

STREPTOCOCCUS GALLOLYTICUS INFECTION IN PIGEONS: PATHOGENICITY AND ANTIBIOTIC SUSCEPTABILITY

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ABSTRACT

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Streptococcus gallolyticus infection is considered an important septicemic disease in pigeons especially in squabs. In this study the pathogenicity and antimicrobial sensitivity of *St. gallolyticus* were searched. 141 samples of diseased and recently dead pigeons of different ages were collected and subjected to bacterial examination in order to isolate *St. gallolyticus*. Sixteen strains were isolated and identified at recovery rate of 11.35%. All isolated strains found following biotype I. The strains were tested *In-Vitro* for antimicrobial sensitivity to 9 types of antimicrobial drugs. All strains were found highly sensitive to enrofloxacin and flumequine, followed by streptomycin and florphenicol then doxycyclin and lincomycin. A half of strains were sensitive to trimethoprim / sulphmethoxazole, while most strains show high resistance to ampicillin and erythromycin. The test indicates that, most isolated strains (11/16) have multi-drug resistance. Experimental infection with a strain of *St. gallolyticus* was studied by 2 routes (Intravenous inoculation or crop inoculation) in clinically healthy pigeons. The inoculated microbe dose not cause deaths by any of the 2 routes used for inoculation. Clinical signs as well as post-mortem lesions of experimentally inoculated pigeons by both two routes were recorded and discussed. The inoculated organism was re-isolated from inoculated birds.

Key words: *Streptococcus, Gallolyticus, Pigeons, Antibiotic susceptibility.*

INTRODUCTION

Streptococcosis is an important septicemic disease in pigeons, it is caused by *Streptococcus gallolyticus* which was formerly identified as *St. bovis* (Devriese *et al.*, 1990b; De Herdt *et al.*, 1994a and Devriese *et al.*, 1998).

Madej, (1961) described an outbreaks of streptococcal disease in five small breeding lofts in Polish.

Devriese *et al.* (1990a) isolated *St. gallolyticus* strains from different lesions in pigeons. *St. gallolyticus* was only infrequently found in the gut and faeces of pigeons without streptococcal disease. It was also isolated from the crop and the pharynx of a minority of healthy pigeons. They mentioned that, *St. gallolyticus* infection in pigeons is an unusual finding because *St. gallolyticus* appears to be rare in other birds. It was not found in the faeces of wild birds (Mundt, 1963) or in the caeca of chickens (Barnes, 1958) or turkeys (Harrison and Hansen, 1950). *St. gallolyticus* is an important component of

the intestinal flora of many mammals, especially farm animals and less often of humans. *St. gallolyticus* (*St. bovis*) can act as a pathogen and may be involved in septicemic disease and endocarditis in man and ruminants (Parker, 1978). Chadfield *et al.* (2007) investigated an outbreak of *St. gallolyticus* in a broiler flock. These investigations also demonstrated a clear heterogeneity with pigeon isolates.

De Herdt *et al.* (1992a) and Vanrobaeys *et al.* (1996) reported that, different types have been recognized within the *St. gallolyticus* species in healthy pigeons and pigeons that died from septicemia in equal frequency (five biotypes, two sub-biotypes, five serotypes and six supernatant-phenotypes). This indicates that *St. gallolyticus* is a facultative pathogen which can belong to the intestinal flora of healthy pigeons. The conditions leading to septicemia and disease are unknown.

Devriese *et al.* (1990b) mentioned that, streptococcal septicemia, is known to exist in pigeons, mainly in squabs, commonly the disease was peracute or acute

and rarely chronic. The mortality rate in untreated pigeons reached 78% and was highest in short-beak pigeons of Belgian race. Pure cultures of *Streptococci* were isolated from the parenchymatous organs and the blood in the majority of the cases. They diagnosed *St. gallolyticus* infection in 20 pigeon lofts submitted for post mortem investigation. Clinical signs were variable and ranged from hyperacute death to chronic lameness with arthritis. Lesions were generally unspecific except for single cases of muscle necrosis with purulent myositis. They found that, *St. gallolyticus* infection was an important etiology ranking next to *Salmonella*. Intravenous inoculations of *St. gallolyticus* resulted in prostration, long lasting loss of weight and polyuria. After oral inoculation no clinical signs were seen.

St. gallolyticus has been reported with septicemia in pigeons and is associated with significant lesions including extensive areas of multifocal necrosis in different organs (Devriese *et al.*, 1990a; De Herdt *et al.*, 1992b; De Herdt *et al.*, 1994a).

De Herdt *et al.* (1994b) described the most important clinical signs of *St. gallolyticus* septicaemia that include inability to fly, lameness, emaciation, polyuria and production of slimy, green droppings, and it may result in sudden death in pigeons of all ages. Also, They studied the prevalence of *St. gallolyticus* in 1056 pigeons. The microbe was isolated from the organs or joints of 106 pigeons (10 %). Other bacterial pathogens were not isolated from the samples that were positive for *S. gallolyticus*.

Van der Toorn and Lumeij, (2001) stated that, *St. gallolyticus*, formerly known as *St. bovis* is known since 1988 as a facultative pathogen of racing pigeons. In one study, *St. gallolyticus* septicaemia was diagnosed in 10% of necropsied pigeons. Since *St. gallolyticus* was also isolated from nearly 40% of clinical healthy pigeons, it is regarded as a facultative pathogen. Experimentally ampicillin, doxycycline and erythromycin have shown therapeutic effects. For the treatment of clinical cases the use of ampicillin is advocated, together with hygienic measures, such as the use of grid floors and avoiding overcrowding.

De Herdt *et al.* (1993) studied antibiotic susceptibility pattern of *St. gallolyticus* isolated from pigeons *in vitro*. *St. gallolyticus* strains were sensitive to penicillins, macrolides, lincomycin, tetracyclines, chloramphenicol and nitrofurans. However, the prevalence of acquired resistance against tetracyclines was approximately 40%. Sulphonamides and trimethoprim had little activity against *St. gallolyticus* while activity of the quinolone enrofloxacin and the aminoglycoside

antibiotics, neomycin and gentamicin were in or near to the intermediate range. The comparative efficacy of 5 antimicrobials administered via the drinking water for the treatment of experimental *St. gallolyticus* infection in pigeons was also tested. Morbidity after intravenous inoculation of *St. gallolyticus* in groups of pigeons treated with ampicillin, erythromycin, doxycycline, enrofloxacin and trimethoprim was 20%, 30%, 20%, 70% and 90%, respectively. Morbidity in an untreated control group was 90%.

Kimpe *et al.* (2002a) reported that, little information is available on antimicrobial resistance in pigeon pathogens. They studied the susceptibility of thirty-three *St. gallolyticus* strains isolated from internal organs of homing pigeons (*Columba livia*) to the antimicrobials that is most commonly used to treat pigeons. Aminoglycosides (gentamicin and kanamycin), trimethoprim and flumequine were relatively inactive against the tested *streptococci*. Acquired tetracycline resistance amounted to 85%, and lincomycin and macrolide (erythromycin) resistance to 48% and 45%, respectively, all erythromycin-resistant strains, except one, were also resistance to lincomycin. Fluoroquinolone (enrofloxacin) resistance was found in four *St. gallolyticus* strains. All strains were susceptible to ampicillin.

As far as Egypt is concerned, Mohamed and Abd El-Motelib, (2007) were able to isolate *St. gallolyticus* from 22.5% of healthy and diseased pigeons. Intravenous inoculation showed signs of inappetance, loss of weight, slimy green drops and shivering and post-mortem lesions of airsacculitis, liver abscess, mottled pancreas and spleen, ulcers and gasses in intestine, nephritis and distended heart. Orally inoculation showed signs of depression, inability to fly, loss of body weight, slimy green drops and lameness and with post-mortem lesions of congested lungs, flaccid heart, mottled pancreas, congested liver, spleen and kidneys, enteritis and arthritis in hock joint. The author noticed that, *St. gallolyticus* isolates were highly sensitive to ampicillin, enrofloxacin, erythromycin, sensitive to gentamycin, neomycin, penicillin, less sensitive to chloramphenicol, tetracycline and resistant to lincomycin and trimethoprim. Mohamed *et al.* (2009) were successful in isolation *St. gallolyticus* with ratio of 21.4% from the examined pigeons.

The present study was designed to investigate the incidence of *St. gallolyticus* in homing pigeons, antimicrobial drug sensitivity *In Vitro* to detect the most effective drugs on these isolates and to detect the pathological effects of this pathogen on healthy pigeons during experimental infection.

MATERIALS and METHODS

*** Samples:**

Specimens from liver, heart, kidneys, spleen and joints of 141 diseased or recently dead homing pigeons that expressed affection in these organs were collected for bacteriological examination to study the prevalence of *St. gallolyticus*.

*** Bacteriological examination:**

Samples were inoculated onto Slanetz and Bartley (S&B) Agar, Columbia Agar (Oxoid, Basingstoke, England) or Columbia CAN (colistine and nalidixic acid) agar (Gibco, Paisley, Scotland) with 5 % bovine blood and Brilliant Green Agar as described by Devriese *et al.* (1998). Cultures on S&B Agar and Columbia Agar were incubated for 24 h at 37°C in an atmosphere enriched with 5% CO₂. Brilliant Green Agar plates were incubated aerobically at 37°C. Uniformly pink colonies on S&B Agar were tested for resistance to NaCl by inoculation in Brain Heart Infusion broth (Oxoid) with 6.5% NaCl and for resistance to bile on Bile Aesculin Agar (Difco, Detroit, USA). Amylase production was tested by spot inoculation on Mueller Hinton Agar (Difco) or on Columbia Agar base in which the starch content was increased to 1.5%. The plates were flooded with iodine solution after overnight incubation. Ability to grow anaerobically on Rogosa Agar (Oxoid) in a H₂ and CO₂ atmosphere (Gaskit, Oxoid) was investigated. Haemolysis was looked for on Columbia Agar with ox blood. All strains were identified biochemically without difficulty as *St. gallolyticus* according to (Devriese *et al.*, 1990a, Devriese *et al.*, 1993 and Devriese *et al.*, 1998). Biotyping identification was made as described by De Herdt *et al.* (1992a). For preservation, isolates were stored frozen on brain heart infusion broth with 30% glycerol at -20°C according to Baele *et al.* (2002).

***Antimicrobial susceptibility pattern of *St. gallolyticus* strains isolated from pigeons:**

In-vitro antimicrobial susceptibility determination was tested by the single-disc diffusion method. Muller-Hinton agar (Oxoid, Basingstoke, UK) was prepared in a uniform thickness (4 mm) for testing of *St. gallolyticus* isolates. The *St. gallolyticus* strains were tested against 9 antimicrobial agents (Bioanalyse – Turkey) including: ampicillin (10 µg), florphenicol (30 µg), doxycycline (30 µg), erythromycin (15 µg), lincomycin (10 µg), enrofloxacin (10 µg), streptomycin (10 µg), flumequine (30 µg) and trimethoprim-sulphamethoxazole (1.25µg /23.75 µg). The diameters of the zones of inhibition were interpreted by referring to the table which represents the

NCCLS subcommittee's recommendation (NCCLS, 2001).

*** Experimental infections:**

*** Birds:** 30 clinically healthy pigeons at 4 to 12 months old, free of bacterial diseases, vaccinated against *Paramyxovirus 1* were collected from different owners to use in experimental infection. Two groups (10 birds for each group plus 5 birds for each group as control) were formed. Each group was housed separately in wire floored cages and received water and food *ad libitum*.

***Preparation of inoculum:** A strain of *St. gallolyticus* was grown overnight on Columbia blood agar and suspensions with a density of 0.5 McFarland scale was prepared in buffered physiological saline. The pigeons received 0.5 ml I/V containing 3.5 x 10⁷ colony forming units (cfu) according to Kimpe *et al.* (2003). The orally infected birds received in the crop 2 ml of a fully grown overnight Brain Heart Infusion broth (Oxoid) culture containing 1 x 10⁸ cfu as described by Devriese *et al.* (1990b).

***Experimental design:** Ten pigeons were inoculated intravenously (I/V) with *St. gallolyticus* strain. The strain was also used in a second experiment in which ten pigeons were inoculated orally. Two of 5 pigeons control groups, one injected I/V by 0.5 ml of sterile saline and kept as control for I/V inoculated group. The other control group of orally inoculated group was received 2 ml of sterile saline orally. Swabs from the droppings and the throats were inoculated onto Slanetz and Bartley agar in order to detect *St. gallolyticus* carriage and excretion for re-isolation the pathogen and also from heart of necropsied pigeons. The birds were observed for deaths, clinical signs, and lesions. At days 7, 14, 21, 28, and 35 post-inoculation 2 birds from each group plus one from its control were necropsied after slaughtering for observing lesions in internal organs.

RESULTS

*** Identification of *St. gallolyticus* strains:**

Sixteen strains of *St. gallolyticus* were isolated from infected homing pigeons. All *St. gallolyticus* strains appear as uniformly pink colonies on S&B Agar (Fig.1), grow on Bile Aesculin Agar and blackened this medium. None was able to grow in 6.5% NaCl. They produced a very characteristic strong amylase reaction (Fig., 2) and were able to grow anaerobically on Rogosa agar. They were not haemolytic. The biochemical characteristics of the strains were typical of the species *St. gallolyticus*. All isolated strains found following biotype I.

Table 1: Incidence of *St. gallolyticus* isolation in naturally infected pigeons.

Adult pigeons		Pigeon squabs		Total pigeons	
No.	%	No.	%	No.	%
3/32	9.38	13/109	11.93	16/141	11.35

Table 2: Incidence of *St. gallolyticus* isolates in relation to status of homing pigeons. (n=141)

Case	Adults		Squabs		Total	
	No. of +ve	%	No. of +ve	%	No. of +ve	%
Diseased	3	2.13	4	2.84	7	4.97
Dead	0	0	9	6.38	9	6.38
Total	3	2.13	13	9.22	16	11.35

Table 3: *In-Vitro* antimicrobial sensitivity of *St. gallolyticus* isolates.

Antimicrobial agent (9)	No. and % of sensitive & resistant strains(n = 16)	
	Sensitive	Resistant
Ampicillin	2 (12.5%)	14 (87.5%)
Florophenicol	13 (81.3%)	3 (18.7%)
Doxycycline	12 (75%)	4 (25%)
Erythromycin	2 (12.5%)	14 (87.5%)
Flumequine	16 (100%)	0 (0%)
Lincomycin	12 (75%)	4 (25%)
Enrofloxacin	16 (100%)	0 (0%)
Trimethoprim/ Sulfamethoxazole	8 (50%)	8 (50%)
Streptomycin	13 (81.3%)	3 (18.75%)

Table 4: Antimicrobial resistance pattern of *St. gallolyticus* (n=61).

Antimicrobial resistance	No. of sensitive strains
Enro., Flu., Floro., Str., Linc.,Dox., STX, Amp., Ery.	(Resist 0 antibiotics) 2
Enro., Flu., Floro., Str., Linc.,Dox., STX.	(Resist 2 antibiotics) 3
Enro., Flu., Floro., Str., Linc.,Dox	(Resist3 antibiotics) 5
Enro., Flu., Floro., Linc.,Dox.	(Resist 4 antibiotics) 3
Enro., Flu., Str., STX.	(Resist 5 antibiotics) 3

Enro.= Enrofloxacin, Flu.= Flumequine, Floro. = Florophenicol, Str. = Streptomycin, Linc. = Lincomycin, Dox. = Doxycyclin, STX = trimethoprim / Sulfamethoxazole, Amp. = Ampicillin and Ery. = Erythromycin.

Table 5: Experimental infection using *St. gallolyticus*.

Experiment	No. of birds	Dose	Time	Clinical signs post inoculation	Post mortem lesions	Mortality	Re-isolation of <i>St. gallolyticus</i> post-inoculation				
							7 days	14 days	21 days	28 days	35 days
Groups											
Controls	10 (5 I/V and 5 orally)	0.5 ml. of 3.5×10^7 sterile saline I/V or 2 ml of 1×10^8 sterile saline orally	35 days.	No clinical signs of disease.	No gross lesions	No mortality	-ve	-ve	-ve	-ve	-ve
							for	for	for	for	for
							all	all	all	all	all
I/V inoculation	10	0.5 ml of 3.5×10^7 cfu	35 days	Ruffled feathers, loss of weights, wing drop (Fig., 3), greenish white diarrhea and polyurea	Cloudy air-sacs (Fig., 3) hepatitis, enteritis, kidneys are enlarged in some birds (Fig., 5) and atrophied in others. round shape heart. (Fig., 6)	No mortality	+ve T., +ve C., +ve H.	+ve T., +ve C., +ve H.	+ve T., +ve C., +ve H.	-ve T., +ve C., -ve H.	-ve T., -ve C., -ve H.
Oral (crop) inoculation	10	2 ml of 1×10^8 cfu	35 days	Ruffled feathers on some birds. very slight loss in weight in two birds.	Cloudy air-sacs in one bird and no other signs.	No mortality	+veT., +veC., -ve H.	+veT., +veC., -ve H.	-ve T., +veC., -ve H.	-ve T., -ve C., -ve H.	-ve T., -ve C., -ve H.

I/V = Intravenous, T = throat, C = Cloacae and H = Heart.

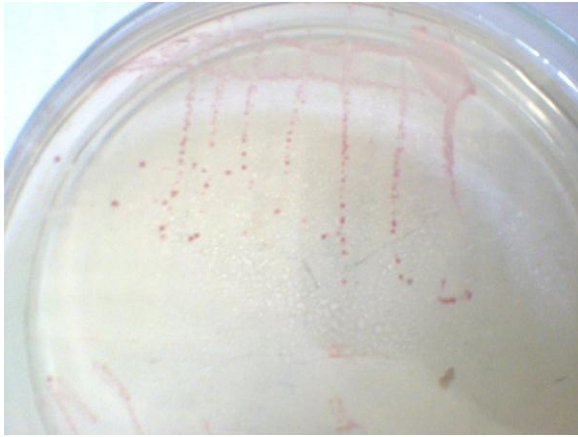


Fig. 1: *St. gallolyticus* (Pink colony on S. & B. medium).



Fig. 2: Amylase test, hallow zone around colony of *St. gallolyticus* after iodine flooding.



Fig. 3: Drop of right wing due to arthritis caused by *St. gallolyticus* inoculation.

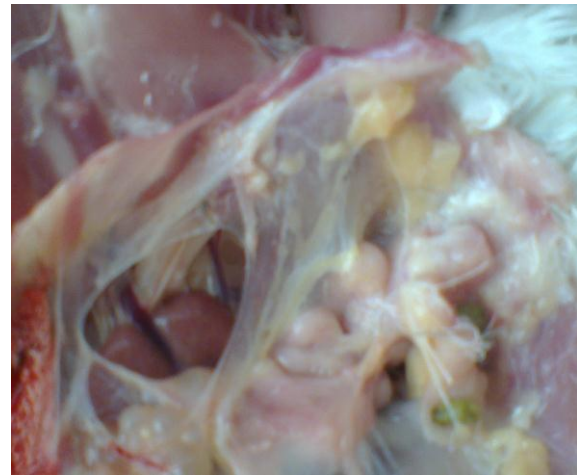


Fig. 4: Cloudy air-sacs as a result of *St. gallolyticus* inoculation.

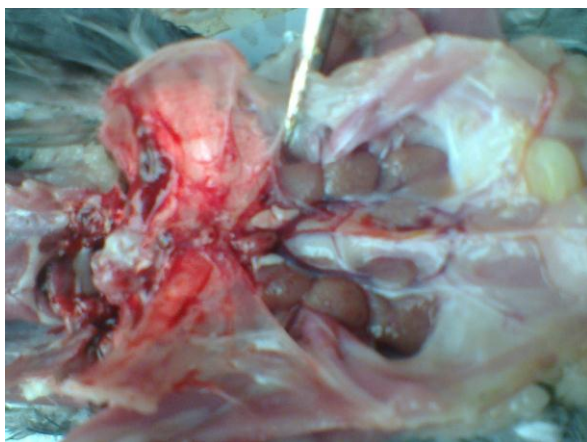


Fig. 5: Enlarged dark kidney as a result of *St. gallolyticus* inoculation.



Fig. 6: round due to *St. gallolyticus* inoculation.

DISCUSSION

1- Isolation of *St. gallolyticus* strains from the infected homing pigeons: In this study Table (1) records the total incidence of *St. gallolyticus* in examined pigeon in relation to number of adults and squabs. It revealed isolation of 16 strains of the *St. gallolyticus* with a percentage of 11.35%. This result nearly was obtained by De Herdt *et al.* (1994b) and Van der Toorn and Lumeij, (2001) who detected the organism in organs and joints of 10% of the infected pigeons. The incidence was higher in the examined squabs (13/109) with percentage of 11.93% than in adults (3/32) that isolated with percentage of 9.38%. Streptococcal septicemia is known to exist in pigeons mainly in squabs (Devriese *et al.*, 1990b). Table (2) shows the incidence of *St. gallolyticus* isolated from 141 infected pigeons in relation to status (diseased or dead) of pigeons. The disease was most prevalent in dead pigeons (6.38%) than in diseased ones (4.97%) and the organism isolated from diseased squabs (2.84%) and adults (2.13%) but isolated only from dead squabs (6.38%) and not detected in dead adults. De Herdt *et al.* (1994b) found that, isolation of *St. gallolyticus* from young pigeons is more higher than that in adults. This may be attributed to low immunity in young pigeons in comparison to that in adults. All isolated strains of *St. gallolyticus* found following biotype I. The same result was recorded by Mohamed and Abd Ell-Motelib, (2007).

2- Antimicrobial sensitivity pattern of *St. gallolyticus* strains isolated from homing pigeons: *In-Vitro* all isolated strains of *St. gallolyticus* in the present work were tested for 9 antimicrobial drugs that usually used in the treatment of pigeon diseases using disc diffusion method. The results are recorded in tables (3) & (4). Table (3) shows that all strains were highly sensitive to flumequine (100%). The same percent was recorded by Kimpe *et al.* (2002a). Also, 100% of the tested isolates were sensitive to enrofloxacin. High sensitivity of enrofloxacin was also reported by Mohamed and Abd Ell-Motelib, (2007) and with ratio of 88% sensitivity by Kimpe *et al.* (2002a) but its sensitivity was found near to intermediate rang by De Herdt *et al.* (1993). The isolated strains show high percent of sensitivity to streptomycin (81.25%), while the result was found near 50% by De Herdt *et al.* (1993). Also, the strains have high rate of sensitivity to florphenicol (81.25%), chloramphenicol as a same drug group of florphenicol was found sensitive by De Herdt *et al.* (1993) but with low sensitivity as described by Mohamed and Abd Ell-Motelib, (2007). *St. gallolyticus* strains were found sensitive to lincomycin and doxycycline with percent of (75% for each). The result for lincomycin agrees with these results recorded by De Herdt *et al.* (1993), but

Kimpe *et al.* (2002a) recorded 52% for lincomycin and acquired resistance to oxytetracycline by rate of 85%. Half of the strains (50%) were found sensitive to trimethoprim/ sulphmethoxazole, while 100% resistance was reported by Kimpe *et al.* (2002a) and Mohamed and Abd Ell-Motelib, (2007). High rate of our isolates resist ampicillin and erythromycin (87.5% for each). This result is reversed in results of Kimpe *et al.* (2002a) who detect (100%) sensitivity to ampicillin and (52%) to erythromycin, also, Mohamed and Abd Ell-Motelib, (2007) reported high sensitivity to ampicillin and erythromycin. Table (5) shows a multi-drug resistance for 11 out of 16 strains of *St. gallolyticus*. These strains show resistance for 3 or more than 3 antimicrobial drugs. The difference in results of *St. gallolyticus* drug sensitivity in different studies may be attributed to the types of drugs that used in pigeon treatment in each study.

3- Experimental infection of healthy pigeons using *St. gallolyticus* strain.

I-Intravenous inoculation (I/V) method: The I/V inoculated birds showed signs of disease after 5-7 days post-inoculation in comparison to controls. These signs were prostration, ruffled feathers, loss of body weights, drop in wings due to arthritis (Fig., 3) polyurea and greenish diarrhea. Mortality was not recorded in inoculated birds. Post-mortem lesions observed were cloudy airsacs (Fig., 4), congested liver, enteritis, enlarged kidney with nephritis in some birds (Fig., 5) and kidney atrophy in some few birds plus changes in heart of some birds (rounded heart as in Fig.,6). Re-isolation of inoculated pathogen from experimentally infected birds was successful from throats till 21 day, from hearts of necropsied birds till 21 day and from cloacae till 28 day. These results nearly agree with these recorded by Devriese *et al.* (1990b) but differ with results of Mohamed and Abd Ell-Motelib, (2007) who reported mortality and septicemia in I/V inoculated pigeons but share in changes that occurs in the heart. On the other hand Kimpe *et al.* (2002b) found that, the birds did not develop clinical disease but shed *St. gallolyticus* in their faeces after I/V inoculation by low virulence strain of *St. gallolyticus*.

II- Orally inoculated methods: Birds expressed no deaths and no signs with an exception of slight ruffled feathers and slight loss in weights in two birds. No post-mortem lesions was observed at necropsy except slight cloudiness of air-sacs in one bird. Re-isolation of inoculated pathogen was detected for 14 days post-inoculation in throats and till 21 days from cloacae. Re-isolated from heart was unsuccessful. These results go hand in hand with that of Devriese *et al.* (1990b) and differ with these results of Mohamed and Abd Ell-Motelib, (2007),

they reported body weight losses, greenish diarrhea and lameness.

Conclusion: *Streptococcus gallolyticus* infection in pigeons is a septicemic disease especially in squabs. The disease infect about 11.35% of examined pigeons. This pathogen shows gradual increase in multi-drug resistance for the traditional antimicrobial agents. So, attention must be taken in consideration to multi-drug resistance development.

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عدوى بكتيريا المكورات السبحية نوع جالوليتيكس في الحمام: التأثير المرضي والحساسية للمضادات الحيوية

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تعتبر الإصابة بعدوى بكتيريا المكورات السبحية نوع جالوليتيكس من أهم الأمراض البكتيرية التي تسبب تسمم دموي في الحمام خصوصا في الزغاليل. تم عمل دراسة على التأثير المرضي للميكروب و حساسيته للمضادات الميكروبية. وبفحص ١٤١ عينة من الحمام المريض والنافق حديثا والذي يعاني من مشاكل مرضية في الأعمار المختلفة تم عزل و تعريف ١٦ عترة من بكتيريا المكورات السبحية نوع جالوليتيكس وتبين أن كل العترات تتبع التصنيف البيوكيميائي للنوع Biotype I . تم اختبار العترات المعزولة لتحديد مدى حساسيتها لتسع أنواع من المضادات الميكروبية. وجد أن كل العترات شديدة الحساسية لـ اسينل، لاذروف، لوكس والفوموكوين يليهم الاستريبتومايسين والفلوروفينيكول ثم الدوكسيسيكلين واللينكوميسين، وتبين أن نصف العترات كانت حساسة لمادة الترايميثوبريم/سلفاكوينوأكساليين، ومن ناحية أخرى وجد أن معظم العترات مقاومة للأميسيلين والارثروميسين. أظهر الاختبار أن ١١ عترة من أصل ١٦ عترة معزولة لها مقاومات متعددة للمضادات الميكروبية. بإجراء اختبار العدوى الاصطناعية بطريق الحقن الوريدي أو حقن الحوصلة وجد أن الميكروب لا يسبب نفوق في أى من طريقتي العدوى. هذا وقد تم تسجيل الأعراض الاكلينيكية والافات التشريحية في الحمام المحقون بالطريقتين مع اعادة عزل الميكروب في كل منهما.