

Animal Health Research Institute,
Assiut Provincial Laboratory.

STUDIES ON GRAM – POSITIVE ORGANISMS IN SICK PIGEONS

(With One Table and 4 Figures)

By

HEBAT-ALLAH A. MOHAMED

and T.Y. ABD EL-MOTELIB*

*Dept. of Poultry Diseases, Fac. Vet. Med., Assiut Univ.

(Received at 17/6/2007)

دراسات علي الميكروبات الموجبة لصبغة الجرام
في الحمام المريض

هبة الله عبد الحليم محمد ، طلبه يونس عبد المطالب

تم جمع ٦٠ حمامة مريضة من أعمار ومصادر مختلفة بمحافظة أسيوط وبالفحص الداخلي لها وجد في بعضها احتقان بالكبد والطحال والتهاب بالرئة والأمعاء ووجود ديدان داخلية (اسطوانية وشريطية) في بعض الحالات. تم أخذ ٦٠ عينة من كلا من الحوصلة والكبد والأمعاء وذلك لعزل بعض الميكروبات موجبة الصبغة التي قد تصيب الحمام وقد أسفر هذا العزل بعد عمل الاختبارات البيوكيميائية عن تواجد ميكروب *Streptococcus bovis* بنسبة ٢٥% وميكروب *Staph.aureus* بنسبة ١٢,٧% وميكروب *Corynbacterium pyogenes* بنسبة ٥%. وقد صنّف ميكروب *Streptococcus bovis* أنه biotype I , serotype 2. تم إجراء العدوى الصناعية لحمام سليم عمر ٧ شهور بميكروب *Streptococcus bovis* (وذلك لارتفاع نسبة عزله) عن طريق الحقن في الوريد وعن طريق الفم. أظهرت العدوى عن طريق الحقن في الوريد حدوث تسمم (٦٠%) من الطيور في خلال ثلاثة أيام من بداية الحقن مع احتقان بكل جسم الطائر أما (٤٠%) من الطيور الباقية فقد ظهر عليهم انكماش وفقدان الشهية ونقص في الوزن مع وجود براز أخضر لزج وبعد أسبوعين من بداية العدوى الصناعية أظهر الفحص الداخلي وجود التهاب بالرئة والأكياس الهوائية واحتقان بالكبد والطحال والكلبي مع وجود برقشة بالبنكرياس وانتفاخ بالأمعاء مع وجود تقرحات بها أما العدوى عن طريق الفم فقد أظهرت نفس الأعراض السابقة بعد شهر من بداية العدوى مع وجود عرج وعدم القدرة علي الطيران وقد أمكن إعادة عزل الميكروب من الحمام المعدي صناعياً. أظهر اختبار الحساسية لميكروب *Streptococcus bovis* أن الأميسيلين والأنثروفلوكساسين والأثرثروميسين هي الأدوية الأكثر تأثيراً.

SUMMARY

Sixty sick pigeons of different ages and sources were collected from Assiut Governorate. 60 samples from crop, 60 samples from liver and 60

samples from intestine were taken for isolation of some gram positive organisms which can affect pigeons. After biochemical tests, this survey revealed the presence of *Streptococcus bovis*, *Staph.aureus* and *Corynbacterium pyogenes* at rates of: 25%, 12.7% and 5% respectively. Serotyping of isolated *Streptococcus bovis* revealed that it was biotype I, serotype 2. Experimental infection of 7 month old healthy pigeons with *Streptococcus bovis* intravenously showed septicaemia for 60% of birds through 3 days after infection and congestion of all carcasses, while the other 40% of birds revealed depression, loss of appetite, decrease of body weight, slimy green dropping. 2 weeks postinoculation the postmortem examination revealed pneumonia, airsacculitis, congestion of liver, spleen and kidneys, mottling of the pancreas with the presence of ulcer and gases in the intestine. While the orally infected pigeons showed the same previous lesions but after one month of infection, beside the presence of lameness and inability for flying. Reisolation of *Streptococcus bovis* from experimentally infected birds was succeeded. In vitro sensitivity test showed that ampicillin, enrofloxacin and erythromycin are the most effective drugs against *Streptococcus bovis*.

Key words: *Pigeons, Streptococcus bovis, Staph.aureus, Corynbacterium pyogenes, pathogenicity.*

INTRODUCTION

Streptococcus bovis (*S. bovis*) is one of gram positive organisms which isolated from pigeons and infection was diagnosed in pigeons from 20 lofts submitted for postmortem investigation. Clinical signs very variable and ranged from hyperacute death to chronic lameness with arthritis. (Devriese *et al.*, 1990)

De Herdt *et al.* (1993) reported that *S. bovis* is an important cause of septicaemia in pigeons. The clinical signs included sudden death in pigeons of all ages, inability to fly, lameness, emaciation, polyuria and production of slimy, green droppings. Lesions were found in various organs and typically included extensive well circumscribed areas of necrosis in the deep and superficial pectoral muscles, tenosynovitis of the tendon of the deep pectoral muscle and arthritis of the stifle, hock or shoulder joints.

Bailey and Scott's (1994) cited that the genus *Corynebacterium* is composed of species of bacteria which are found in man and animals, many of them are nonpathogenic and a few are pathogenic and are associated with both acute and chronic diseases. The members of this

genus other than *Corynebacterium diphtheriae* are commonly called “diphtheroids“ and the genus *Corynebacterium* is closely related to the genera *Mycobacterium* and *Nocardia* according to chemical and serological similarities in their principal cell wall antigens. The cell wall of some species is weaker at the ends, resulting in a club-like shape and during division the daughter cell can remain attached on one side resulting in Land V arrangements that are referred to as “Chinese letters“ in groups.

Chamanza *et al.* (1998) and Lu and Walker (2001) noticed that both experimental and naturally occurring infections of *S. bovis* causing acute septicemia and joint infections in racing pigeons and produced acute onset of mortality with occasional lameness, inappetence, diarrhea and the inability to fly. The postmortem examination revealed congestion of the spleen and liver with accumulations of fluid around the pectoral muscles which has been observed. In experimental inoculation of pigeons with *S. bovis*, leptomeningitis and encephalitis are common.

The principal pathogen of the genus *Staphylococcus* is *Staph.aureus*. The pathogenesis of *Staph.aureus* is complex and probably involves the synthesis of surface associated protein along with the secretion of exotoxins resulting in damaging effect on host cell. (Takeuchi *et al.*, 2001).

Kimpe *et al.* (2003) stated that *S. gallolyticus*, formerly known as *S. bovis* is the etiologic agent of Streptococcosis in pigeons which is an important septicemic disease.

The aim of this work is designed to cover the following points:-

- Survey about gram positive bacteria which affect pigeons.
- Experimental infection of the healthy pigeons by the most prevalent isolated organism to study its effect on pigeons.
- In vitro sensitivity test to show the most effective drugs on this isolated organism.

MATERIALS and METHODS

Materials:

Samples:

A total of 60 pigeons were collected (60 samples from crop, 60 samples from liver and 60 samples from intestine) from Assiut governorate and were subjected for bacteriological examination.

Media: Sheep and ox blood agar, brain heart infusion broth, nutrient agar, sugar fermentation media, mannitol salt agar, Baird-Parker agar, christensen's media and gelatin media.

Reagents and solutions: 30% Hydrogen peroxide, Voges–proskauer, rabbit plasma, 6.5% Nacl, iodine solution and starch.

stain used: Gram's Stain.

Experimental Birds:

Thirty, 7 months healthy pigeons were used in our experiment.

Antibiotic Sensitivity discs:

Penicillin (5µg), Lincomycin (2µg), tetracycline (30µg), chloramphenicol (30µg), trimethoprim (5µg), enrofloxacin (5µg), neomycin (30µg), gentamycin (10µg), ampicillin (10µg) erythromycin (15µg).

Methods:

Isolation:

Samples:

Samples from clinically healthy pigeons were taken from crop, liver and intestine and cultured on to blood agar and incubated in both aerobic environment and in 5% CO₂ for 24 hr. at 37° C.

Identification of the isolated organism:

The suspected colonies were examined for their morphology. (shape–colour–odour) and films from suspected colonies were stained by Gram's stain.

Biochemical reactions:

Differential reactions of Streptococcus species were done according to Peter *et al.* (1986) and De Herdt *et al.* (1992b) and the most important biochemical reaction of Staph. species were done according to Ellen *et al.* (1994) and Kirk Skeeles (1997), while for Corynebacteria species the biochemical tests were done according to Merchant and Packer (1961) and to Bailey and Scott's (1994).

Serotyping of S.bovis was done according to De Hrdt *et al.* (1994a).

Pathogenicity test:-

Thirty, 7 months old healthy pigeons were used and 5 birds from them were tested before experiment to prove that they free from infection and other birds were divided as follow:

1st group: ten, 7 months old pigeons inoculated with 0.5 ml of S.bovis broth containing 6.5×10^6 CFU (McFarland tube N. 0.5) according to Devriese *et al.* (1990) and De Herdt *et al.* (1992_a).

2nd Group: ten, 7 months old pigeons inoculated orally with 2 ml of fully grown overnight brain heart infusion broth culture containing 1.2×10^8 CFU (according to Devriese *et al.*, 1990).

3rd group: Five, 7months old pigeons were left as control.
Reisolation of *S. bovis* from experimental pigeons were carried out.

In vitro sensitivity test:

The determination of sensitivity of the isolated organism against different antibiotic discs was done according to the technique of De Herdt *et al.* (1993).

RESULTS

Postmortem examination of the collected pigeons revealed pneumonia, congestion of liver, spleen and kidneys and the presence of some internal parasites.

Bacteriological examination showed 3 different colonies of organisms. The first type of colony was grey white in colour and Gram's stain revealed gram-positive, spherical bacteria occurring in pairs or short chain. Biochemical reactions showed that the organism was positive for Voges Proskauer, ferment lactose, glucose, galactose, fructose and maltose. The organism was not able to grow on 6.5 % NaCl. But it produced a very characteristic amylase reaction. The second type of colony was opaque white to gray pinpoint and haemolytic. Gram's stain revealed gram-positive pleomorphic bacilli and tendency to form clumps. Palisade arrangements are frequently observed. Biochemical tests showed that the organism non motile, catalase positive, liquefied gelatin and lactose, galactose, dextrose and maltose positive and litmus milk was acidified and coagulated. The organism was negative for Voges Proskauer and methyl red tests. The third colony produce beta haemolysis and pigmented white to orange on nutrient agar. On Baird. Parker agar media, the colony was black surrounded by faint yellow zone and on mannitol salt agar, the colony appears yellow in colour. Gram's stain appeared gram-positive cocci found in clusters. Biochemical reactions revealed: coagulase positive, liquefied gelatine, ferment mannitol, maltose, sucrose and glucose, catalase and Voges Proskauer positive.

Serotyping of *S. bovis* revealed that the isolates belong to biotype I, serotype 2.

According to the cellular and colonial morphology and biochemical reactions, the frequency of the isolates was: *S. bovis* 46/180 isolates (25 %), *Corynebacterium pyogenes* 19/180 isolates (5 %), and *Staph. aureus* 23/180 isolates (12.7 %)

So we used *S. bovis* for pathogenicity test because it was more prevalence.

Pathogenicity test:

6 pigeons in group I which inoculated intravenously showed septicaemia during first 3 days postinoculation (P.I) and death with congestion of all carcasses (Fig. 1) while the other 4 birds showing inappetance, depression, decrease in the body weight, slimy green droppings and 2 pigeons showing shivering. 2nd week PI, the postmortem examination of these birds showing abscess in the liver (Fig. 2), mottling of the pancreas and spleen, presence of ulcer and gases in the intestine (Fig. 3), congestion of kidneys, distension of the heart, airsacculitis (Fig. 4).

Pigeons in group II inoculated orally showing depression and decrease in body weight, slimy green droppig at the 2nd week PI. At the end of the experiment (one month), the birds showed lamness and couldn't fly. Postmortem examination showed congestion of lungs, flaccid heart, mottling of the pancreas, congestion of liver, spleen and kidneys, enteritis and inflammation of hock joint.

Neither signs nor lesions or deaths were recorded in birds of group III.

Reisolation of *S. bovis* from crop, liver, spleen, kidney and intestine from experimentally infected birds was successful.

In vitro sensitivity test:

The effect of the different antibiotics on the isolated *S. bovis* is illustrated in Table 1.

Table 1: Illustrated in vitro sensitivity test:-

Antibiotic discs	Sensitivity of <i>S. bovis</i> isolates
Ampicillin	+++
Enrofloxacin	+++
Erythromycin	+++
Gentamycin	++
Neomycin	++
Penicillin	++
Chloramphenicol	+
Tetracycline	+
Lincomycin	-
Trimethoprim	-

+++ sensitive
++ weak sensitive

++ moderate sensitive
- resistant.

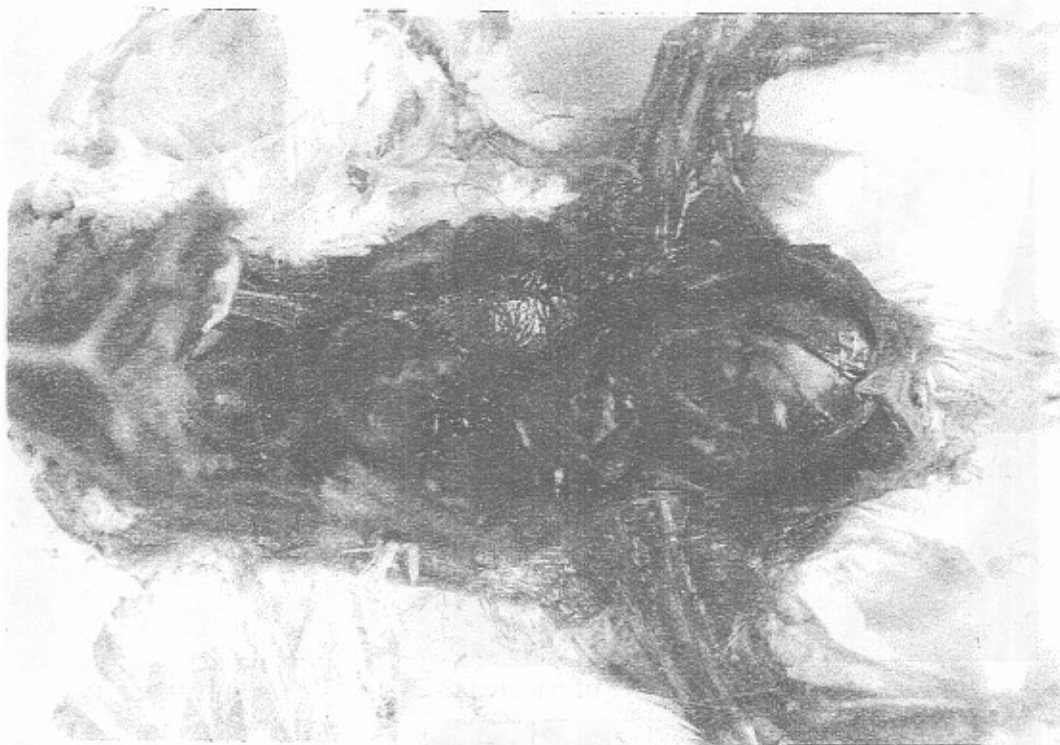


Fig 1: Congestion of all carcass.

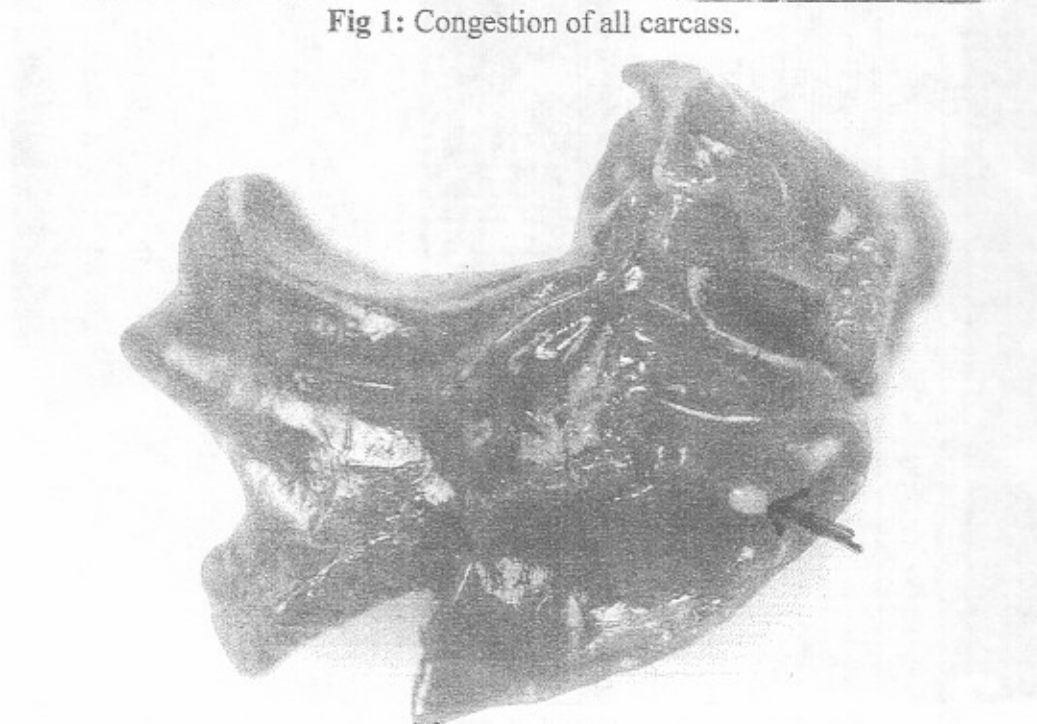


Fig 2: Abscess formation in liver after 2 weeks of infection.

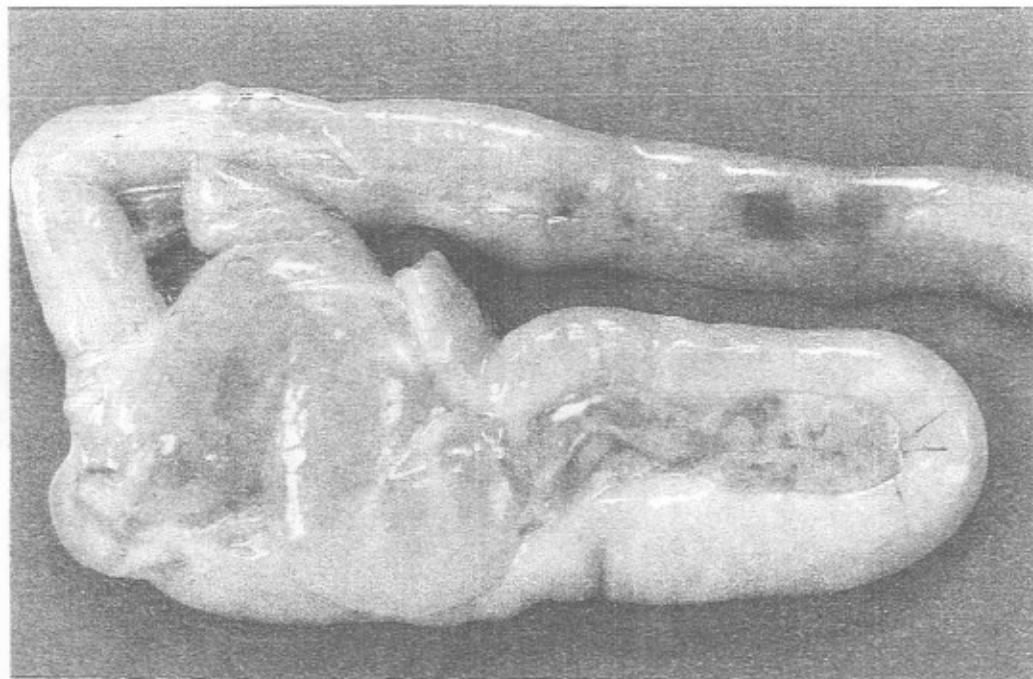


Fig. 3: Showing mottling of pancreas and ulcer in the intestine.



Fig. 4: Slight fibrinous pericarditis, perihepatitis and air sacculitis.

DISCUSSION

In this study we make survey on some gram-positive bacteria which can affect pigeons.

During a systematic and detailed study of pigeons, septicemic cases presented during 1988, found that *Streptococcus bovis* infection was an important aetiology ranking next to *Salmonella* (Devriese *et al.*, 1990).

Streptococcus gallolyticus, formerly known as *S. bovis*, is considered a facultatively pathogenic agent and may be part of the normal intestinal flora in pigeons. Predisposing factors which are hitherto largely unknown may cause some of the *S. gallolyticus* strains to be break through the intestinal tract and enter the blood stream (Vanrobaeys *et al.*, 2000 and Kimpe *et al.*, 2003).

In our study, post mortem examination of the sick pigeons showed pneumonia, congestion of liver, spleen and kidneys, enteritis and some birds have internal parasites.

Bacteriological examination of these pigeons revealed isolation of *S. bovis* (25%), *Staph aureus* (12.7%) and *Corynbacterium pyogens* (5%). The rate of *S. bovis* (25%) in our study is less than that recorded by De Herdt *et al.* (1993) who isolated *S. bovis* at rate of 40% from the apparently healthy pigeons of all ages.

Experimental infection of *S. bovis* isolates to 7 months old pigeons intravenously revealed septicemia, congestion of all carcass and slimy green dropping, this result is inagreement with that observed by De Herdt *et al.* (1994_b). The gross lesions appeared abscess in the liver, distension of the heart, congestion of kidneys, mottling of the spleen, airsacculitis and enteritis, our result is resemble that observed by Devriese *et al.* (1990) but we also noticed beside these lesions mottling of the pancreas and gases filled the intestinal tract.

Orally infected pigeons showed depression, decrease in body weight, lameness and inability for flying, our result is resemble more or less that occurs in naturally infected pigeons. But on the other side is differ with that observed by Devriese *et al.* (1990) who mentioned that no clinical signs were observed in their inoculated piogeons.

We succeeded to reisolate *S. bovis* from experimentally infected birds and this result is inagreement with that observed by Devriese *et al.* (1990), De Herdt *et al.* (1993) and Kimpe *et al.* (2003).

In vitro sensitivity test we found that ampicillin, enrofloxacin and erythromycin are the most effective drugs against *S. bovis*, our

result is the same as that result noticed by De Herdt *et al.* (1994) who showed that ampicillin, doxycycline and erythromycin are the most effective drugs.

Our conclusion in this study proved that *S.bovis* is a normal intestinal flora of clinically healthy pigeons and factors predisposing to the disease are not known but some indications were found for a possible relationship between the occurrence of *S.bovis* septicemia and poor hygiene on pigeon-lofts and also any stress factors as intestinal parasites causing *S.bovis* break through the intestinal tract and enter the blood stream.

So intravenous inoculation of *S. bovis* resulted in signs and lesions which resembled those of the chronic forms of the spontaneous disease.

Prevention of infection requires reducing the stresses and preventing immunodepressive diseases and conditions. Proper cleaning and disinfection can also reduce environmental Streptococcal resident flora to minimize external exposure reduces the total count of *Streptococcus* spp.

Also inability to fly is a fairly common problem in female pigeons during the breeding season and is often attributed to hypocalcaemia so must be differentiated from *S. bovis* infection.

REFERENCES

- Bailey and Scott's (1994):* Diagnostic Microbiology 9th Edition. p. 462-466 (part 4).
- Chamanza, R.; Febri, T.H.F. and Van Veen, E. (1998):* Enterococcus-associated encephalomalacia in one week-old chicks. *Vet. Rec.* 143: 450-451.
- De Herdt, P.; Desmidt, M.; Haesebrouck, F. and Devriese, L.A. (1992a):* Experimental *Streptococcus bovis* infections in pigeons. *Avian Dis.*, 36: 916-925.
- De Herdt, P.H.; Aesebrouck, F.; Devriese, L.A. and Ducatelle, R. (1992b):* Biochemical and antigenic properties of *Streptococcus bovis* isolated from pigeons. *J. Clinical Microbiol.* 30: 2432-2434.
- De Herdt, P.; Devriese, L.A.; Brigitte De Grootte; Ducatelle, R. and Haesebrouck, F. (1993):* Antibiotic treatment of *Streptococcus bovis* infection in pigeons. *Avian pathology*, 22, 605-615.

- De Herdt, P.; Haesebrouck, F.; Ducatelle, R.; Groote, B. and Devriese, L.A. (1994a):* Streptococcus bovis infections in pigeons: Virulence of different serotypes. *Vet. Microbiol.* 41: 321–332.
- De Herdt, P.; Ducatelle, R.; Haesebrouck, F.; Devriese, L.A.; Brigitte De Groote and Roels, S. (1994b):* An unusual outbreak of Streptococcus bovis septicaemia in racing pigeons (*Columba Livia*). *Veterinary Record* 134, 42–43.
- Devriese, L.A.; Uyttebroek, E.; Gevaert, D.; Vandekerckhoue, P. and Ceysens, K. (1990):* Streptococcus bovis infection in pigeons. *Avian Pathology.* 19: 429–434.
- Ellen, J.O.; Baron Lance, Peterson, R. and Sydney, M. Finegold (1994):* Diagnostic Microbiology P. 321–331 9th Ed.
- Lu, L. and Walker, W.A. (2001):* Pathologic and Physiologic interactions of bacteria with the gastrointestinal epithelium. *Am. J. Clin. Nutr.* 73: 1124 S–1130 S.
- Kimpe, A.; Decostere, A.; Hermans, K.; Mast, J. and Haesebrouck, F. (2003):* Association of Streptococcus gallolyticus strains of high and low virulence with the intestinal tract of pigeons. *Avian Diseases* 47: 559–565.
- Kirk Skeeles, J. (1997):* Staphylococcosis. *Diseases of Poultry* 10th Ed. B.W. Calnek, ed. Iowa State Univ. Press, Ames, I.A. P.P. 247–253.
- Merchant, I.A. and Packer, R.A. (1961):* *Veterinary Bacteriology and virology.*
- Peter, H.A.; Smith, Micholas, S.; Marir, S.; Elisabeth Sharpe, M. and John, G. Holt (1986):* *Bergey's Manual of Systematic Bacteriology, Vol. 2.*
- Takeuchi, S.; Meada, T. and Hayakawa, Y. (2001):* Variation of the agr locus in Staphylococcus aureus isolates from cows with mastitis. *Vet. Microbiology.* 79, 267–274.
- Vanrobaeys, M.; Haesebrouck, F.; Ducatelle, R. and De Herdt, P. (2000):* Adhesion of Streptococcus gallolyticus strains to extracellular matrix proteins. *Vet. Microbiol.* 74: 273–280.