

RAPID DETECTION OF CHLAMYDIA IN THE GENITAL TRACT OF EWES AND WOMEN

BY

HADIA A. MOUSSA, H. B. HASSAN*, S. A. SALEM,
A. A. EL-MONLA** and N. A. M. MAHMOUD

Animal Health Research Inst., Dokki, Giza, Egypt

* Microbiol. Dept., Fac. Vet. Med. (Kafr El-Sheikh), Tanta Univ.

**Hyg. Det. Albaath Univ., Fac. Vet. MED., Syria

SUMMARY

Vaginal swabs from ewes and women were examined for the presence of Chlamydia by clearview Chlamydia test. The results indicated the applicability of this test for the rapid diagnosis of chlamydiosis in sheep (enzootic abortion in ewes) as well as its use in human for the detection of Chlamydia carrier.

INTRODUCTION

The family Chlamydiaceae includes obligate intracellular microorganisms, to which belongs two Chlamydia species; *C. psittaci* and *C. trachomatis*. Moreover, a third species, *C. pneumoniae* has been proposed for those *C. psittaci* isolates from human referred to as TWAR strain (Quinn et al., 1994). Chlamydiae do not show a strict host specificity and are responsible for a variety of disease syndromes in animals and man (Pierce et al., 1964; Page, 1966 and Buxton, 1986). In sheep, *C. psittaci* causes abortion, still-birth and weakly developed lambs in pregnant ewes (Storz, 1971 and Johnson et al., 1983). The organism is transmitted to pregnant women and causes abortion (Giroud et al., 1956 and Buxton, 1986). The laboratory diagnosis of Chlamydial infection depends on microscopic

examination of stained smears from the cotyledons or chorion for evidence of elementary bodies in case of abortion or in smears made from vaginal swabs or from the surface of the aborted fetuses for the detection of Chlamydial elementary bodies by chemical stains (Dagnall and Wilshire, 1990), immunofluorescence (Perez-Martinez and Storz, 1985), enzyme linked immunosorbent assay (Elisa) and immunoperoxidase (Wuinn et al., 1994). Morphologically, all the abortion isolates appear as round or oval compact deeply stained inclusions and by cultivation of the organism in 6-7 day old developing embryonated hen's eggs via the yolk sac route and the tissue cultures of BHK-12, Vero 1929 and McCoy's cells (Johnson et al., 1983 and Woodland et al., 1987). Improvements in the tissue culture systems have resulted in this use to quantify infectivity, to study the metabolism of the parasite and the infected host cell and to analyze host-parasite interactions at the cellular level to isolate organisms from clinical materials and to obtain purified preparation of elementary or reticular bodies. All these techniques have been used to isolate and cultivate Chlamydiae and these methods are still the most reliable and widely used of propagation of *C. psittaci* strains (Storz, 1971). Not only because of its greater convenience and sensitivity but also because it allows detailed observation of

Chlamydial growth. This work was carried out as a trial for rapid detection of *Chlamydia psittaci* in the genital tract of apparently healthy ewes and women by using the commercially developed clearview Chlamydia test produced essentially for rapid detection of *Chlamydia trachomatis* in human beings.

MATERIAL AND METHODS

Vaginal swabs:

Endocervical swabs were collected by obstetricians from hospitalized women. Swabs from ewes were taken from ewes intended to be Slaughtered at Muniebb Abbatoir, Giza, according to the instruction manual of the test Kits, where excess mucus from the endocervix was first removed by a separate swab, and another sterile swab was introduced into the endocervix for 10-30 seconds, then transferred to the laboratory in sterile tubes without transport medium, stored at 4°C and examined within 5 days.

Clearview Chlamydia test:

The test was developed by Unipath. Bed mainly for the detection of *C. trachomatis* human. According to the instruction manual Chlamydial antigen was extracted from the s by heating in 0.6 ml extraction buffer contain 0.1% sodium azide solution at 80°C in water for 10-12 minutes. The swabs were allowed to cool for 5 minutes at room temperature. 1 drop of the extraction buffer were applied to sample window of the test unit and the test read after 15 minutes. Positive result is indicated by the presence of blue line in the result window which remains clear in negative samples. Positive control window swabs clear blue line with positive control chlamydia antigen.

RESULTS AND DISCUSSION

In the present study, the clearview test was applied for the detection of Chlamydial antigen in vaginal swabs taken from 77 ewes and 24 women. The results are presented in Table (1) and Figure 1.

Table (1): Results of clearview Chlamydia test for rapid detection of Chlamydial antigen in endocervical swabs from ewes and women.

Species	Total examined samples	Positive	Negative	%
Human	23	7	16	30.43
Sheep	77	24	53	31.16

(1&2). The antigen was detectable in 23 and 7 cases of the swabs taken from ewes and women, respectively. The test is based on genus specific monoclonal antibody against Chlamydial lipopolysaccharide antigen linked to latex beads that when mixed with the antigen in the mixture migrates from the sample window to the result window along a strip and forms blue line in the result window. Accordingly, positive cases are indicated by clear blue line in the result window of the kit, which is compared with the positive control, whereas negative cases show a clear view in the result window. The clearview test is mainly produced for the detection of *C. trachomatis* in human. It is an ELISA test which was applied by Saintagne et al., (1992) for the detection of Chlamydial antigen in enzootic sheep abortion,

arthritis and pneumonia in lambs as well as conjunctivitis in goats. Commercially available other ELISA tests have been developed and widely used in man and animals for the detection of Chlamydial antigen using either polyclonal or monoclonal antibodies (Chernesky et al., 1986; Ryan et al., 1986 and Weigler et al., 1988). However, the culture of Chlamydia is generally considered to be a standard against which other tests are evaluated. Comparing the clearview test with the culture. Davidson and Wilsmore (1991) proved that the clearview test was highly sensitive (96.3%) and specific (91.1%). The same authors stated that the culture itself is not 100% sensitive and can only detect viable organisms, whereas the clearview test has the advantage of detecting Chlamydial antigen in denaturated material.

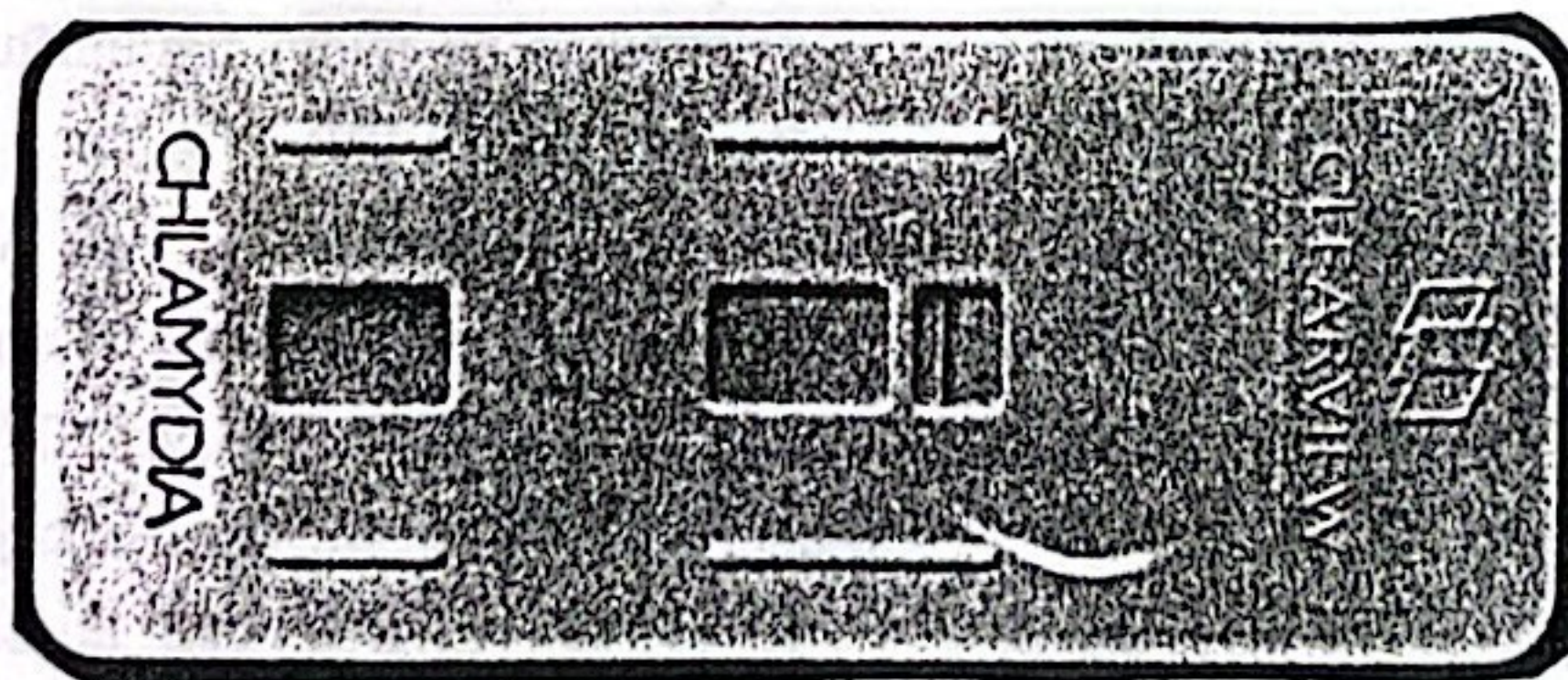


Fig. (1): Clearview Chlamydia test showed negative reaction.

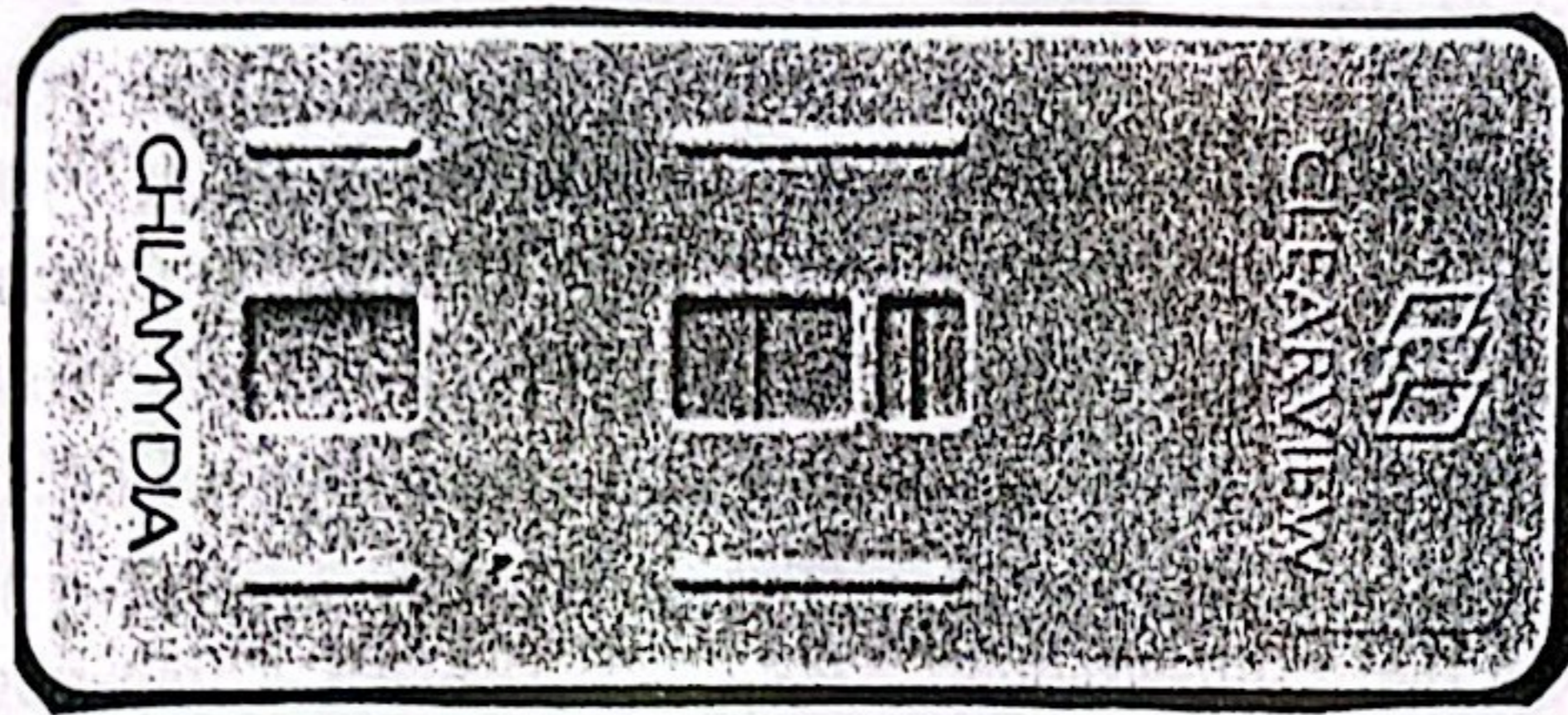


Fig. (2): Clearview Chlamydia test revealed positive reaction.

REFERENCES

- Buxton, D. (1980): Potential danger to pregnant women of *Chlamydia psittaci* from sheep. *Vet. Rec.* 118, 510-511.
- Chernesky, M. A.; Mahony, J. B., Castriciano, S.; Mores, M.; Stewart, I. O.; Landis, S. J.; Seideman, W.; Sargeant, E. J. and Leman, C. (1986): Detection of *Chlamydia trachomatis* antigen by enzyme immunoassay and immunofluorescence in genital specimens from symptomatic and asymptomatic man and women. *J. Infec. Dis.*, 154, 141-148.
- Dagnall, G. J. R. and Wilsmore, A. J. (1990): A simple staining method for the identification of Chlamydial elementary bodies in the foetal membranes of sheep affected by ovine enzootic abortion. *Vet. Microbiol.*, 21, 232-239.
- Giroud, P. roger, F. and Dumas, N. (1956): Resultats concernant I, avortement de la femme du agent du groupe de la psittacose. *C. r. Acad. Sci. (Paris)* 242, 697-698.
- Johnson, F. W. A.; Clarkson, M. J. and Spensor, W. N. (1983): Direct isolation of the agent of enzootic abortion of ewes (*Chlamydia psittaci*) in cell culture, *Vet. Rec.*, 113, 413-414.
- Page, I. A. (1966): Interspecies transfer of psittacosis-LGV-trachoma agents. Pathogenicity of two avian and two mammalian strains for eight species of birds and mammals. *Am. J. Vet. Res.*, 27, 397-407.
- Perez-Martinez, J. A. and Storz, J. (1985): Antigenic diversity of *Chlamydia psittaci* of mammalian origin determined by microimmunofluorescence. *Infect. & Immun.*, 50, 905-910.
- Pierce, K. R.; Careli, I. H. and Moore, R. W. (1964): Experimental transmission of ornithosis from sheep to turkeys. *Am. J. Vet. Res.*, 25, 977-980.
- Quinn, P. J.; Carter, M. W.; Markey, B. K. and Curter, G. R. (1994): *Clinical vet. micro.*, Mosby-Yearbook, Printed by Crofos-Spain.
- Ryan, R. W.; Kwasnik, I.; Steingrimsson, O.; Gudmundson, J.; Thorarinsson, H. and Tilton, R. C. (1986): Rapid detection of *Chlamydia trachomatis* by an enzyme immune assay method. *Diagn. Microbiol. Infect. Dis.*, 5, 225-234.
- Saintagne, J.; Chabanet, D.; Poncelet, J. L.; Inguibert, J. L. and Joannard, C. (1991): Demonstration of evidence of chlamydia in Veterinary samples by clearview Chlamydia test. *Bull. des, G. T. V.*, 4, 51-58.
- Storz, J. (1971): *Chlamydia* and *Chlamydia* induced

diseases. Springfield II, Ellinois, Charles C. Thomas, pp. 5-358.

Weigler, B. J.; Baldock, F. C.; Girjes, A. A.; Carrick, F. N. and Lavin, M. F. (1988): Evaluation of an enzyme immunoassay test for the diagnosis of Chlamydia psittaci infection in free ranging koala (Phascolarctes cinereus) in south-eastern Queensland Australia, J. Wildlife, Dis., 24, 259-263.

Wilsmore, A. J. and Davidson, I. (1991): Clearview rapid test compared with other methods to diagnosis Chlamydial infection. Vet. Rec., 128, 503-504.

Woodland, R. M.; Kirton, R. P. and Darougar, S. (1987): Sensitivity of mitomycin-C treated McCoy cells for the isolation of Chlamydia trachomatis from genital specimens. Eur. J. Clin. Microbiol., 6, 653-656.

SUMMARY

The effect of the broad spectrum antiviral ribavirin has been tested against the replication of rinderpest virus (RPV) in bovine kidney cells (BK) as primary culture and vero cell line culture.

Ribavirin showed no effect on growth and viability of both cell cultures in a range of 0.1-200 µg/ml. When such ribavirin concentrations were applied to BK and vero cell cultures, infected with RPV, no cytopathic effects were detected for 12 days of incubation. The efficacy of ribavirin against RPV in vitro has been confirmed by the plaque titration test.

INTRODUCTION

Ribavirin (1-B-D- Ribofuranosyl-1,2,4-oxadiazole-3,5-carboxamide) had been shown to exhibit a potent antiviral effect against many DNA and RNA viruses in vitro and in vivo (Sidwell et al., 1972 and 1973). Ribavirin has been used in clinical trials in the United States of America, Mexico and Brazil in promising results in treating measles (A. G. and Anaya, 1977), herpes gingivostomatitis (Dih et al., 1977), hepatitis A (Galvez and Castro 1973), influenza (Maguiness et al., 1975) and genital infections caused by herpes virus-2 (Sidwell et al., 1973).

Toxicological studies (Huffman et al., 1971; Lowell et al., 1972 and 1973) have presented data for the safety and efficacy of ribavirin both in vitro and in vivo.

Rinderpest virus (RPV) is a member of the Paramyxoviridae family and causes fatal disease in cattle. Egypt was considered to be free from Rinderpest (Abdel-Ghaffar et al., 1977), until the devastating epidemic which commenced in 1987. In addition, the disease is still endemic and epidemic in most of the Near and Far East countries and most of the African countries.

The present work aimed to study the possibility of the antiviral ribavirin (Virazole) on rinderpest virus (Live attenuated vaccinal strain) in vitro.

MATERIAL AND METHODS

1- Ribavirin:

Ribavirin was obtained from Daitoberic (Virazole, Inc. U.S.A.) Stock solution of ribavirin was prepared by dissolving 100 mg in 10 ml distilled water. The stock solution was stored at 4°C and sterilized by autoclaving at 121°C for 15 min. (Miller, 1977). The stock solution was diluted in sterile distilled water to give a concentration of 100 µg/ml.