Assiut Vet.Med. J. Vol. 51 No. 106 July 2005

Dept. of Poultry Diseases,
Fac. of Vet. Medicine, Assiut University

RIEMERELLA ANATIPESTIFER INFECTION ACCOUNTS FOR MAJOR ECONOMIC LOSSES TO MEAT DUCKS IN UPPER EGYPT

(With 4 Tables and 6 Figures)

By

R.S. IBRAHIM and M.W. ABD AL-AZEEM*

*Dept. of Vet. Microbiology, Fac. of Veterinary Medicine, South Valley University

(Received at 18/6/2005)

عدوى ميكروب الرايميريلا أناتيبيستيفر كأحد أهم مسببات الخسائر الاقتصادية لبط التسمين فى مصر العليا

رجب سيد ابراهيم ، محمد وائل عبد العظيم

تمت دراسة الاصابه بميكروب الرايميريلا أناتيبيستيفر فى البط فى الفترة منذ عام ٢٠٠٢ وحتى عام ٢٠٠٤ فى محافظتى أسيوط والمنيا. لوحظت الاعراض المرضية على البط الصغير فى شكل افرازات من الانف والعين، ضعف النمو، خمول، أعراض عصبية مع عدم القدرة على الحركة. بينما البط الاكبر من ١٠ أسابيع سجلت الاعراض على شكل التهاب وتضخم الجيوب الانفية وضعف النمو واضطراب الحركة. عند اجراء الصفة التشريحية ظهرت درجات متفاوتة من التهاب الأغشية السيروزيه مثل غشاء التامور، الغشاء الكبدى والأكياس الهوائية مع التهاب المفاصل. أظهر العزل البكتيرى نسبة اصابه تراوحت من ١٠-١٢% من البط الصغير، بينما كانت النسبة أقل فى الاعمار الاكبر وتراوحت من ٣,٣ - ٦%. باجراء الفحص السيرولوجى باختبار الترسيب فى الأجار تم التعرف على الأنواع السيرولوجية ٢، ٥. تراوحت نسبة النوع السيرولوجى ٢ من العزلات الايجابية بين ٣٤,٦٩% فى البط الصغير و٤٣,٧٥% فى البط الكبير، بينما تراوحت نسبة النوع السيرولوجى ٥ من العزلات الايجابية بين ١٤,٢٨% فى البط الصغير و٩,٣٧% فى البط الكبير. أما الأنواع التى لم يتم تصنيفها سيرولوجيا فمثلت نسبة ٥١% فى البط الصغير و٤٦,٨٧% فى البط الكبير. عند اجراء العدوى التجريبية عن طريق الحقن العضلى سجلت نسبة الاصابة ١٠٠% ونسبة النفوق ٩٠% بعد اليوم السابع من الحقن. لوحظ نفوق عدد ٢ من الطيور بعد ٢٤ ساعة من الحقن بشكل فوق حاد، أما عند الاعداء عن طريق الأنف كانت نسبة الاصابة ٨٠% ونسبة النفوق ٢٠%، أما عند العدوى عن طريق الفم فكانت نسبة الاصابة والنفوق ٤٠%، ١٠% على التوالى. تشابهت الأعراض الاكلينيكية والأفات التشريحية مع الصورة الطبيعية للمرض باستثناء التهاب الجيوب الأنفية. بالنسبة للنفوق فوق الحاد لوحظ الأنزفة على القلب ، الكبد، الطحال والرئتين. عند دراسة مدى تأثير أقل جرعة مثبطة من المضادات الميكروبية على ميكروب الرايميريلا أناتيبيستيفر وجد أن البنسللين، الأموكساسيللين، الانروفلوكساسين، اللينكوسبكتين، الأوكسي

تتراسيكللين والسيفالوسبورين هى الأكثر تأثيرا، بينما لم تؤثر مركبات الاستريبتوميسين، الجنتاميسين والسلفادايميثوكسين. تم توصيف البلاسميد فى العترات التى أظهرت مقاومة ضد المضادات الميكروبية باستخدام طريقة الاستخلاص القلوى. لوحظ تشابه فى البلاسميد المعزول من النوع السيرولوجى ٢ وكان الوزن الجزيئى ٤ ميجادالتون. كما تم عزل عدد ٢ بلاسميد من النوع السيرولوجى ٥ وزنهم الجزيئى ٢,٤، ٣,٥ ميجادالتون. فقط عترة واحدة من العترات التى لم يتم تصنيفها سيرولوجيا اكتسبت عدد ٢ بلاسميد وزنهم الجزيئى ٩، ١٠,٤ ميجادالتون.

SUMMARY

Riemerella anatipestifer (*R. anatipestifer*) infection in ducks was studied in Assiut and El-Menia Governorates since year 2002-2004. Clinical picture of the infection was reported in ducklings aged 1-8 weeks old as occulonasal discharges, poor growth, anorexia while nervous manifestation and ataxia. In older ages (10-18 weeks), signs noticed as sinusitis, poor growth and incoordination. Bacteriological examination of clinically diseased ducklings revealed 10-12% positive cases, while in older ages the percentage of positive cases was lower (3.3-6%). At necropsy, lesions showed variable degrees of serositis (pericarditis, perihepatitis, and airsacculitis), and swollen joints. Serotyping using agar gel precipitation test (AGPT) revealed isolation of serotypes 2 and 5. Serotype 2 represented 34.69% and 43.75% of positive cultures isolated from ducklings and older age respectively. Serotype 5 represented 14.28% and 9.37% of positive cultures isolated from ducklings and older age respectively. Untypable strains represented 51.0% and 46.87% of positive cultures isolated from ducklings and older age respectively. On experimental infection via the intramuscular (I/M) route, 100% morbidity and, 90% mortality rate by day 7 post inoculation were recorded. Two birds died peracutely with septicemia within 24 hours post inoculation. In case of intranasal (I/N) route of infection, 80% morbidity and 20% mortality rates were recorded by day 7 post inoculation. Oral route challenge displayed lower morbidity and mortality rates (40% and 10%). The challenged birds showed clinical picture and necropsy lesions similar to natural infection with exception of sinusitis after 48 hours post inoculation. Peracutely dead birds showed progressed hemorrhages on the heart, liver, spleen and lung. Minimum inhibitory concentration (MIC) of tested antimicrobials showed susceptibility of *R. anatipestifer* isolates to penicillin, amoxicillin, enrofloxacin, lincospectin (lincomycin-spectinomycin), oxytetracycline, and cephalosporin. Complete

resistance to aminoglycosides (streptomycin,gentamicin) and sulfadimethoxine was demonstrated. Plasmid profile analysis of antimicrobial resistant isolates showed high rate of plasmid acquisition. Similar plasmid was detected in isolates of serotype 2 with molecular weight (MW) of 4 Megadaltons (MDa). Plasmids of MW 2.4 and 3.5 MDa were detected in two isolates of serotype 5. Only one strain of tested untypable isolates possessed two plasmids with high MW of 9 and 10.4 MDa.

***Key words**: Riemerella anatipestifer, Infectious serositis, ducklings, sinusitis, plasmid.*

INTRODUCTION

Riemerella antatipestifer (*R. antatipestifer)* is a gram negative bacterium that causes disease in a wide variety of wild and domestic birds. This disease has been reported mainly in ducks, turkeys, chickens, quails, pheasants and water fowl Pierce and Vorhies, (1973); Smith *et al.*,(1987) and Sandhu and Rimler, (1997). In Egypt, Ibrahim (1991) reported on isolation of *P. anatipestifer* from native breed of ducks with sinusitis and respiratory infection. Ibrahim and Sohair (2000) recovered *R. antatipestifer* from duckling and turkeys as one of multifactor causative agents causing sinusitis. The exact route of *R. antatipestifer* infection is unknown. Hendrickson and Hilbert (1932) produced infections via the inravenous route. Graham *et al.*, (1938) reproduced the infection via the intraperitoneal, intravenous and interatracheal routes. Hatfield and Morris (1988) also reproduced infection via oral, nasal and intramuscular routes. *R. antatipestifer* infection is a contagious disease of domestic ducks, turkeys and various birds. It occurs as an acute or chronic septicemia characterized by fibrinous pericarditis, perihepatitis, airsacculitis, caseous salpingitis and meningitis. The respiratory tract may also be infected without showing clinical signs. *R. antatipestifer* infection incriminated in major economic losses to the duck industry due to high mortality, weight loss and condemnations (Sandhu and Rimler, 1997). The causative bacterium was isolated and characterized by Hendrickson and Hilbert (1932) who called it *Pfeferella antatipestifer*. Burner and Fabricant (1954) concluded that the organism had more in common with Moroxella species. Then classified as genus *Pasteurella* according to genetic and DNA homology (Mannheim,1984). Segers *et al.*.(1993)

reported significant differences suggested placing this organism in a separate genus *Riemerella.*

MATERIALS and METHODS

Surveying *R. antatipestifer* infection:

This survey was carried out in Assiut and El-Menia Governorates during years 2002 - 2004. A total of 1070 ducks were examined, 450 young ducklings at age ranged from 1-7 weeks suffered from variable mortalities, ataxia, respiratory sings, and 620 ducks at age ranged from 10-18 weeks suffering from sinusitis and occulonasal discharges. Young birds usually displayed high mortalities, especially at 1-3 weeks of age that may extend to 6 weeks of age. Necropsy examination of moribund birds revealed pericarditis, perihepatitis and airsacculitis. Such cases were subjected for bacteriological examination of *R. anatipestifer*.

Isolation, bacteriological examination and biochemical reactions:

Living birds with suspected sings and sinusitis were examined by nasal, ocular and tracheal swabbing and subsequent culturing on tryptone soy agar (TSA) supplemented with yeast extract, at 37°C in candle jar for 24-48 hours. Dead birds with serositis were examined bacteriologically by isolation from liver, heart, spleen, kidneys, lung and air sacs. Tissue impression smears were carried out for bipolar Intercellular bacilli as a guide for process of isolation. The produced colonies were identified morphologically, biochemically as well as sugar fermentation reactions.

Serotyping:

The bacteriologically suspected isolates were selected for serological identification using agar gel precipitation test (AGPT) according to Brogden *et al.*, (1982). Antigen was prepared from whole bacterial cells heavily seeded on TSA plates by heat extraction (autoclaving of formalized saline suspension) and tested against antisera prepared by rabbit hyper immunization with serotypes 1,2,3 and 5 according to Ibrahim (1991).

Experimental infection:

A group of 45 day-old white pekin ducklings were obtained from conventional source and reared to 14 days of age. One isolate representing the isolated serotypes (serotype 2) was used for experimental infection through three routes (I/M, I/N and oral). The challenge isolate were grown on TSA plate at 37°C for 24 hours in

candle jar. The produced colonies were inoculated in brain heart infusion broth (BHI, DIFCO lab.Detroit,MI), incubated at 37°C in shaking water bath (120 rpm). The final inoculum contained 10^6 CFU/ml. The control group was treated by sterile BHI broth via the three applied routes.

For this study 45 ducks aged 14 days were divided into 4 groups. The first three groups each of 10 birds while the 4th one of 15 birds and subgrouped into three groups each of 5 birds (control group) first group was inoculated intramuscularly with 0.5 ml of broth culture containing 10^6 CFU/ml of *R. antatipestifer*. Second group was intranasally infected with the same culture, while the 3rd group was given 0.5 ml of broth culture orally. In parallel way control birds were treated by sterile broth via I/M, I/N and oral routes. Ducks were visually observed for clinical sings. Dead birds were necropsies and sample was monitored for 7 days for postmortem and bacteriological examination. Results are recorded in table 3.

Minimum inhibitory concentration(MIC):

MIC of 9 antimicrobials as listed in table 4., were determined by agar dilution method (Ishiyama *et al.*,1968). A 10^{-2} dilution of 10 hours trypticase soy broth (TSB) supplemented with 0.3% yeast extract was inoculated by micropipette on Muller Hinton agar, (Difco) containing serial two fold dilution of the tested antigens. The agar plates were incubated at 37°C in candle jar for 24 hours. The MIC was defined as the lowest concentration of antimicrobials that prevented bacterial growth.

Plasmid profile:

Alkaline lysis method of Birnboim and Doly (1979) were used for plasmid DNA extraction. The strains used were the resistant strains to antimicrobials to make the correlation between antibiotic resistance and plasmid acquisition. Electrophoresis was done in 0.7% agarose combined with ethidium bromide. DNA ladder marker (Supercoild DNA Laddar, Sigma) was used with MW (1.3; 1.9; 2.6; 3.2; 3.9; 4.5; 5.1; 6.5; 7.8; 9 and 10.4 Mda).

RESULTS

Surveying *R. antatipestifer* infection:

Ducklings (1-7 weeks of age), mortality rates about 18-30%. About 200 native ducks and 250 pekin duckling were examined for *R. antatipestifer* infection through bacteriological examinations, 24 and 25

positive cases were recorded with percentage of 12 and 10 % respectively.

Out of 300 ducks (native breed), 120 muscovy ducks and 200 white pekin ducks suffering from sinusitis (Fig.A), decreased body weight, locomotor disturbances and arthritis 18, 4 and 10 bacteriological positive cases for *R. antatipestifer* was isolated with percentage of 6, 3.3 and 5% respectively. The ages were ranged from 10-18 weeks (Results are shown in table 1). Died birds showed pericarditis, perihepatitis, and airsacculitis. Clinical sings appeared as poor growth, locomotor disturbances, ruffled feathers, anorexia, hunched up, and respiratory sings, nasal discharges and ocular secretions. Results are illustrated in table (1).

Bacteriological examination:

Produced colonies were transparent, glistening and butyrous on TSA and iridescent. Microscopic examination demonstrated gram negative, short bacilli. In recent culture *R. antatipestifer* was bipolar. The growth was enhanced by reduced O_2 tension and addition of yeast extract. It is non motile on semisolid agar tubes.

Biochemical reactions:

Slow alkaline change of litmus milk, negative growth on MacConky's agar, non-hemolytic on blood agar. Nitrate reduction and indole production were negative, while urease, oxidase and catalase tests were positive. Most of isolates could not ferment sugars (glucose, fructose, maltose, sucrose and lactose).

Serotyping:

A panel of antisera used included antisera against serotypes 1,2,3, and 5. None of isolated strains belonged to serotypes 1 and 3. Serotyping using AGPT revealed isolation of serotypes 2 and 5. Serotype 2 represented 34.69% and 43.75% of positive cultures isolated from ducklings and older age respectively. Serotype 5 represented 14.28% and 9.37% of positive cultures isolated from ducklings and older age respectively. Untypable strains represented 51.0% and 46.87% of positive cultures isolated from ducklings and older age respectively.

Percentage of isolates in relation to totally examined birds; Serotype 2 represented 17/450 (3.77%) and 14/620 (2.25%) from ducklings and older ages respectively. Serotype 5 was identified at lower rate from both young and adult duck with percentage of 1.55% (7/450) and 0.48% (3/620) respectively. Twenty-five isolates out of 450 (5.55%) from

duckling and 15 out of 620 (2.41%) from ducks aged more than 8 weeks (10-18) were untypable strains.

Totally 10.88% (49/450) were identified from duckling aged from 1-8 weeks while 5.2% (32/620) were identified from ducks aged more than 8 weeks until 18 weeks of age. Results are listed in table 2.

Experimental infection:

Three routes were used for experimental challenges I/M, I/N installation and oral route. In case of I/M infection morbidity rate was higher than other two routes and reached 100% while it was 80% and 40% in case of I/N and oral routes respectively. Two birds died peracutely after intramuscular infection with septiceamic lesions on heart muscle, coronary fat and subserosal area of liver as well as spleen (Fig.D). Mortality rates by day 7-post infection were 90%, 20% and 10% respectively. Clinical picture produced were sticky ocular discharges due to intranasal instillation (Fig.B), diarrhea, and ataxia as well as, decrease in body weight with ruffled feathers, anorexia, and hunched up (Fig.C).

Necropsy findings included as septicemia in rapid onset death (peracute death shortly after 24 hours post inoculation). After 48 hours post inoculation pericarditis, perihepatitis, airsacculitis, and congested liver were recorded (Fig.E). No signs or lesions were noticed in control birds. Reisolation was at higher rates from liver, followed by heart and spleen then kidney and lower Percentage of isolation was from lung. Results are listed in Table (3)

Minimum inhibitory concentration:

Different antimicrobials used for determination of MIC against 10 antimicrobial resistant isolates of *R. antatipestifer*. Complete resistance to aminoglycosides (streptomycin, gentamicin) and sulfadimethoxin was demonstrated. While complete susceptibility to penicillin, amoxacillin, enrofloxacin, lincospectin, oxytetracycline and cephalosporin, was recorded.

Plasmid profile:

Similar plasmids with molecular weight of 4 MDa were detected in 4 tested isolates belonged to serotype 2. Plasmid of molecular weight of 2.4 and 3.5MDa were detected in isolates of serotype 5.The untypable strains (3 isolates tested) only one isolate showed two high molecular weight plasmid of 9 and 10.4 Mda.

DISCUSSION

R. anatipestifer infection is an important disease in water fowl, especially in ducks. Though various studies have looked at the transmission of *R. anatipestifer* (Asplin, 1956; Graham *et al.*, 1938; and Hatfield *et al.*, 1988), the exact route of infection is still debatable.

In this study *R. anatipestifer* infection in ducks at Assiut and El-Menia Governorates were investigated from two age groups (ducklings of 1-8 weeks & ducks of 10-18 weeks) since year 2002-2004 during summer season. The clinical picture was observed as occulonasal discharges, increased mortalities, ataxia and poor growth rate in ducklings, while the frequent clinical picture of older ages noticed as sinusitis, locomotor disturbances and poor growth. Postmortem lesions appeared as serositis and arthritis. These results agreed with Ibrahim, (1991); Sandhu and Rimler, (1997) and Ibrahim and Sohair, (2000). Nervous signs were reported by Sandhu, (2001).

During this study, bacteriological examination of clinically diseased ducklings revealed 10-12% positive cases, while in older ages the percentage of positive cases was lower (3.3-6%). Serotyping using AGPT revealed isolation of serotypes 2 and 5. Serotype 2 represented 34.69% and 43.75% of positive cultures isolated from ducklings and older age respectively. Serotype 5 represented 14.28% and 9.37% of positive cultures isolated from ducklings and older age ducks respectively. Untypable strains represented 51.0% and 46.87% of positive cultures isolated from ducklings and older age ducks respectively. Regarding to this point, Ibrahim, (1991) isolated *Pasteurella anatipestifer* serotypes 2, 3 and 5. Subramaniam *et al.*, (2000) stated that serotypes 1,2,3,5 and 15 are most prevalent using agglutination test. On contrast Pathanasophon *et al.*, (1994) reported that serotype 1 was the most prevalent followed by serotype 6. The occurrence of more than one serotype in infected ducks at any one time and changes in serotypes from year to year within a single farm have been described by Subramaniam *et al.*, (2000). They stated that *R. anatipestifer* infection has been continued problem in the intensive production of meat ducks since 1982.

In this study, the experimental infection revealed that I/M route was the most severe route of infection followed by I/N and oral routes where morbidity rates were reached 100%, 80% and 40%, and mortality rates were reached 90%, 20% and 10% respectively. Two birds were died peracutely with septicaemic picture. Very close clinical signs and

lesions to the natural infection were reproduced by experimental infection. Nearly the same results were reported by Ibrahim, (1991); Sandhu and Rimler, (1997) and Sarver *et al.*, (2004). Sinusitis was not reproduced experimentally even by I/N infection, this may be explained by the multifactor etiology of this affection (Ibrahim and Sohair, 2000).

The studying of susceptibility of *R. anatipestifer* to different antimicrobials using MIC resulted in complete susceptibility to penicillin, amoxacillin, enrofloxacin, lincospectin, oxytetracycline and cephalosporin. Complete resistance to aminoglycosides (streptomycin, gentamicin) and sulfadimethoxin was demonstrated. In agreement with the present results, Pathanasophon *et al.*, (1994) and Ibrahim and Sohair, (2000). On contrast Sandhu, (2001) stated that sulfadimethoxine-oretopim are effective in reducing mortality and agreed with our results concerning penicillin and enrofloxacin susceptibility. On the same side Sandhu and Rimler, (1997) reported on high degree of *in vivo* susceptibility to lincomycin-spectinomycin, penicillin or combination of penicillin and dihydrostreptomycin. They reported that sulfamethazine could prevent the onset of clinical signs.
Trial for plasmid profiling was carried out in this study and showed similar plasmid in tested isolates of serotype 2 with molecular weight (MW) of 4 MDa. Plasmids of MW 2.4 and 3.5 MDa were detected in two isolates of serotype 5. Only one strain of tested untypable isolates possessed two plasmids with high MW of 9 and 10.4 MDa. These plasmids have several functions such as virulence, toxin production and bacterial antimicrobial resistance (Price *et al.*, 1993 and Lee and Wooly, 1995).

It is concluded that *R. anatipestifer* infection considered as one of important pathogens affecting ducklings and causing severe economic losses for meat duck farms.

REFERENCES

Asplin, W.J. (1956): Experiments on the transmission of a septicaemic disease of ducklings. Vet Rec., 68: 588-590.

Birnboim, H.C. and Doyl, J. (1979): A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acid Res., 7: 1513-1523.

Brogden, K.A. Rhoades, K.R. and Rimler, R.B. (1982): Serologic types and physiologic characteristics of 46 avian *Pasteurella anatipestifer* cultures. Avian Dis., 26: 891-896.

Bruner, D.W. and Fabricant, J. (1954): A strain of *Moraxella anatipestifer* (*Pfeifferella anatipestifer*) isolated from ducks. Cornell Vet., 44: 461-464.

Graham, R.; Brandly, C.A. and Dunlap, G.L. (1938): Studies on duck septicemia. Cornell Vet., 28: 1-8.

Hatfield, R.M. and Morris, B.A. (1988): Influence of the route of infection of *Pasteurella anatipestifer* on the clinical and immune responses of white pekin ducks. Res. Vet. Sci., 44: 208-214.

Hatfield, R.M.; Morris, B.A. and Henry, R.R. (1988): Development of an enzyme-linked immunosorbent assay for detection of humoral antibody to *Pasteurella anatipestifer.* Avian Path., 16: 123-140.

Hendrickson, J.M. and Hilbert, K.F. (1932): A new and serious septicaemic disease of young ducks with a description of the causative organism. *Pfeifferella anatipestifer.* Cornell Vet., 22: 239-252.

Ibrahim, R.S. (1991): Some studies on avian pasteurellosis. M.V.Sc., Thesis presented to Dept. Poultry Diseases, Fac. Vet. Med., Assiut University.

Ibrahim, R.S. and Sohair, Z. Hussein (2000): Bacterial agents associated with sinusitis in waterfowls and turkeys in Assiut and El-Menia Governorates. Assiut Vet. Med. J., 44(87): 185-195.

Ishiyama, S.; Ueda, Y.; Kuwabara, S.; Kosakai, N.; Koya, N.; Konno, M. and Fugii, R. (1968): On the standardization of method for determination of minimum inhibitory concentrations. Chemotherapy (Tokyo), 16: 98-99.

Lee, M.D. and Wooly, R.E. (1995): The effect of plasmid acquisition on potential virulence attributes of *Pasteurella multocida.* Avian Dis., 39: 451-457.

Mannheim, W. (1984): Family III *Pasteurellacae* Poh 1981a. 382. In: N.R. Krieg and J.G. Holt (eds Bergey's Mannual of Systemic Bacteriology, 9th ed., vol. 1. Williams & Wilkins, Baltimore. MD, pp. 550-557.

Pathanasophon, P.; Tanticharoenyos, T. and Sawada, T. (1994): Physiological characteristics, antimicrobial susceptibility and serotypes of *Pasteurella anatipestifer* isolated from ducks in Thailand. Vet. Microbiol., 39: 179-185.

Pierce, R.L. and Vorhies, M.W. (1973): Pasteurella anatipestifer infection in geese. Avian Dis., 17: 868-870.

Price, S.B.; Freeman, M.D. and MacEwen, M.W. (1993): Molecular analysis of a cryptic plasmid isolated from avian strains of *Pasteurella multocida*. Vet. Microbiol., 37: 31-43.

Sandhu, T.S. (2001): Duck health care. International Duck Research Cooperative Inc., pp. 1-6.

Sandhu, T.S. and Rimler, R.B. (1997): Riemerella anatipestifer infection. In: Diseases of Poultry, 10th ed., B.W. Calnek, H.J. Barnes, C.W. Beard, L.R. McDougald and Y.M. Saif, eds. Iowa State Univ. Press, Ames IA. Pp.161-166.

Sarver, C.F.; Morishita, T.Y. and Nersession, B. (2004): The effect of route of inoculation and challenge dosage on *Riemerella anatipestifer* infection in pekin ducks (Anas Platyrhynchos). Avian Dis., 49(1): 104-107.

Segers, P.; Mannheim, W.; Vancanneyt, M.; De-Brandt, K.; Hinz, K.H.; Kersters, K. and Vandamme, P. (1993): Riemerella anatipestifer gen. nov., comb. Nov., the causative agent of septicemia anserum exsudativa, and its phylogenitic affiliation within the *Flavobacterium-Cytophaga* r RNA homology group. Int. J. Syst. Bacteriol., 43: 768-776.

Smith, J.M.; Frame, D.D.; cooper, G.; Bickford, A.A.; Ghazikhanian, G.Y. and Kelly, B.J. (1987): Pasteurella anatipestifer infection in commercial meat type turkeys in California. Avian Dis., 31: 913-917.

Subramaniam, S.; Huang, B.; Hida, L.; Kwang, J.; Hai-Meng, Tan; Kim-Lee Chua and Frey, J. (2000): Characterization of a predominant immunogenic outer membrane protein of *Riemerella anatipestifer*. Clinical and Diagnostic Lab. Immunol., 7(2): 168-174.

Table 1: Isolation of *R. anatipestifer* from ducks in Assiut and El-Menia Governorates

Species	Age in weeks	No. Examined	No. Positive	No. Positive
Native	10-18	300	18	6%
Muscovy	10-12	120	4	3.3%
White Pekin	10-14	200	10	5%
Native	1-6	200	24	12%
White Pekin	1-7	250	25	10%

N.B. All ducks selected for isolation of *R. antatipestifer* showed clinical signs as sinusitis, ocular nasal discharges, and ataxia or retarded growth.

Table 2: Results of serotyping of *R. anatipestifer* isolates obtained from two different age groups using AGPT.

Serotype	Duckling (1-8 weeks) N=450			Ducks (10-18) weeks N=620		
	Positive No.	No/450 %	No/49 %	Positive No.	No/620 %	No/32 %
1	-	-	-	-	-	-
2	17	3.77	34.69	14	2.25	43.75
3	-	-	-	-	-	-
5	7	1.55	14.28	3	0.48	9.37
Untypable	25	5.55	51	15	2.41	46.87
Total	49	10.87		32	5.14	

Table 3: Results of experimental infection with *R. anatipestifer* serotype 2 in 14-day-old white pekin ducks

Group	Birds No.	Dose CFU/ml	Challenge route	Morbidity %	Mortality %	Reisolation
1	10	10^6	I/M	100%	90%	+
2	10	10^6	I/N	80%	20%	+
3	10	10^6	Oral	40%	10%	+
4a	5	S. broth	I/M	0.0%	0.0%	-
4b	5	S. broth	I/N	0.0%	0.0%	-
4c	5	S. broth	Oral	0.0%	0.0%	-

Table 4: Results of MIC of different antimicrobials against 9 isolates of *R. anatipestifer*

Antimicrobial	No. of isolates with MIC (µg/ml)								
	≤ 0.2	0.39	0.78	1.56	3.13	6.25	12.5	25	≥ 50
OTC			3	5	2				
Sulfadimethoxine								2	8
Penicillin	6	2	2						
Amoxicillin	8	2							
Cephalosporin		1	2	5	1	1			
Streptomycin						1	3	4	2
Gentamicin									10
Enrofloxacin		3	7						
Lincospectin		8	2						

Fig. A: Sinusitis in ducks (natural infection)

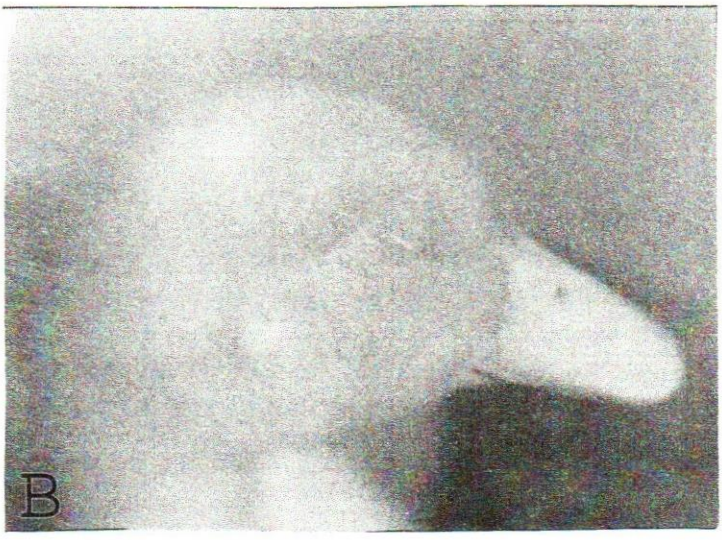

Fig. B: Experimental infection in 14-day-old ducks shows sticky ocular discharges due to intranasal infection with *R. anatipestifer*.

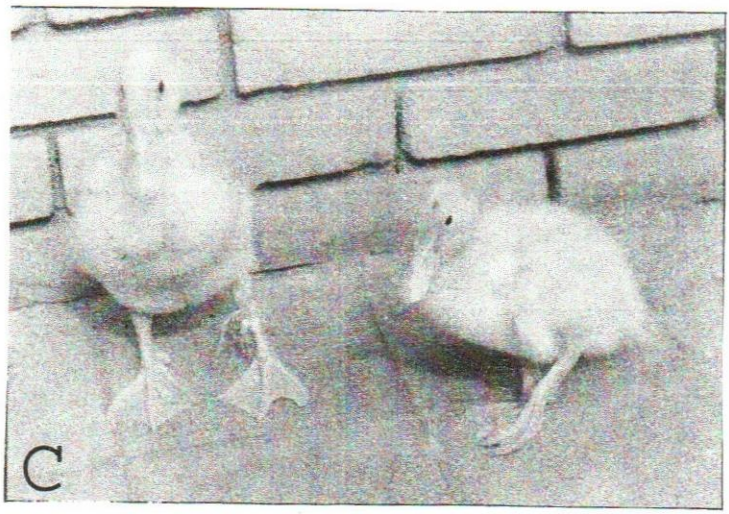

Fig. C: Experimental infection in 14-day-old ducks shows incoordination, poor growth with ruffled feathers, anorexia, and hunched up after I/M inoculation of 10^6 CFU/ml of *R. anatipestifer*.

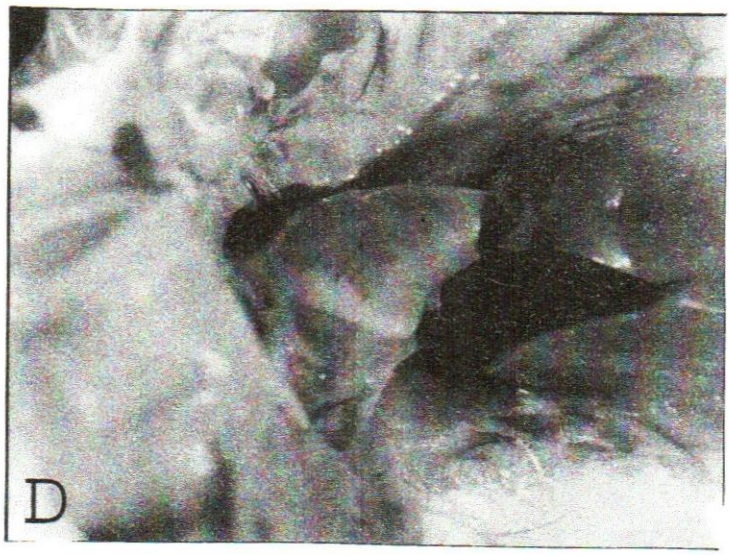

Fig. D: Septiceamic lesions on heart muscle, coronary fat and subserosal area of liver 24 hours postinoculation of 10^6 CFU/ml of *R. anatipestifer*.

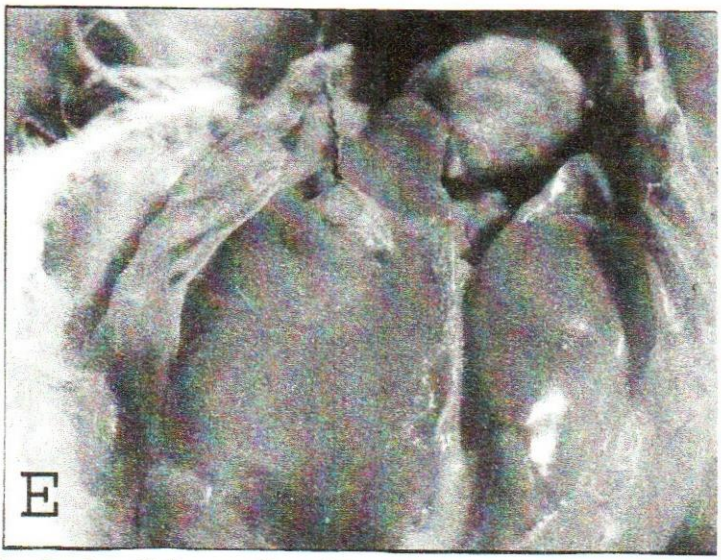

Fig. E: Pericarditis, perihpatitis, air sacculitis after 48 hours postinoculation of 10^6 CFU/ml of *R. anatipestifer* postinoculation.

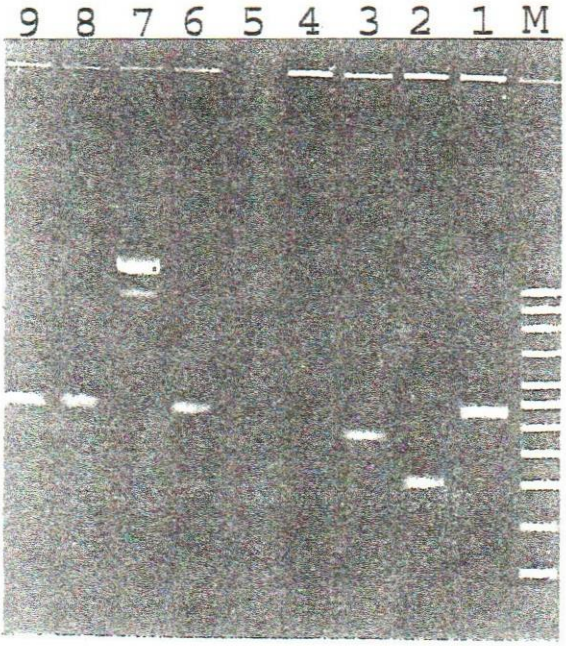

Fig. F: Plasmid profile analysis; leans 1, 6, 8 and 9 (serotype 2); leans 2 and 3 (serotype 5); leans 4, 5 and 7 (untypable strains); M: Supercoild DNA Laddar,Sigma, with ascending MW (1.3; 1.9; 2.6; 3.2; 3.9; 4.5; 5.1; 6.5; 7.8; 9 and 10.4 Mda).