GROWTH CHARACTERISTICS OF *CHLAMYDIA PSITTACI (CHLAMYDOPHILA ABORTUS)* ISOLATES IN McCOY CELL LINE *

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Abstract

A better understanding of Chlamydia psittaci is required to appreciate and highlight the epidemiology of chlamydiosis. Chlamydial organisms are obligate intracellular pathogens that do not grow on artificial media. Different cell lines have been found to be efficient for the isolation of C. psittaci. This study was conducted to identify the growth characteristics of C. psittaci in McCoy cell lines. Marked CPE was exhibited by the isolates in McCoy cell lines. In stained preparations rounding and clumping of cells, syncytia formation and intracytoplasmic inclusion bodies were noticed. To confirm the identity of the isolates, fluorescent antibody staining technique was used. Specific apple green fluorescing inclusions were observed which were very prominent by 96 h PI.

Keywords : *Chlamydophila abortus, growth characteristics, McCoy cell line.*

Chlamydiae are obligate intracellular prokaryotic pathogen of animals, birds and man, exhibiting a wide variety of clinical symptoms. The diverse diseases caused by the organisms are having both public health importance and economic significance.

Realizing the ability of *Chlamydia psittaci* in the causation of a wide spectrum of diseases, the very need for sensitive, reliable and definitive laboratory diagnosis is badly felt. Early diagnosis of *C. psittaci (Chlamydophila abortus)* is mainly based on direct demonstration of the organism in clinical materials and confirmatory diagnosis is through cultural recovery of the organism in cell culture and embryonated eggs.

The uniformity in susceptibility of cultured cells has potential advantage over the chick embryo technique for the isolation and propagation of the organism. Several cell lines support the growth of a variety of strains of chlamydia. Johnson *et al.* (1983) described McCoy cells to be more sensitive for isolation of *C. psittaci* from abortion cases.

The present study was undertaken to propagate chick embryo adapted *C. psittaci* isolates in McCoy cell line and to determine the changes produced in McCoy cells using May-Grunwald Giemsa staining and by indirect immunofluorescence examination.

Materials and Methods

Chlamydial isolates obtained from caprine and bovine abortion which were preserved in the Department of Microbiology, were used in this study. The isolates were revived by passaging in six to seven day-old embryonated eggs through yolk sac route.

Twenty per cent suspension of heavily infected yolk sac from all four isolates were suspended in sucrose-phosphate glutamate

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(SPG) buffer with antibiotics and was centrifuged at 3800 rpm for 15 min. The sediment and the fat layers were discarded and the middle layer was collected. It was diluted with maintenance medium at the rate of 1:100 and used as the inoculum. Test tubes containing glass cover slip were seeded with McCoy cell and incubated at 37°C for the formation of monolayer. The monolayer was inoculated with 0.2 ml of inoculum and incubated at 37°C for an hour. The monolayer was washed with maintenance medium (MM) and added MM containing cycloheximide at a concentration of 0.1 ml per 100 ml at the rate of 2 ml per bottle. Control coverslips were prepared simultaneously in which instead of inoculum MM was added. These were incubated at 37°C and were collected at 24 h intervals for a period of 96 h and stained using May-Grunwald Giemsa stain as well as indirect immunofluroscence technique to appreciate the cytopathic effect and/or inclusion bodies.

For staining with May-Grunwald Giemsa stain, the cover slips were fixed overnight in methanol. They were stained for 10 min in May-Grunwald stain and for 20 min in 1 in 10 diluted Giemsa stain. The cover slips were rinsed rapidly in two changes of acetone and then in two parts of acetone and one part of xylene for five sec. They were then placed in one part of acetone and two parts of xylene for one minute, cleared in two changes of xylene, two minutes each, dried and mounted with DPX on a clean grease free glass slide and examined for CPE or inclusion bodies under a microscope.

The infected monolayers on glass cover slips were subjected to indirect immuno-fluorescence examination. The cover slips with monolayer were fixed in ice-cold acetone for five minutes. Antichlamydial 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 for one hour. Monolayer was then thoroughly rinsed with phosphate buffered saline (PBS) and triple distilled water thrice respectively. It was allowed to react with 0.1 ml of fluorescein isothiocyanate conjugated antimouse antiglobulins produced in rabbit at a dilution of 1: 30 in PBS and incubated at 37°C for one hour. Nine parts of the conjugated antiglobulin was mixed with one part of 0.5 % Evans blue before it was used to react with the monolayer culture. They were again rinsed well with PBS and triple distilled water. The cover slips were mounted in glycerol saline to demonstrate chlamydial inclusion bodies.

Results and Discussion

The isolates passaged in McCoy cells showed marked CPE from 24 h of incubation onwards. Rounding and swelling of cells were produced by all the isolates. Formation of syncytia also started by 24 h in the monolayer (Fig. 1). By 48 h, syncytia formation were more pronounced (Fig. 2). By 72 h, intra cytoplasmic inclusion bodies started appearing and were prominent by 96 h. Cells were soon to get

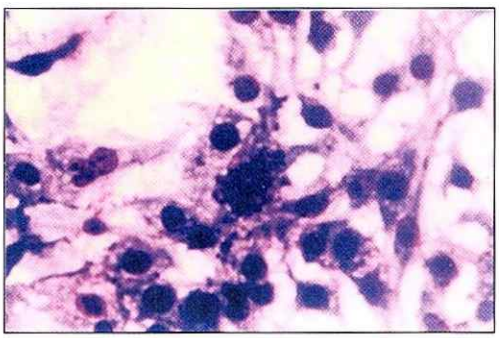


Fig. 1

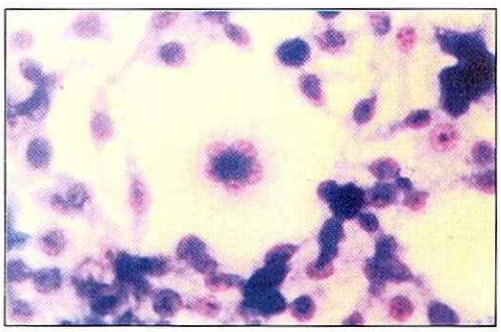


Fig. 2

detached from the adjacent ones (Fig.3). Using May-Grunwald Giemsa staining, compact deeply pink staining intra cellular inclusion bodies were noticed 72 h post inoculation (PI), which became prominent by 96 h. Elementary bodies, which were lightly pink, were also noticed by 96 h PI. These bodies were extracellular in nature (Fig. 4). Complete dislodgment of cells from the glass surface occurred at the end of 96 h. The control tubes showed no changes during the

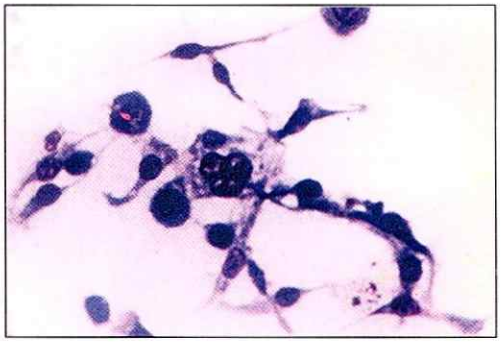


Fig. 3

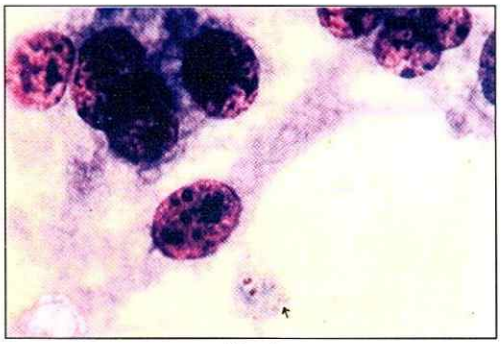


Fig. 4

observation period and remained as confluent monolayers (Fig. 5). Page (1981) reported similar CPE in cell cultures infected with *C. psittaci* in two to six days of incubation. Katoch (1997) and Nagal *et al.* (1997) described intracytoplasmic inclusions in McCoy cell line 72 h PI.

In the present study our attempt to demonstrate *C. psittaci* in McCoy cell lines employing monoclonal antibodies revealed apple green fluorescing inclusions 96 h PI in sufficient number to be of diagnostic level (Fig.6).These observations were in confirmation with those of Vanrampay *et al.* (1994) and Nagal *et al.* (1997).

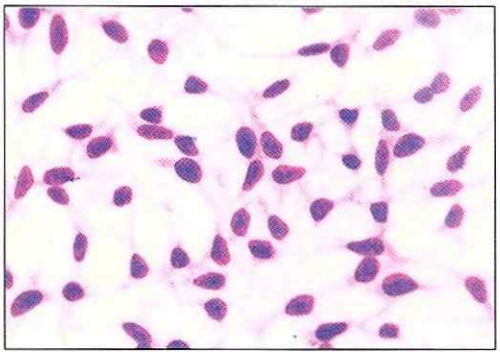


Fig. 5

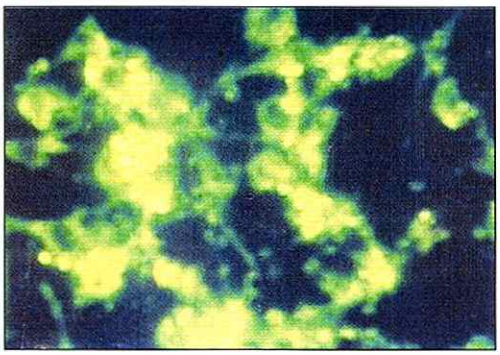


Fig. 6

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