Incidence of *Chlamydophila psittaci* In Domestic Birds in Sharkia Governorate, Egypt

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ABSTRACT

Chlamydia infections are occurring in domesticated birds. Efforts to detect and identify chlamydiae are important, because it is often accompanied with concurrent infections and variable outbreaks. Human being in contact with birds and slaughtering houses are exposed to hazards of infection. In the present study, smears from liver, lung, heart and spleen from suspected birds were examined by Giemsa stain to demonstrate the presence of chlamydia inclusions. Pooling of internal organs were inoculated in embroynated chicken eggs via yolk sac route for isolation of *Cp.psittaci* and smears from yolk sacs were subjected to Gimenez stain. The high incidence of chlamydia (92%) was detected in chickens followed by ducks (88%), turkeys (76%) and pigeons (72%).Polymerase chain reaction (PCR) and direct fluorescence antibody test (FAT) were done on 12 positive Gimenez stain samples from each species and the results revealed that the high percentage was in chickens and turkeys with percentage 91.6% in PCR reaction and (83.3%,75%) in direct immunofluorscent test in chickens and turkeys respectively followed by ducks and pigeons. Complement fixation test (CFT) was carried out on a total of 48 fecal swabs; the percentage of Chlamydia shedding in chickens, pigeons ,ducks and turkeys was 91.6%, 83.3%, 75% and 66.6% respectively. In conclusion, the high incidence of chlamydiosis in domestic birds calls for more efforts to safeguard humans from this infection.

Key words: *Chlamydophila psittaci*, isolation, PCR and direct immunofluorscent test

INTRODUCTION

Ornithosis is a naturally occurring systemic disease of domestic birds caused by *chlamydia psittaci* (Family chlamydiacaea, order chlamydiales) *(1)*. ***Everett et al., (2)*** Proposed a new classification of chlamydia into two genera and nine species based on ribosomal RNA sequence. Avian chlamydiosis can be economically devastating to producers and act as a serious health problem *(3)*. *Chlamydia psittaci* lead to outbreaks in domestic bird *(4)*.Major outbreaks have occurred in turkey and duck farms *(5)*. ***Sachse et al.*** *(6)* detected *C.psittaci* in pigeons. Natural infections have been reported in breeder, broiler and layer flocks *(7)*.Clinical presentation of *C.psittaci* infection in birds range from asymptomatic to systemic illness with severe respiratory and enteric signs. Efforts to detect and identify chlamydiae are important because chlamydiae not only cause disease but also interact synergistically with viruses as avian pneumovirus and pox virus or with other bacteria as *Campylobacter, Salmonellae* and *E.coli* which exacerbating the clinical manifestation of these organisms *(2)*. Human infections are common following handling or processing of infected turkeys or ducks. Most infections are through inhalation of infectious aerosols; thus, processing plant employees are especially at risk, as are farm workers and poultry inspectors at processing plants *(8,9)* therefore, awareness of the danger and early diagnosis are important *(10)*.Studying the importance of chlamydiosis

in poultry to ascertain its spread and to know the extent of their contribution to the economic losses to the poultry industry and if domestic birds have a role in the transmission of the disease to humans so, the study was carried out to investigate the presence of *Chlamydophila psittaci* in domestic birds from different farms at Sharkia Governorate, Egypt with emphasis on the use of cytological examination and serological investigation of this organism.

MATERIAL AND METHODS

Birds

Two hundred birds from four species (chickens, turkeys, ducks, and pigeons) were collected from different localities at Sharkia Governorate. Fifty birds from each species were tested.

Specimens

Organs (lung, liver, heart and spleen) from each bird were tested. Forty eight fecal swabs (12 from each species) were collected for complement fixation test.

Impression smears

Impression smears were prepared from internal organs and stained with Giemsa stain to demonstrate the presence of chlamydia inclusions ***(11)***

Bacterial isolation ***(12)***

Tissue homogenates were prepared and processed for inoculation via yolk sac of 6-7 day old embroynated chicken eggs according to ***(13)*** and the harvested yolk sac membranes were stained with Gimenez stain.

Direct immunofluorescence test (Biomeriux) (Ref 55321)

Direct fluorescence antibody test (FAT) enabled the detection of Chlamydia in impression smears from the inoculated yolk sac membrane according to ***(14)***. A specimen was considered positive if there were characteristic fluorescent chlamydial bodies (elementary or reticulate) over the whole surface of the smear.

Detection of *C. psittaci* antigens in the collected faecal samples by complement fixation test (CFT)

Complement fixation test was conducted according to ***(12)*** using Amboceptor (Anti-sheep red blood cell); reference antiserum of Chlamydiae for CFT (*Cp.psittaci* CF test Reagent''Seiken'') supplied commercially from Denka Sieken Co., Ltd., Tokyo, Japan. Controls were included throughout the entire tests (complement control, positive known serum control and antigen control ***(15)***.

Identification of Chlamydia species using PCR

Chromosomal DNA was extracted from the infected yolk sac membranes using QIA amp DNA mini Kit (Qiagen # 51304) ***(16)***.

Primer pairs Forward C-Psitt 5-GCTACGGGTTCCGCTCT-3 and reverseC-Psitt5 TTTGTTGATYTGAATCGAAGC-3 specific for Chlamydiales Dream Taq ™ PCR Master Mix (2X) (Thermo Scientific # K1081), Molecular weight marker: Gene Ruler ™, 100 bp ladder DNA marker (Thermo Scientific) .The PCR was run according to ***(17)***.

RESULTS

Detection of chlamydiae inclusions using Giemsa stain

From 50 birds of chickens, ducks, pigeons and turkeys impression smears stained with Giemsa stain prepared from organ samples. Chlamydial inclusions were demonstrated in 44(88%) in chickens; 41 (82%) in ducks; 34 (68%) in pigeons and in turkeys 32(64%) as shown in (Table 1).The characteristic chlamydial inclusions demonstrated in smears of different organs (liver, lung, heart and spleen) stained with Giemsa stain appeared as small, rounded reddish purple inclusions (Fig.1).

Detection of chlamydiae inclusions using Gimenez stain

Gimenez stain of membranes of yolk sac smears revealed Chlamydia inclusions as small, rounded red dots (Fig.1). The infected embryos appeared dwarfed with presence of hemorrhagic spots in the head and toes (Fig.2).Results of impression smears prepared from yolk sac of inoculated chicken embryo infected with *Cp.psittaci* are as shown in Table (1).

Detection of chlamydiae using direct fluorescence test

Chlamydia inclusions were demonstrated in impression smears from the infected yolk sac membranes using FAT technique with percentages of 83.3%, 66.6%,75% and 58.3% from 12chickens, 12ducks, 12 turkeys and 12pigeons respectively (Table 3 and Fig. 3).

Detection of C. psittaci antigens in the collected faecal samples by complement fixation test (CFT)

As shown in Table 2, eleven out of twelve (91.6%) , ten out of twelve (83.6%), nine out of twelve (75%) , and eight out of twelve (66.6%) fecal swabs collected from chickens, pigeons, ducks and turkeys respectively were positive for the presence of *C. psittaci* antigen using CFT.

Detection of *Chlamydophila psittaci* using PCR

The expected amplified product of ompA gene specific for *Chlamydophila psittaci* at 1041 bp was detected (Fig. 4). The results of PCR were 91.6% in chickens and turkeys while 83.3% in ducks and pigeons as shown in Table (3).

Table 1. Direct detection of chlamydial inclusion bodies within the examined samples

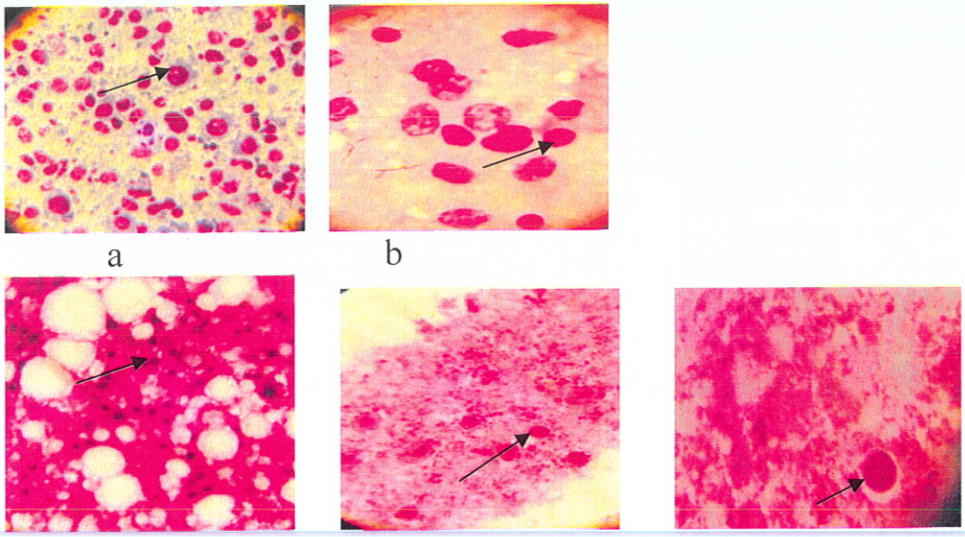
Domestic birds	Different organs Giemsa stain smears	Yolk sac Gimenez stain smears
	Positive (%)	Positive (%)
Chickens	44(88%)	46(92%)
Ducks	41(82%)	44(88%)
Pigeons	34(68%)	38(76%)
Turkeys	32(64%)	36(72%)
Total	151(75.5%)	164(82%)

Fifty bird were examined from each species

Table 2. Detection of Chlamydia psittaci antigens in the collected faecal samples of chickens, ducks, pigeons and turkeys by complement fixation test (CFT)

Type of birds	Negative titer						Positive titer						Total positive	
	1/2		1/4		1/8		1/16		1/32		1/64			
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Chickens	-	-	-	-	2	16.6	-		3	16.6	8	66.6	11	91.6
Ducks	1	8.3	1	8.3	-	-	2	16.6	4	33.3	3	25	9	75
Pigeons	-	-	-	-	-	-	-	-	1	8.3	9	75	10	83.3
Turkeys	-	-	-	-	1	8.3	-	-	-	-	8	66.6	8	66.6

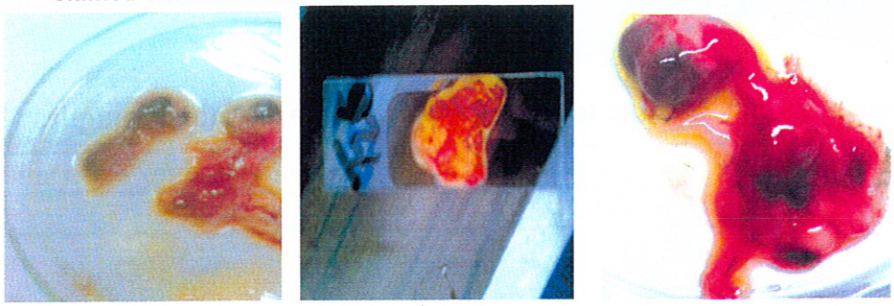
Forty eight swabs were collected 12 from each species



a b

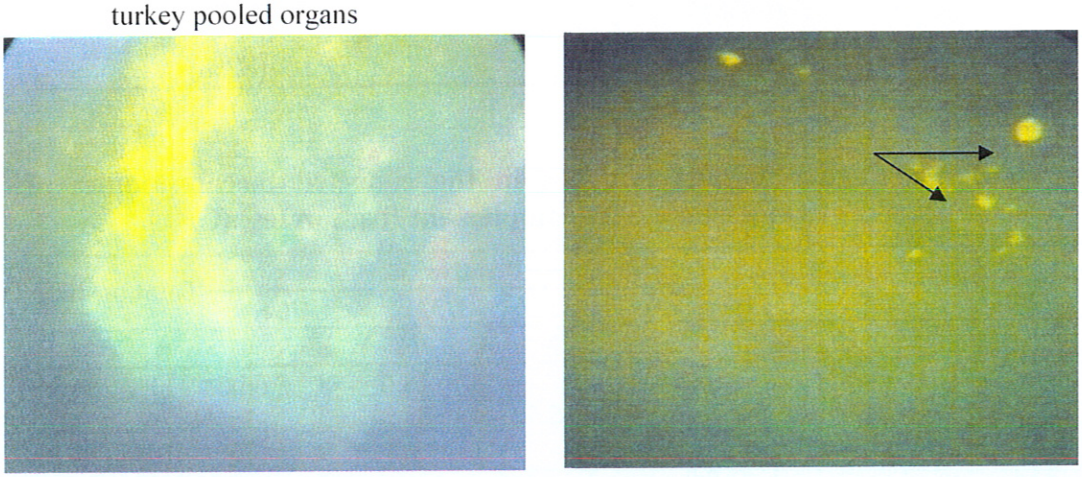
c d e

Fig. 1 Chlamydial inclusion bodies in the impression smears (a–d) Chlamydia inclusions in spleen, liver, lung, and heart impression smears, respectively of internal organs of the examined birds stained with Giemsa stain. (e) Chlamydial inclusions in the infected yolk sac membrane stained with Gimenez stain.



a b c

Fig. 2 (a and c) chicken embryos growth abnormalities: dwarfism and congestion of chicken embryos among the inoculated eggs, (b) Congested yolk sac membranes after 3 days of inoculation from turkey pooled organs



a b

Fig. 3 Chlamydial inclusions in the infected yolk sac membrane stained with FA. (a) Negative impression smear for Chlamydia after staining with FA. (b) Positive impression smears for Chlamydia using FA.

Comparative study between sero-diagnosis (CFT and FAT) and molecular identification (PCR) as shown in Table (3)

The results of PCR, FAT and CFT among the examined 12 chickens,12 turkeys,12 ducks and 12 pigeons are shown in Table (3). The expected amplified product of 16S rRNA gene specific for family Chlamydiacaea at 1041 bp was detected (Fig. 4). It is obvious that eleven (91.6%), ten (83.3%), eleven(91.6%) in chickens ,eleven (91.6%), nine (75%), eight(66.6%) in turkeys ,ten (83.3%), seven (58.3%),ten (83.3%) in pigeons and ten (83.3%),eight (66.6%), nine (75%) in ducks were positive for chlamydiosis using PCR, FAT and CFT respectively.

Comparative study between conventional methods (Giemsa stain and Gimenez stain) as shown in Table (3)

The results of Giemsa stain and Gimenez stain were compared among 50 chickens, 50 turkeys, 50 pigeons and 50 ducks. The results were forty four (88%), thirty two(64%), thirty four (68%) and forty one (82%); forty six (92%), thirty eight (76%), thirty six (72%) and forty four (88%)were positive for chlamydiosis using Giemsa stain and Gimenez stain respectively as shown in Table (4)

Table 3. Comparison of the positive percentages yielded by different diagnostic methods used for detection of chlamydiae in domestic birds samples

Test	Chickens	Ducks	Pigeons	Turkeys	Total %
PCR	11(91.6%)	10(83.3%)	10(83.3%)	11(91.6%)	87.5
FAT	10(83.3%)	8(66.6%)	7(58.3%)	9(75%)	70.8
CFT	11(91.6%)	9(75%)	10(83.3%)	8(66.6%)	79.1

Forty eight swabs were collected 12 from each species

Table 4. Comparison of the positive percentages yielded by different staining methods used for detection of chlamydiae

Test	Chickens	Ducks	Pigeons	Turkeys	Total %
Giemsa stain	44(88%)	41(82%)	34(68%)	32(64%)	75.5
Gimenez stain	46(92%)	44(88%)	36(72%)	38(76%)	82

Fifty bird were examined from each species

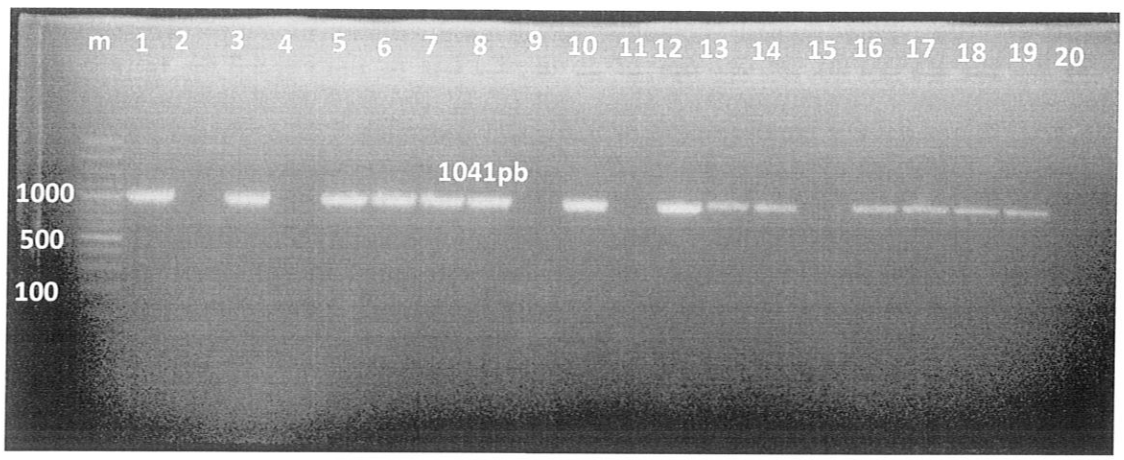


Figure 4. Molecular identification of Chlamydophila psittaci using PCR: Lane "L": 100 bp ladder (marker), **lane "1"** positive control, **lanes "3,5,6,7,8,10,12,13,14,16,17,18 and 19"** positive samples showed bands at 1041 bp **lane "2,4,9,11,15 and 20" negative samples**

DISCUSSION

Avian chlamydiosis is a zoonotic, infectious and occasionally fatal disease in domestic and wild birds ***(18-19)***.*Chlamydophila psittaci* infects many domestic birds; chickens, turkeys, ducks and pigeons ***(5,20)***. Chlamydiosis can be transmitted mainly to humans by asymptomatic birds or by birds that are obviously sick. In the present study living birds from four domestic species (chickens, ducks, pigeons and turkeys) were investigated for chlamydiosis. Using Giemsa stain chlamydial inclusions were demonstrated in chickens, ducks, pigeons and turkeys samples with percentage 88%, 82%, 68%and 64% respectively. Results showed that higher percentage of *Chlamydophila psittaci* was recorded in chickens and ducks and this could be referred to poor hygienic conditions and/or secondary bacterial infections. These results agreed with ***(21)***, who recorded the percentage of *Cp.psittaci* in chicken samples, was 77.3% using Giemsa stain and ***(22)*** who recorded the percentage of *Cp.psittaci* in ducks was 55.28%.

Culture of chlamydiae is difficult and infrequent because of the obligate intracellular nature of the bacteria ***(23)***.Cell culture or egg inoculation is the gold standard for diagnosis of chlamydiae. Isolation of viable chlamydiae requires infection of embryonated eggs or cell cultures ***(24)***. In present study positive cases were confirmed by pathological lesions encountered in the embryonic membranes of the infected embroynated chicken eggs in the form of congestion and severe engorgement of the blood vessels. Embryos appeared dwarfed with presence of hemorrhagic spots in the head and toes. Chlamydial inclusions were demonstrated in the impression smears of collected yolk sac membranes stained with Gimenez stain. Out of 50 chickens organ samples, forty six were positive (92%). out of 50 ducks forty four (88%), out of 50 pigeons thirty six (72%) and out of 50 turkeys thirty eight (76%).The higher percentage was in chickens and ducks which in ducks the high prevalence rate an indication of the bad hygienic conditions under which these birds allotted. Our results disagree with ***(25)*** who studied the prevalence of chlamydiosis among chickens by examining 125 pooled visceral organs, the isolation % was 12.0% and agree with ***(22)*** who isolated *Chlamydophila psittaci* in ducks in a percentage of 62.67% also with ***(26)*** who recorded the percentage of chlamydiosis in domestic ducks was 69.23%while in turkeys ***(27)*** recorded out of 96 pools of internal organs 79.16% were positive by Gimenez stain.

The positive Gimenez impression smears were examined by direct immunofluorscent test for detection of *Chlamydophila psittaci* antigen. Direct immunofluorescence test has been used by many researchers for diagnosis of *Chlamydophila psittaci* infection ***(28-31)***. Positive immunofluorscent test was (83.3%) in chickens, (66.6%) in ducks, (58.3%) in pigeons and (75%) in turkeys. Our results in turkeys were more or less in agreement with ***(27)*** who investigated the presence of *Chlamydophila psittaci* in turkeys in a percentage 89.4% and ***(32)*** who isolated *Cp. psittaci* antigen in turkeys in a percentage 73% using direct immunofluorescence (DIF) test.

Complement fixation test was the sero-diagnostic tool to study the shedding of *Cp. psittaci* antigen prepared from infected fecal swabs following the method of ***(12)***. Our findings after using CFT on fecal swabs the results were in chickens eleven out of 12 (91.6%). In ducks nine out of 12 (75%).Ten out of 12 in pigeons (83.3%). In turkeys eight out of 12 (66.6%) fecal swabs were positive for the presence of *Cp. psittaci* antigen using CFT. The highest percentage in chickens may be attributed to secondary bacterial infection and/or unhygienic conditions. Our results in ducks were more in agreement with ***(22)*** who made detection of *Chlamydophila psittaci* antigen from fecal swabs using CFT in a percentage (69.84%).

The ompA gene was investigated as a target DNA sequence among Chlamydiacaea ***(33)***. Most of the examined samples showed the expected amplified product specific for *Chlamydophila psittaci* at (1041 bp). Out of

the examined forty eight positive Gimenez samples twelve from each species, chlamydial ompA gene was demonstrated in eleven samples from chickens and turkeys (91.6%) and ten samples was recorded in pigeons and ducks in a percentage 83.3%.

In previous researches claimed that Gimenez stain is more practical than ELIZA and cell culture for detecting chlamydiae in birds *(34)* while FAT reported by *(35)* to be more sensitive than Gimenez stain in diagnosing chlamydiosis. The polymerase chain reaction (PCR) test reported by *(36)* has become the test of choice due to the much greater sensitivity than cell culture, immune histology, micro immunofluorescence, enzyme immunoassays *(37,38)* and embroynated chicken egg inoculation procedures *(39)*. However, isolation is known as the gold standard in the diagnosis of Chlamydiosis, even though other methods are popular *(40)*.

The present study clearly indicated the need for great awareness by laboratory diagnostic veterinarian, technicians and quarantine workers in abattoir to take great precautions against possible hazards of *Chlamydophila psittaci* infections to avoid severe respiratory manifestations. Also to prevent problems associated with this zoonostic disease as high risk from severe respiratory signs and may lead to death.

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الملخص العربي

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عدوي الكلاميديا متواجده في الطيور الداجنه ومن المهم الكشف والتعرف علي الكلاميديا لانها غالبا ما ماتترافق مع عدوى متزامنة وتتفشي بطريقه متغيره وايضا اتصال الانسان مع الطيور المصابه في المجازر يعرضه للعدوي. في دراستنا، تم الفحص بصبغه الجيمسا مسحات من الكبد والرئة والقلب والطحال من طيور تشتبه بالمرض للتعرف علي الاجسام المحورية للكلاميديا.وتم عزل الكلاميدوفيلا سيتاسي من تجمع الاحشاء عن طريق الحقن في أجنة البيض وتم عمل بصمات نسيجية من الكيس المحي وصبغت بصبغة الجمينيس. تم الكشف عن ارتفاع حالات الكلاميديا بنسبة ٩٢٪ في الدجاج تليها البط والديوك الرومية والحمام بنسبة ٨٨٪، ٧٦٪ و ٧٢٪ على التوالي. وقد أجريت اختبارات تفاعل البلمرة المتسلسل واختبار الفلوريسينت المناعي المباشر على ١٢ عينة إيجابية مصبوغه بصبغه الجمينيس من كل انواع الطيورالمصابة وكشفت النتائج أن اعلي نسبة كانت في الدجاج والديك الرومي بنسبة ٩١,٦٪في تفاعل البلمرة المتسلسل و٨٣,٣%،٧٥% في اختبار الفلوريسينت المناعي المباشر يليه علي التوالي البط والحمام. وقد تم عمل اختبار المثبت المكمل علي ٤٨ مسحه شرجيه مجمعة من الطيورالمصابه وكانت نسبه العزل في الدجاج والحمام و البط والرومي ٩١,٦% و٨٣,٣%و٧٥% و٦٦,٦% بالتتابع. وفي الختام فان ارتفاع نسبة الاصابة بالكلاميدوفيلا سيتاسي في الطيور الداجنة يدعو الي بذل المزيد من الجهد لحماية الانسان من هذة العدوي.