

## THE ROLE OF CHLAMYDIOSIS IN DIARRHOEA OF NEWBORN CALVES

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### Abstract

Eighty-four faecal samples were collected from diarrheic newborn calves. Chlamydial isolation was done in yolk sacs of embryonated chicken eggs. Impression smears were made from yolk sac of infected chicken eggs, and stained with Gimenez stain to demonstrate chlamydial inclusion bodies. Complement fixation test (CFT) was applied on the collected sample suspension and 41 positive cases out of 84 were given at a percentage of 48.80%. Meanwhile, ELISA technique also was applied which figured in and, 46 positive cases out of 84 were figured at a percentage of 54.76%.

### INTRODUCTION

Diarrhoea remains one of the most important causes of calves mortality. The economic significance of the disease varies among herds, and to some extent depends on the system of management and the degree of intensification. Tzipori (1981) mentioned that diarrhoea of young calves is a clinical entity with variable aetiology involving three components, micro-organisms, environment and host. Doughri *et al.* (1994) also, said that bovine chlamydial strains were isolated from the faeces of newly born calves with diarrhoea and polyarthritis from other view. Coetzer and Thomson (1994) described enteritis syndrome as characterized by mucoid to watery or bloody diarrhoea of variable severity in young calves and lambs, also, mentioned that the onset of the disease is sudden with moderate fever. In addition, calves showed anorexia, depression, stiffness, staggering, salivation, cough and the animal may become recumbent and paralyzed. In this study, we are dealing with Chlamydia which causes diarrhoea in newborn calves by isolation of the causative agents from the faeces of diarrheic calves in the yolk sac of embryonated chicken eggs and applying CFT and ELISA techniques as serological tests to identify the chlamydial agents in the collected faecal samples.

## MATERIALS AND METHODS

### 1. Samples

Eighty-four faecal samples were collected from diarrheic newborn calves. PBS (Phosphate buffer saline) were added and centrifuged. The supernatants were collected & streptomycin was added at 500 µg/ml according to Eugster and Storze (1971) then, recentrifuged, and the collected supernatant of each sample was kept at -70°C till use.

### 2. Chlamydial isolation

According to Edwine and Nathalie (1979), each prepared sample was inoculated into 3 embryonated hens eggs 5-7 days old through yolk sac route at a dose of 0.2 ml/egg, then, incubated at 35°C and candled daily. The embryos, which died through the first 3 days after inoculation, were discarded, while, those died after that, yolk sacs of each sample were collected separately. Impression smears were made and stained with Geminize stain.

### 3. Histopathological examination

As described by Schachter and Dawson (1978), impression smears were made from yolk sacs of infected embryonated chicken eggs and stained with Geminize stain to demonstrate the cytoplasmic inclusion bodies of Chlamydia by examination under oil immersion lens.

### 4. Serological techniques

**A) Complement fixation test (CFT)** was applied on supernatant faecal samples as described by Edwine and Nathalie (1979) by using reference chlamydial serum and antigen from Denka Seiken Co. Ltd.

#### B. ELISA technique

**Serum preparation** as described by Armel *et al.* (1994). Chlamydial antiserum was obtained from aborted goat. Serum collection was done after 3 weeks from abortion.

**Production of antiserum in mice.** Mice received two inoculations by *C.psittaci* strain I/V one week apart. Antisera were obtained 5 days after second inoculation according to Armel *et al.* (1994).

#### Capture ELISA technique

The technique was applied according to Grouch *et al.* (1984). Microtiter plates (96 wells) were sensitized with *Chlamydia psittaci* goat antisera diluted in carbonate/bicarbonate coating buffer pH 9.6). The plates were washed 3 times with PBS containing 0.1% tween 20 and blocked with 1% bovine serum albumin in PBS, then incubated for 2 hours and washed 3 times. Tested samples were diluted two fold dilution in 0.01MPBS containing 0.05% tween 20 and 100  $\mu$ l of each dilution was added to duplicate wells. Negative control faecal samples for *Chlamydia psittaci* were inoculated to test at the same dilution of test samples. Plates were incubated at 37°C for one hour and washed. *Chlamydia psittaci* mice antiserum was diluted with diluting buffer, and 100  $\mu$ l were added to each well, then, the plates were incubated at 37°C for one hour, washed with washing buffer 3 times. Anti-mouse (IgGAM peroxidase-conjugate) was diluted in diluting buffer, PBS-tween 20-BSA 2%) was added and left for about 30 minutes and was read at optical density (OD) 490 nm.

## RESULTS

Out of 84 samples obtained from calves suffering from diarrhoea, 37 samples were positive for chlamydial inclusion bodies in the impression smears stained with Geminize stain in percentage of 44.04% (Fig.1).

By application of Complement fixation test (CFT), 41 samples out of 84 samples were positive to chlamydia in a percentage of 48.80% (Table 1). Meanwhile, by using ELISA technique, 46 positive cases out of 84 samples were positive to chlamydia in percentage of 54.76% as shown in Table 2.

Table 1. Results of Complement fixation Test Applied on the Antigen prepared from faecal samples of calves.

Name of Farm	No. of faecal Samples	Titers							+Ve cases	-Ve cases
				1/8	1/16	1/32	1/64	1/128		
Nobaria	32	2	4	2	1	2	5	1	17	15
Damitta	29	4	-	2	2	1	1	2	12	17
Sharkia	23	5	2	-	2	1	1	1	12	11
Total	84	11	6	4	5	3	7	4	41	43

Positive cases 41 samples

Negative cases 43

Positive cases percentage = 48.80%

Negative cases percentage = 51.19%

## DISCUSSION

Diarrhoea was incriminated as one of the most important causes of calves mortality. The economic significance of the disease varies among herds as mentioned by Tzipore (1981). There are several infectious agents involving, environmental, immunological and possibly genetic factors which are responsible in precipitating the disease.

The infectious agents include *E.coli* (k99) campylobacter species, in addition to viral agents as corona virus, rotavirus, calcivirus, parvovirus ... etc. and also parasitic agents such as *Cryptosporidium* and protozoa.

In our investigation, we dealt with *Chlamydia psittaci* which causes intestinal infection and enteritis in young calves as mentioned by Doughri *et al.* (1974) who added that, bovine chlamydial strains were first isolated from the intestinal tract of clinically normal young calves, and also, isolated from faeces of newborn calves with diarrhoea and polyarthritis.

In this study, 84 faecal samples were collected from diarrheic newborn calves. The results revealed that, out of 84 impression smears that showed inclusion bodies of chlamydia, 37 samples were positive. Serological tests were applied on the prepared faecal samples to identify the chlamydial agents by using CFT. The results showed that, 41 cases out of 84 faecal samples were positive in percentage of 48.80% (Table 1). Also, ELISA technique was applied and resulted in 46 positive cases from 84 samples in percentage of 54.76% (Table 2).

Table 2. Results of ELISA Technique Applied for Detection of Chlamydial Agent in Faecal Samples of calves.

Name of Farm	No.of faecal Samples	Positive cases	Negative cases
Nobaria	32	21	11
Damitta	29	11	18
Sharkia	23	14	9
Total	84	46	38

Positive cases percentage = 54.76 %  
 Negative cases percentage = 47.61 %

The impression smears proved positive cases lower than those obtained by CFT and ELISA which might be due to that some samples containing dead agent or the infected particles are very fine and failed to produce infection in the inoculated embryonated eggs.

The investigation is in agreement with that mentioned previously by Rose *et al.* (1990) who found that 17 out of 19 samples were positive for chlamydiosis when tested with ELISA, while, 15 out of 19 were positive when cultured. On the other hand, the results showed that ELISA technique is more sensitive than CFT. This result was accepted with Coetzer and Thomson (1994) who mentioned that CFT is most commonly used for serological diagnosis but is replaced by ELISA which is more sensitive and rapid and can easily be automated.

Reviewing the present literature, chlamydiosis revealed a significant role in diarrhoea of newborn calves. For accurate identification of *Chlamydia psittaci*, ELISA technique and Complement fixation test are recommended in addition to isolation and impression smears.

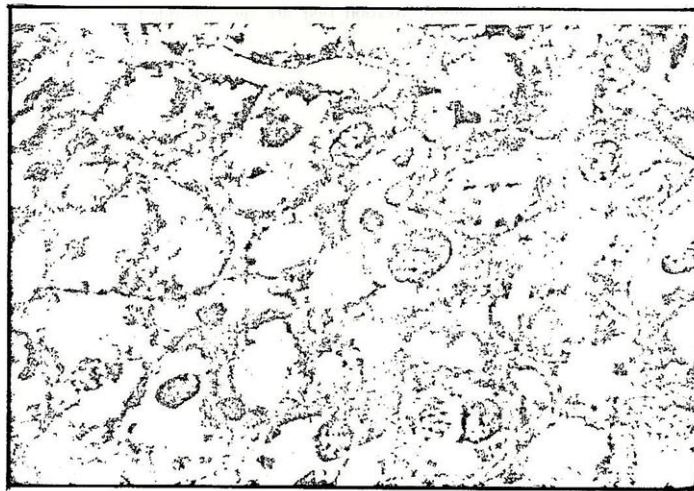


Fig. 1. Impression smears from infected yolk sac stained with Geminize stain showing inclusion bodies of *Chlamydia psittaci*.

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## دور الكلاميديا كأحد أسباب حدوث الاسهال فى العجول حديثة الولادة

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تم جمع عدد ٨٤ عينة براز من عجول حديثة الولادة مصابة بحالات اسهال. وتم عزل ميكروب الكلاميديا على أجنة البيض المخصب عمر ٥ - ٧ أيام فى المخ. وأخذت مسحة من غشاء المخ وصبغت بصيغة الجمينيز للاستدلال على وجود الأجسام المحورية لميكروب الكلاميديا فى سيتوبلازم الخلايا وكانت النتيجة ايجابية لوجود الأجسام المحورية فى عدد ٣٧ عينة بنسبة اصابه ٤٤,٠٤% وكذلك تم استخدام اختبارى المثبت المكمل والليزا لتحديد المسبب المرضى وكانت نسبة تواجد الكلاميديا فى اختبار المثبت المكمل عدد ٤١ عينة ايجابية بنسبة ٤٨,٨% بينما كانت نتيجة اختبار الاليزا تواجد عدد ٤٦ عينة ايجابية بنسبة ٥٤,٧٦% حيث كان اختبار الاليزا أكثر حساسية فى التعرف على المسبب المرضى من اختبار المثبت المكمل.