thus it is a specific hazard in pregnant women.

Clinical diagnosis is often difficult as the signs and lesions are observed in other abortions also. Early diagnosis of the condition is based on direct demonstration of the organism in clinical material and through the cultural recovery of the organism in embryonated chicken egg or in cell culture. (Longbottom *et al.* 2004). Confirmatory diagnosis of *C. abortus* infection can be achieved by the various antigen detection methods like immunohistochemistry, PCR and serological techniques like indirect immunofluorescence, complement fixation or ELISA.

The present study is aimed at the confirmatory diagnosis of the infection by indirect immunofluorescence technique following the isolation of the organism in cell culture.

Materials and Methods

Chlamydial isolates obtained from caprine and bovine abortion which were preserved in the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, were used in this study (Table 1). The isolates were revived in Mc Coy cell lines. All the isolates revealed marked CPE characteristic of *C. abortus* which included rounding and swelling of cells, formation of syncytia by 24 hours which became pronounced by 48 hours; intra cytoplasmic inclusion bodies started appearing by 72 hours and by 96 hours elementary bodies, were also noticed. For further confirmation they were subjected to indirect immunofluorescence test.

Mc Coy cells were grown as cover slip cultures. These cells were infected with *C. abortus*. At intervals of 24 h, 72 h, and 96 h they were fixed in ice cold

Table-1. Details of *Chlymydophila abortus* isolates

Isolate	Source
M-28	Liver of an aborted caprine foetus
M-430	Lung of an aborted caprine foetus
M-121	Liver of an aborted bovine foetus
P-156	Infected yolk sac material obtained from Department of Microbiology, Veterinary College, Palampur, Himachal Pradesh as reference isolate

acetone for five minutes. Anti-chlamydial group specific monoclonal antibodies developed in mice was diluted to 1:10 and added to each monolayer at the rate of 0.1ml and incubated at 37°C for one hour. Monolayer was then thoroughly rinsed with phosphate buffered saline (PBS) and triple distilled water. It was allowed to react with 0.1ml of fluorescein isothiocyanate conjugated antimouse antiglobulin produced in rabbit at a dilution of 1:30 in PBS and incubated for one hour. Nine parts of the conjugated antiglobulin was mixed with one part of 0.5 percent Evans Blue before it was used to react with the monolayer culture. They were again rinsed well in PBS and triple distilled water. The cover slips were mounted in glycerol saline to demonstrate chlamydial inclusion bodies. Uninfected cover slip cultures were also processed as above, as control.

Results and Discussion

Specific apple green fluorescence was detected from 72 h post infection (PI) with M-28 and M-430 isolates and maximum fluorescence was noticed only by 96 h PI. In the cells infected with P-156 and M-121 fluorescence was noticed only by 96 h PI. In control cover slip cultures no fluorescence was noticed.

In this study we were able to demonstrate *C. abortus* in McCoy cell lines employing monoclonal antibodies which revealed apple green fluorescing inclusions from 72 h to 96 hour PI giving a confirmatory diagnosis. This observation is in agreement with that of Nagal *et al*. (1997).

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References

1. Everett, K.D.E., Bush, R.M. and Andersen, A.A. (1999). Amended description of the order *Chlamydiales*, proposal of *Parachlamydiaceae* fam. Nov. and *Simkaniaceae* fam. Nov., each containing one monotypic genus, revised taxonomy of the family *Chlamydiaceae*, including a new genus and five new species, and standards for the identification of organisms. *Int. J. Syst. Becteriol*. 49: 415-440.
2. Jee, J.B., Degraves, F.J., Kim, T.Y and Kaltenboeck, B. (2004). High prevalence of natural *Chlamydophila* species infection in calves. *J. Clin. Microbiol*. 42: 5664-5672.
3. Longbottom, D., Fairly, S., Chapman, S., Psarrou, E., Vretou, E. and Livingstone, M., (2004). Serological diagnosis of ovine enzootic abortion by enzyme-linked immunosorbent assay with a recombinant protein fragment of the polymorphic outer membrane protein POMP90 of *Chlamydophila abortus*. *J. Clin. Microbiol*, 40: 4235-4243.
4. Nagal, K. B., Katoch, R.C., Joshi, V.B., Batta, M.K., Sharma, M. (1997). Infectivity of *Chlamydia psittaci* in Mc Coy cells. *Indian J. Anim. Sci*. 67(5): 402-408.

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