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**INVESTIGATION ON PROBLEM OF CHLAMYDIOSIS
AMONG DOMESTIC AND WILD SHEEP**
(With 7 Tables and 1 Photo)

By

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فحوصات عن الكلاميديا في الأغنام المستأنسة والبرية

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أجريت هذه الدراسة على الأغنام المستأنسة (٣٩٥ حيوان) والأغنام البرية (٩٠ حيوان) وذلك لإلقاء الضوء على مرض الكلاميديا في هذه الحيوانات من حيث الأعراض ومدى انتشار المرض وتشخيصه وعلاجه ومحاولة الوقاية منه. وبإجراء الفحوصات الأكلينيكية لهذه الأغنام وجد عدد من الأغنام المريضة (١٧٥ مستأنس ، ١٦ بري من نوع أرابي) حيث كانت الأعراض المرضية في الأغنام المستأنسة هي أجهاض في الفترة الأخيرة من الحمل أو ولادة حملان ميتة أو ولادة حملان ضعيفة ومريضة بالإضافة إلى أعراض تنفسية وأسهالات في البعض الآخر وأحيانا التهابات في العين أو المفاصل بينما كانت الأعراض في الأغنام البرية هي أعراض تنفسية وأسهالات. تم أخذ العينات اللازمة لعزل المسبب المرضي من الحيوانات المريضة وكذلك من الحيوانات السليمة ظاهريا والمخالطة للحيوانات المريضة حيث تم حقن هذه العينات في أجنة البيض المخصب وفي القتران وكذلك تم إجراء اختبار التثبيت المكمل. أظهرت النتائج وجود أجسام الكلاميديا وعزلها عن طريق الاختبارات المعملية السابقة من كل من الحيوانات المريضة والحيوانات المخالطة والسليمة ظاهريا وبمضب متفاوتة في كل من الأغنام المستأنسة والبرية. وبإجراء اختبار التثبيت المكمل على عينات السيرم المجمع من الأغنام المستأنسة المريضة والمخالطة تبين وجود أجسام مناعية للكلاميديا في الأغنام المستأنسة المريضة بنسبة ١٦,٦% وفي الأغنام المستأنسة المخالطة بنسبة ٤٦%. علما بأنه قد تم علاج الحيوانات المريضة باستخدام التتراسيكلين.

SUMMARY

This study was designed to tackle the problem of chlamydia among 395 domestic and 90 wild sheep. Clinical examination of the animals investigation declared variable clinical manifestation to the disease. Clinical signs exhibited on 175 domestic sheep represented mainly by abortion at late stage of pregnancy, stillbirth or birth of weak unthrifty

lambs, which show pneumonic or enteric signs. While the prominent signs appeared on 16 wild sheep was diarrhea and respiratory distress. Chlamydial bodies were detected and identify laboratory from suspected materials by chicken embryo inoculation, mice injection, Giemsa stained smears examination and CFT. The incidence of chlamydiosis relying on the above techniques was recorded and discussed. The specific chlamydial complement fixing antibodies was detected in both diseased (16.6%) and contact clinically normal domestic sheep (6.4%). The treatment regimens for the diseased animals was applied and discussed. The current results suggested that, chlamydia psittaci is wide spread, chlamydiosis may have a wide range of clinical manifestation or occurs as latent infection and the latency was cleared from detection of complement fixing antibodies against chlamydial infection in apparently healthy sheep. Risk of transmission of chlamydia species between wild and domestic sheep anticipated; so avoid or minimizing close contact of wild sheep with other domestic animals particularly sheep.

Key words: Chlamydiosis, sheep.

INTRODUCTION

Chlamydiosis is a contagious disease infect sheep and goats transmit the organism to uninfected susceptible animals. The classic symptoms of the disease are abortion, stillbirth, or premature delivery of weak lambs. Aborting animals are subsequently immune and will usually not abort with a chlamydial infection again. These animals remain chronically infected and transmit the organism to further susceptible sheep or goats, first through the placenta and vaginal discharges, later as chronic intestinal carriers (Milon *et al.*, 1983; Shalaby *et al.*, 1987; Coetzer *et al.*, 1994; Asrani *et al.*, 1996; Batta *et al.*, 1997; Radostits *et al.*, 2000 and Rekiki *et al.*, 2002). Chlamydia psittaci is widely distributed obligate intracellular pathogens, which exhibit a broad pathogenic potential (Fukushi and Hirai., 1992).

All livestock species are infected (not species specific) but sheep and goats are the most commonly affected. Animals frequently become infected but show no signs and stress may precipitate clinical disease either as sporadic cases or as epidemics. It is probable that the fecal oral route is the most common means of transmission although aerosol and contact transmission may occurs. Also venereal transmission and insects spreading are possible. Relying on variable factors such physiological state of the host, virulence of the organism and environmental factors,

the chlamydial infection may be causing abortion, still birth, pneumonia, polyarthritios, gastroenteritis, encephalomyelitis and infertility (Storz., 1971; Sharma *et al.*, 1983; Miller *et al.*, 1990 and Chiocco *et al.*, 1992).

Moreover, Gut-Zangger *et al.* (1999) reported that chlamydia psittaci causes abortion and latent intestinal infection in sheep. Andrews *et al.* (1987) and Meagher *et al.* (1992) diagnosed an outbreak of infectious keratoconjunctivitis among bighorn sheep, also Saint-Agne *et al.*, (1992) found that lambs showing pneumonia, arthritis and conjunctivitis were positive to ELISA antibody against chlamydial infection. Chlamydia psittaci was recognized previously in Egypt as important pathogens in some domestic animals (Farid *et al.*, 1980; Ata., 1982; EL-Sayed., 1993 and Hadia *et al.*, 1998)

Laboratory diagnosis of chlamydial infection among farm animals is based on the serological procedures because the isolation techniques remains more difficult and the staining methods are not totally reliable due to intracellular chlamydia are almost indistinguishable from mycoplasmas and rickettsias (Page, 1978 and Amin *et al.*, 1998) and serological testes can be used to confirm or refute diagnosis (Martin and Aitken, 1999). The main problem in evaluating serotests results is their correlation to the actual infectious process (EL-Sayed, 1993)

The chlamydial CFT is the most widely accepted serodiagnostic method for chlamydial infections in animals (Kaltenboeck *et al.*, 1997). It gave satisfactory results with ovine, caprine and avian serum samples but not with bovine samples (Butty and Nicolet., 1987). There was 88% and 68.04% agreement between the CFT and ELISA as reported by Vitu *et al.* (1982) and EL-Sayed. (1993) respectively.

Control measures of chlamydiosis should be based on serotest findings, the animals excreting the chlamydia but having no detectable antibody posses the risk of spreading the agent. This may contributed to the insufficient amount of antigen or to the early stage of infection (Storz, 1971).

Treatment with long acting tetracycline will reduce the number of chlamydial bodies sheded but it will not eliminate infection nor can it reverse the pathological damage already done to a heavily infected placenta. The most effective way to avoid introducing infection to a clean flock is to keep it close or to obtain replacements from sources known to be free of chlamydial infection (Martin and Aitken, 1999).

The purpose of this manuscript was to throwlight on field problems namely abortion, respiratory distress, diarrhea and

conjunctivitis among either domestic or wild sheep through the following points:

- A- Description of different clinical manifestation
- B- Trial to clarify and identify the etiological determinant from inoculation of suspected materials in embryonated chicken eggs and mice, Giemsa staining smears examination and CFT.
- C- Serological monitoring of both diseased and clinical normal contact animals
- D- Therapeutic trials.

MATERIAL and METHODS

1- Animals and clinical investigation:

This study was performed on total number of 395 domestic sheep belonged to Sharkia and Ismailia governorates and 90 wild sheep from different zoos in Egypt. History and clinical examination of domestic and wild sheep were recorded (Table 1).

2- Antisera:

Reference antisera for chlamydia (chlamydia psittaci CF test reagents, "Seiken"), obtained from Denka Seiken Co., Ltd, 3-4-2 Nihonbashikayaba, Ocho, Chno-Ku, Tokyo., Japan. It was used for detection of chlamydia bodies in the suspected materials

3- Reference chlamydia antigen:

It was obtained from Denka Seiken Co., Ltd, 3-4-2 Nihonbashikayaba, Ocho, Chno-Ku, Tokyo., Japan. It was used in serological detection of antibodies.

4- Complement:

Freeze dried preparation of preserved guinea pig serum (Welcome Reagent Ltd) used in complement fixation technique.

5- Sampling:

The type and numbers of samples was illustrated in table (2).

A- Fecal samples: These were collected freshly in sterile plastic container from domestic and wild sheep, to prepared fecal , diluted to 20% in phosphate buffer saline (pH 7.2-7.4) and centrifuge for 15 minutes at 3000 rpm and the supernatant were taken and exposed to filtration, add 1mg /ml of each of streptomycin sulfate, kanamycin sulfate and vancomycin, and the filtrate stored at -70°C until used in mice or egg inoculation (yolk sac route) and preparation of films stained by Giemsa stain.

B- Nasal and ocular swabs: It were taken from both domestic and wild sheep which used for detection and identification of chlamydia infection as mentioned previously in fecal samples.

C- Aborted fetus and placenta: The aborted fetus and placenta were sampled from 26 domestic sheep. The specimens and swabs from placenta and from lung, stomach and spleen of the aborted fetus were collected.

D- Serum samples: It was collected from diseased as well as apparently healthy domestic sheep (4w-post abortion) for detection of chlamydial antibodies using CFT.

Table 1: Animals under investigation.

Animals	Total number	Diseased sheep	Apparently healthy sheep
Domestic sheep	395	175	220
Wild sheep	90	16	74

Table 2: Type and number of samples collected from domestic and wild sheep.

Samples	No of samples collected from			
	Domestic sheep		Wild sheep	
	Apparently healthy	Diseased	Apparently healthy	Diseased
1- Fecal samples	220	64	74	16
2- Nasal & ocular swabs	-	85	74	16
3- Aborted fetus & placenta	-	26	-	-
4- Serum	220	175	-	-

5- Laboratory analysis:

A- Mice inoculation: 3-4 week old mice were inoculated intraperitoneally with 0.2 ml of fecal filtrate fluid, observed for 7 days post inoculation and mice that succumbed to the infection were autopsied and impression smears made from liver and spleen and stained by Giemsa stain. Also the survival control mice were sacrificed at the same time and handled in the same manner.

B- Egg or chicken embryo inoculation: Chicken embryos were inoculated via yolk sac route by fecal and nasal & ocular swabs filtrate and examined daily and discard embryos that die within 48 hours while those died three to ten days post inoculation were investigated for the

presence of chlamydial elementary bodies by examination of Giemsa stained yolk-sac smears and the yolk sac harvested from positive samples were emulsified and stored at -70°C until further identification (Pierre and Michel, 1993).

C- Giemsa staining films: The films prepared from fecal filtrate, nasal & ocular swabs filtrates, the surface of liver and spleen of inoculated and control mice and from yolk sac of inoculated eggs (Hopkins *et al.*, 1973).

D- Complement fixation test (CFT): It was used in detection of chlamydial bodies in the suspected specimens (Yolk sac of infected embryonated hens eggs) after the method adopted by Edwine and Nathalie (1979). Also it was conducted on sera collected from domestic and wild sheep for detection of complement fixing antibodies against chlamydia according to the method of Edwin *et al.* (1979).

E-Trials of treatment: The treatment of domestic sheep was done using oxytetracycline hydrochloride (oxytetracycline hydrochloride 5%, 1ml/10kg, B.W., IM, Arab Veterinary Industrial Co.) as specific therapy beside symptomatic medicates (Coetzer *et al.*, 1994 and Martin and Aitken., 1999).

RESULTS

1-Clinical investigation:

The observed clinical signs on domestic sheep were abortion at late stage of pregnancy and still birth or birth of weak unthrifty lambs. Few animals showed respiratory distress, arthritis in one or more joint, kerato-conjunctivitis and diarrhea. While the prominent signs exhibited on wild sheep were diarrhea and /or respiratory distress.

2-The incidence of chlamydiosis:

It was determined from:

A-Mice injection and Giemsa staining: The incidence recorded among domestic sheep was 18.2% (26.9% in diseased and 11.4% in apparently healthy), while was 13.3 % in wild sheep (25% in diseased and 10.8% in apparently healthy animals) as in tables (3) & (5).

B- Egg inoculation: The incidence was 17.2% in domestic sheep (25.7% diseased and 10.5% in apparently healthy one) while was 11.1% in wild sheep (18.8% in diseased and 9.5% in apparently healthy animals) as in tables (4) & (6).

3- CFT:

It revealed that, chlamydial antibody was detected in 29 (16.6%) diseased domestic sheep and 14 (6.4%) apparently healthy one as in Table (7).

Table 3: Incidence of chlamydiosis using mice inoculation and Giemsa staining among diseased and apparently healthy domestic sheep.

Total number	T.ve	%	No of diseased sheep			No of apparently healthy sheep		
			No	Positive	%	No	Positive	%
395	68	17.2	175	45	25.7	220	23	10.5

Table 4: Incidence of chlamydiosis using egg inoculation among diseased and apparently healthy domestic sheep.

Total number	T.ve	%	No of diseased sheep			No of apparently healthy sheep		
			No	No. of Positive	%	No	No. of Positive	%
395	72	18.2	175	47	26.9	220	25	11.4

Table 5: Incidence of chlamydiosis using mice inoculation and Giemsa staining among diseased and apparently healthy wild sheep.

Total number	T.ve	%	No of diseased sheep			No of apparently healthy sheep		
			No	Positive	%	No	Positive	%
90	10	11.1	16	3	18.8	74	7	9.5

Table 6: Incidence of chlamydiosis using egg inoculation among diseased and apparently healthy wild sheep.

Total number	T.ve	%	No of diseased sheep			No of apparently healthy sheep		
			No	No. of Positive	%	No	No. of Positive	%
90	12	13.3	16	4	25	74	8	10.8

Table 7: Results of CFT in diseased and apparently healthy domestic sheep.

Total number of sheep	Diseased sheep			sheep		
	No	Positive	%	No	Positive	%
395	175	29	16.6	220	14	6.4

CFT titer ranged from 1/8-1/128

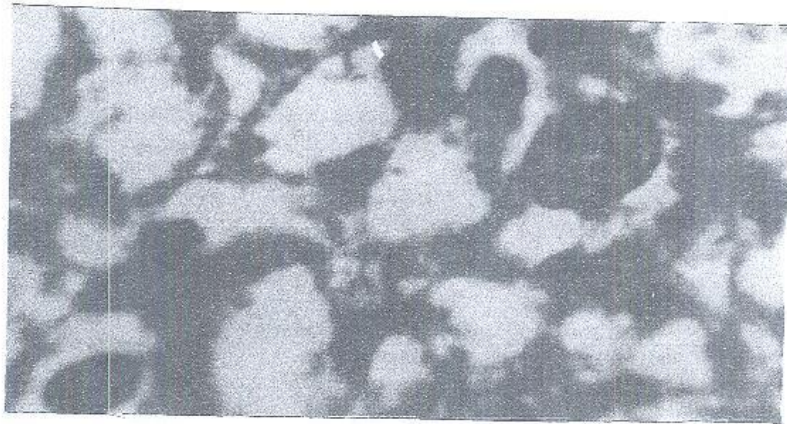


Photo 1: Chlamydial bodies in yolk sac smear of inoculated chicken embryo stained by Giemsa stain

DISCUSSION

Chlamydiosis is infectious disease of all livestock species where sheep, cattle and wild ungulates are the most commonly affected, caused by *Chlamydia psittaci*, characterized clinically by variable clinical syndromes depending on whether the gastrointestinal, respiratory, nervous or reproductive systems and joints or eyes involvement. The infected animals frequently show no apparent clinical signs while stress factors may precipitate clinical disease (Mainar *et al.*, 1998). However naturally occurring diseases caused by chlamydia spp. are relatively few in farm animals (Hungerford, 1990 and Radostits *et al.*, 2000). Chlamydial abortion causes serious reproductive wastage in many sheep producing regions of the world and occurs mostly in flocks that are intensively managed over the parturient period (Martin and Aitken, 1999).

A definitive diagnosis is based on correlation of clinical signs and pathological findings, the isolation and identification of chlamydiae from affected tissues or their presence in smears or exudates or in tissue sections (Coetzer *et al.*, 1994). The clinical signs manifested on domestic sheep were identical to that described previously (Hopkins *et*

al., 1973; Dravecky *et al.*, 1985; Shalaby *et al.*, 1987; EL-Sayed, 1993; Hadia *et al.*, 1998; Quinlan and McGuckin, 1999; Fraile and Berduque, 1999). While those observed on wild sheep similar to that observed by Taylor *et al.* (1996) and Cubero-Pablo *et al.* (2000). The incidence of chlamydiosis regarding mice inoculation and Giemsa staining (table 3 & 5) in investigated sheep was highly augmented by the prior work of Farid *et al.* (1980); Sharma *et al.* (1996); Hadia *et al.* (1998) and Cubero-Pablo *et al.* (2000).

The incidence of the disease using inoculation of egg inoculation (table 4 & 6) were in agreement to that obtained previously by Edwine and Nathalie (1979); Machado *et al.* (1988); EL-Sayed. (1993); Batta *et al.* (1996); Batta *et al.* (1997); Giovannini *et al.* (1998) and Hadia *et al.*, (1998). In this respect, Edwine and Nathalie., (1979) and Butty and Nicolet (1987) concluded that, isolation of chlamydia on embryonated eggs was very sensitive and considered the route of choice in isolation but was time consuming and easily ruined by bacterial contamination or toxicity.

The examination of liver impression smears prepared from inoculated mice and yolksac smears from inoculated chicken embryos after staining by Giemsa stain revealed chlamydial bodies (Photo 1). Such result was in harmony to that obtained previously by Edwine and Nathalie (1979); Krishna and Rajya (1985); EL-Sayed (1993); Batta *et al.* (1996) and Hadia *et al.* (1998).

Serological studies added little information if compared with the isolation results of chlamydia *psittaci*. The group specific complement fixation test has been of little value because inadequate sensitivity and reliability (Schachter *et al.*, 1974). However CFT is commonly used to identify flocks free of infection, it has good sensitivity but is not specific for the agent of enzootic abortion (Radostits *et al.*, 2000). The detection of chlamydial bodies in investigated samples by CFT identical to that obtained by Hadia *et al.* (1998).

The result of CFT in domestic sheep was tabulated in table (7). The current results was nearly identical to that reported by Farid *et al.* (1980); Farina *et al.* (1985); Perini *et al.* (1988); Batta *et al.* (1996); Kalteneboeck *et al.*, (1997); Duman and Durak (1998) and Quinlan and McGuckin (1999). However, interpretation of CFT results can be confounded by false positives, perhaps attributable to concurrent infection with *C.psittaci* and *C.pecorum* (Martin and Aitken, 1999).

The detection of CFT in apparently healthy documented the latency of chlamydial infection in sheep. The above results were similar to that recorded by the prior workers (Ahmed, 1982; Hazarika and Dhingra, 1985 and Ishida *et al.*, 1988 and Cubero-Pablo *et al.*, 2000). Moreover Escalante *et al.* (1996) detected chlamydia psittaci in enteric subclinical infections in adult sheep.

Diagnosis of abortion caused by chlamydia psittaci var bovis could be established if the results of CFT was taken in conjunction with clinical and pathological findings (Shtefan, 1984).

The results of treatment proved marked clinical improvement and the problem ceased within three weeks, this result was in agreement to that recorded by Martin and Aitken (1999). In this context Quinlan and McGuckin (1999) treated all the ewes in a sheep flock manifesting chlamydial abortion with long acting tetracycline at 20 mg/kg, b/w and the problem ceased within two weeks.

The current result suggested that, chlamydia psittaci is wide spread both as a cause of abortion and as latent intestinal infection. The evidence of chlamydial infection among apparently healthy sheep proved the latency.

As well, from epidemiological point of view the system of breeding in Egypt facilitate contact of different animals species enhancing spread of chlamydia sp. Griffiths *et al.* (1995) who mentioned that close contact between ruminant species on the farm suggested that chlamydia psittaci strain might have been transmitted to cattle, traced such opinion from infected sheep. Also migratory sheep flock disseminate the infection along wide area.

Finally, control measures can be therefore based on accurate diagnosis and good management and hygiene as isolation of aborting ewes, cleanup and disinfect infected pens, hygienic disposal of aborted fetus and placenta, avoid crowding at lambing, avoid contact between domestic and wild sheep or other animals, or reduce the risk of intertransmission of chlamydia sp between wild and domestic ruminants through grazing on the same pastures, restriction or avoid migration of sheep flocks and recommended vaccination particularly in flocks with recurrent chlamydiosis.

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