Beni-Suef

BS. VET. MED. J. JULY 2011 VOL.21 NO.1 p.27-32

Veterinary Medical Journal

Studies on pathogenicity of Aeromonas species to native breed (Fayoumi) chickens

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The pathogenic effect of representative local isolates of *Aeromonas* organisms was studied on 1،0 native breed chickens. At 2 weeks of age, one hundred chicks were grouped into four equal groups (1-4); 25 birds each; after collection of fecal from all groups, examined and proved to be free from *Aeromonas* species. All groups were subcutaneously (s.c) inoculated with 0.5 ml containing 9×10^5 cfu /bird. Group 1 of chickens was infected by *A. hydrophila*; while group 2 was infected with *A. caviae*. The third group was infected with *A. sobria*; while, chicks of Group 4 were kept as non infected control. Results were showed mild clinical signs in some birds in the form of alternative diarrhea. Post mortem lesions showed general congestion of all carcasses. In severely emaciated cases the lesions were confined to the intestine, which filled with watery fluid and distended with gas. Results of *Aeromonas* reisolation revealed that all collected weekly fecal swabs were positive. While, percentage of *A. hydrophila* and *A. caviae* was 100% out of all tested organs, while isolation of *A. sobria* was 100%, 100% and 80% from liver, intestine and heart; respectively. Histopathological examination of infected chicken organs showing focal coaggulative necrosis in liver with mononuclear cells infiltration that varied in severity between groups as less severe in *A. hydrophila* (Gr.1) than *A. caviae* (Gr. 2), while *A. sobria* (Gr. 3) was markedly affected showing severe degenerated and dissociated hepatocytes. Intestinal changes were severe in group 3 than 2 while group 1 showing the mildest comparatively the changes was consist of necrosed mucosa, gland with leucocytic infiltration in lamina propria. Under the condition of our study we can conclude that the used *Aeromonas* isolates from field diseased chickens were of mild pathogenicity to s.c. inoculated 12 days old Fayoumi chicks with long course affection.

Aeromonas is a member of family Vibrionaceae genus *Aeromonas* which is a facultative anaerobes Gram negative microorganism which can grow over wide range of environmental conditions as pH values from 4.0 to 10.0 and salt concentrations up to 6.5% (Blair *et al.,* 1999).

Most members of the genus are mesophiles with an optimal growth temperature of 28˚C as some *Aeromonas* can grow at temperatures ranging from 4˚C to 42˚C, the capacity to grow at such extreme temperatures varies among strains and seems to be closely related to the source of isolation, or to environmental adaptation. *Aeromonas* isolates are belonged to 3 major groups according to the biochemical and physiological growth characteristics which are *A. caviae* group includes *A. caviae, A. eucrenophila* and *A. media*; *A. hydrophila* group includes A. hydrophila and a motile biogroup of *A. salmonicida*, while *A. sobria* group includes *A. sobria* and *A. veronii*.

In human *Aeromonas* species were reported to cause gastroenteritis, septicemia, endocarditis and respiratory tract disease (Colwell *et al.,* 1986; Agger and Callister, 1987; Abbott, 1992). In fish farms cause major problem (Austin and Allen-Austin, 1985; Janda and Duffey, 1988; Merino *et al.,* 1995) and can be isolated from a variety of

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foods such as vegetables, raw milk, meat and sea foods (Hird *et al.,* 1981).

Pathogenicty of Aeromonads was recorded in some avian species in the form of septicaemia in turkey (Gerlach and Bitzer, 1971), conjunctivitis in pet parrots (Garcia *et al.,* 1992), salpingitis in ducks (Bisgaard, 1995) and diarrhea and watery feces in water fowls (Efuntoye, 1995). Diarrhea and weight loss were reported in Japanese quails, canaries and cocktails (Rosskof and Woerple, 1996). High mortality in SPZF chicks (Setta, 2004) and out break in farm rabbits (Paniagua *et al.,* 1998).

Pathogenicity of *A. hydrophila* was studied on 12 day-old chicken embryos and adult Japanese quail by Efuntoye (1995) where the results showed depression, ruffled feathers after 2 days post inoculations, severe diarrhea, emaciation, no specific lesions were observed after post mortem (PM) examination. Only congestion and friable livers were evident. In chickens it causes gastrointestinal disturbance (Swift *et al.,* 1999; Lynch *et al.,* 2002). Additionally, Shane and Gifford (1985); El-khashab (2001) reported that *A. hydrophila* was pathogenic to chicks.

Disease pathology and virulence of the pathogen resulted from many factors including stress responses and heat shock proteins (Efuntoye, 1995).

This work was planed to study pathogenicity of local representative *Aeromonas* isolates to subcutaneous inoculation in 2 week old native breed Fayumi chicks.

Materials and Methods

Bacterial strains. Representative Local field isolates of *Aeromonas* organisms including *A. hydrophila, A. caviae* and *A. sobria* were serotyped and molecularly characterized by Dr. Zeinab Gira (2007), Poultry Diseases Department, N. R. C., Dokki, Giza.

Preparation of inoculums. These organisms were isolated from diseased chickens associated with clinical respiratory and/or intestinal affections. The cultures were incubated aerobically at 25˚C for 24-48 hours. Typical *Aeromonas* colonial appearance of species was selected and completely identified morphologically using the methods described by Cruickshank *et al.,* (1975); Krieg and Holt (1984).

Experimental chicks. A total number of 120 one-day old native breeds Fayoumi chicks were obtained from commercial hatchery as hatched to be used in this study.

Ration. The chicks were feed on prepared balanced commercial ration containing anticoccidial drug during the whole experimental period.

Histopathological examinations. It was carried according to Shane and Gifford, (1984). Representative samples from liver, intestine and heart of each group were immersed and fixed in 10% formal saline. These samples were dehydrated, cleared, embedded and cut into 7 µ size then they were transferred to glass slides and stained with hematoxylin and eosin (H&E) then examined by ordinary microscope (X 200).

Reisolation. Weekly fecal swabs were collected from all groups and separately inoculated on *Aeromonas* agar medium. The organ samples were collected from sacrificed 5 chicks/group birds at 1st, 2nd and the 3rd week of infection (the end of observation). From each chick parts of intestine, liver and heart blood were used. The obtained bacterial growth was identified according to Cruickshank *et al.,* (1975); Colwell *et al.,* (1986).

Experimental design. At the 2nd week of age, one hundred chicks were grouped into four equal groups (1-4), 25 birds each, fecal samples were collected from the experimental chicks, cultured on *Aeromonas* media and examined to ensure the freedom of infection. Then 100 chick were randomly collected and divided into 4 equal groups (1-4); 25 chicks each; chicks groups were kept each in a separate cage and treated as follow: Birds of group 1 were infected with *A. hydrophila*, group 2 with *A. Caviae* , while *A. sobria* was inoculated in the birds of group 3. Birds of group 4 kept uninfected as control negative.

All chick groups were kept under daily observation for 3 weeks post infection with recording of clinical signs and mortalities. At weekly intervals (post inoculation) 5 fecal swabs per group were collected for bacteriological examination. At the end of experiment (35 days) 5 birds/group were sacrificed for post mortem examination and the collection of tissue samples including intestine, liver and heart for reisolation of the inoculated organisms. Intestine and liver samples were fixed in formol saline and subjected to histopathological examination.

Results

Moderate clinical signs were observed in few birds in the form of alternative diarrhea allover the observation period. Post mortem lesions showing general congestion of all carcasses in cases, which were severely emaciated. The lesions were confined to the intestine, which filled with watery fluid and distended with gas.

Reisolation of inoculated organisms from heart blood, intestine and liver sample on *Aeromonas* agar media was successful as the organisms produce yellow to dark green colonies. The percentage of reisolation was shown in table (1) and (2). While liver of infected groups showed focal area of coaggulative necrosis infiltrated with mononuclear cells varied in severity between each groups as less severe in group 1 than group 2 while group 3 was markedly affected showing severe degenerated and dissociated hepatocytes (Photos 3, 5 and 7). The same intestinal changes which were severe in group 3 than 2 while group 1 showing the mildest severity comparatively. The changes was consisted of necrosed mucosa, gland with leucocytic infiltration in lamina propria (Photos 4, 6 and 8).

Histopathological examination of control chick's liver showing apparently normal hepatocytes and hepatic tissue also the intestine show normal histological structure (Photo 1 and 2).

Table (1): Results of bacteriological examination of weekly fecal swabs from infected and control groups (n = 5 chicks).

Groups	organism	Weeks post infection		
		1st	2nd	3rd
1	*A. hydrophila*	+ve	+ve	+ve
2	*A. caviae*	+ve	+ve	+ve
3	*A. sobria*	+ve	+ve	+ve
4	-ve control	-ve	-ve	-ve

Table (2): Results of *Aeromonas* spp. reisolation from different organs at the end of experiment (n = 5 chicks).

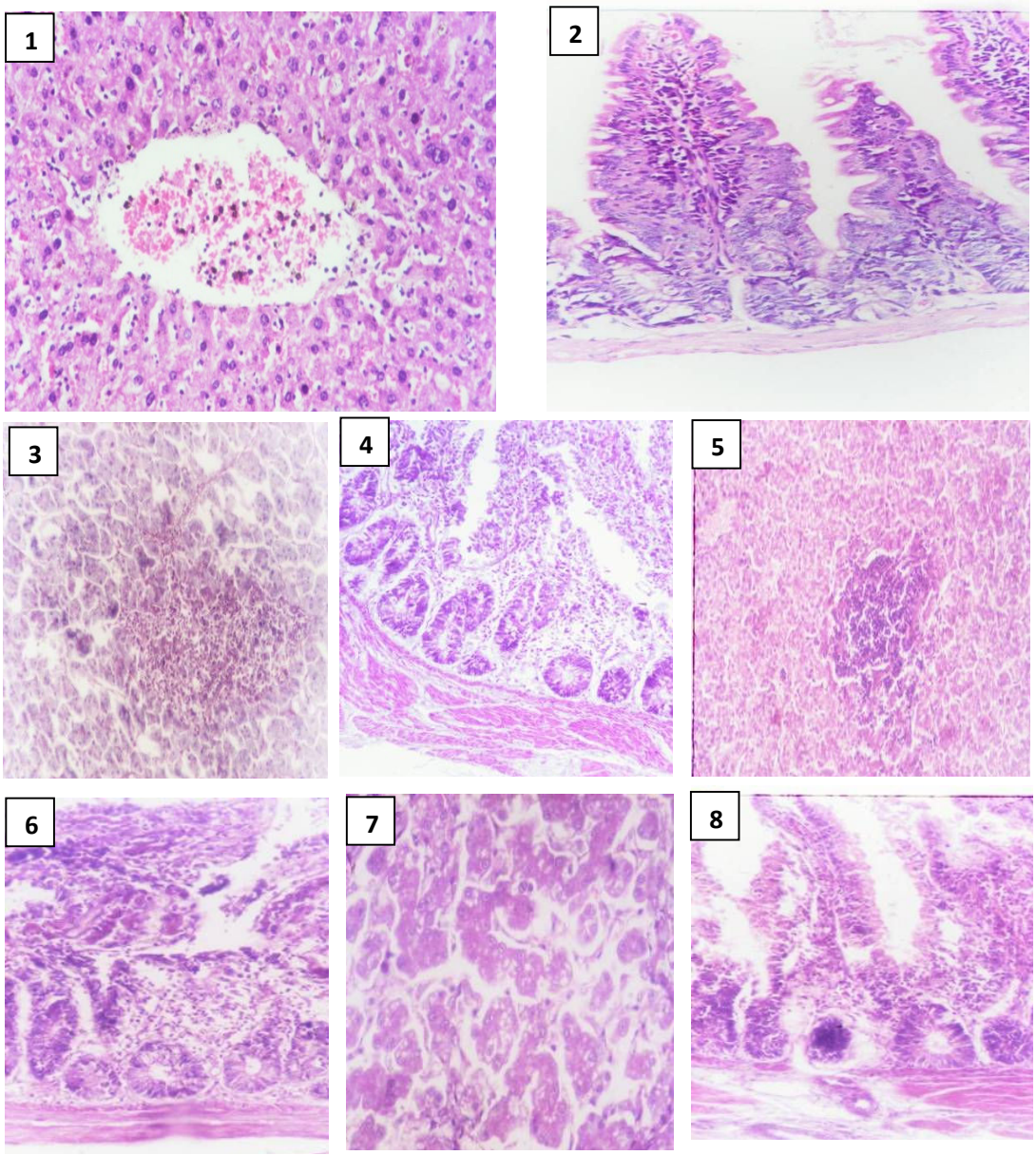
Group no	Type of organism	Organs of re isolation					
		Liver		Intestine		heart	
		No.	%	No.	%	No.	%
1	*A. hydrophila*	5	100	5	100	5	100
2	*A. caviae*	5	100	5	100	4	80
3	*A. sobria*	5	100	5	100	4	80
4	-ve control	0	0	0	0	0	0

Disscussion

Aeromonas species are extremely common in the environment, especially in association with water. There organisms are likely to be ingested with water in many situations, fecal isolation may often simply reflect the organism infection when found in large numbers in association with diarrhea. Possibly this may reflect a disturbance in the intestinal ecology which has permitted their growth to high numbers (Turnbull *et al.,* 1984).

Our results revealed a moderate clinical signs in the form of long alternative diarrhea in some individuals of birds. Post mortem lesions showing general congestion of all carcasses were observed in cases, which were severely emaciated. The lesions were confined to the intestine, which filled with watery fluid and distended with gas. These finding was accorded with that report of Miyazaki and Jo (1985); Miyazaki and Kaige, (1985) who reported that; two species of *Aeromonas (A. hydrophila and A. caviae)* were most commonly associated with diarrhea. These results are some what agree with those of Setta (2004) who reported lesions of septicemia and enteritis in inoculated SPF chicks. While, Shane and Gifford (1985), observed no specific lesions although

generalized congestion was evident. Lesions were also including fecal cerebral plaques and petichial hemorrhage on the mucosa of proventriculus and jejunum. Also pulmonary congestion and hepatic petechiane were recorded. EL-Khashab (2001) observed generalized s/c venous congestion as well as congestion of liver, spleen, lungs, kidneys, intestine especially duodenum showed severe hemorrhage in experimentally infected chicks. *A. hydrophila* either alone or in combination with other organisms can cause localized and systemic infections in poultry (Shane and Gifford, 1985; Glunder, 1988).



Histopathological photos of examined tissue sections stained with H&E under magnification of X200.

Photo 1: Normal hepatocytes of apparently normal chickens
Photo 2: Normal intestine of apparently normal histology of intestinal tissue.
Photo 3: Group 1 liver showing focal area of coagulative necrosis infiltrated with mononuclear cells.
Photo 4: Group 1 intestine showing necrosed mucosa with necrosed gland & leucocytic infiltration.
Photo 5: Group 2 liver showing focal area of necrosed hepatocytes replaced by mononuclear cells.

Photo 6: Group 2 intestine showing necrosed mucosa with desquamated epithelium and leucocytic infiltrated lamina propria.
Photo 7: Group 3 liver showing markedly degenerated and dissociated hepatocytes .
Photo 8: Group 3 intestine showing mucosal degeneration and submucosal edema and congestion.

Regarding to reisolation of *Aeromonas* spp. at the end of the 3rd week (Colwell *et al.,* 1986) it was found that the percentage of *A. hydrophila* and *A. caviae* reisolation was reached 100% while isolation of *A. sobria* was 100%, 100% and 80% from liver; intestine and heart; respectively. On the other hand all collected fecal swabs in the 3 times (table 1) were all positive. This result indicates that the affection may be long lasting. Efuntoye (1995) reported that fecal samples were collected (while the disease lasted) from diarrheic and healthy animals which including chickens. *A. hydrophilia* was reported that the low level of the bacteria in healthy animals and the high recovery rate in diarrheic animals suggested that *A. hydrophila* is closely associated with out breaks of diarrhea in the animals. Setta (2004) reported the reisolation of *A. hydrophila* from liver and hart blood of s.c infected SPF chicks.

The reported histopathological lesions in the examined tissues where liver showing focal area of coagulative necrosis infiltrated with mononuclear cells (Gr. 1); focal area of necrosed hepatocytes replaced by mononuclear cells (Gr. 2) and markedly degenerated and dissociated hepatocytes (Gr. 3). Intestine showing necroses in mucosa with necrosed gland and leucocytic infiltration (Gr. 1), necrosis in mucosa with desquamated epithelium and leucocytic infiltrated lamina propria (Gr.2), while mucosal degeneration and submucosal edema and congestion were recorded in group 3. Similar lesions were reported by Setta (2004).

In conclusion: our results including the reported signs, gross and histopathological lesions proved that the used *Aeromonas* isolates were of mild pathogenicity to s. c. inoculated 2 week old Fayoumi chicks with long course alternative diarrhea. This area needs more investigation.

Referance

Abbott, S.L.; Cheung, W. K. W; Kroske-Bystrom, S.; Malekzadeh, T. and Janda, J.M. (1992): Identification of *Aeromonas* strains to the genospecies level in the clinical laboratory. J.Clin.Microb., 30-1262-1266.

Agger, W. A. and Callister, S. M. (1987): Intestinal infections with *Aeromonas*. Ann. of Inter. med. 106:497.

Austin, B. and Allen-Austin, D. (1985): Hand book of microbiological media. 2nd Ed. CRC presss Boca Raton, NY, London, Tokyo.

Bisgaard, M. (1995): Salpingitis in web-footed birds: prevalence, etiology and significance. Avian pathol.24, 443-452.

Blair, I. S.; Mcmahon, M. A. S. and Mcdowell, D. A. (1999): *Aeromonas* detection by cultural and modern techniques. Frood studies research unit, university of ulster at Jordan stown, Co. Antim, Northern Ireland Academic press, 25-30.

Clowell, R. R.; Macdonell, M.T. and Delay, J. (1986): Proposal to recognize the family *Aeromonadaceae* fam. Nov.int.J.syst.Bacteriol.36-473.

Colwell, R. R.; MacDonell, M.R. and Deley, J. (1986): Proposal to recognize the family *Aeromonadaceae*. Fam. Nov.Int. J. Syst. Bact., 36: 473- 477.

CruickShank, R.; Duguid, J. P.; Marimo, B.R. and Swain, R.H. (1975): Medical Microbiology, 2nd Ed.,Vol.11,living stone, London, newyork.

Efuntoye, M.O. (1995): Diarrhea disease in live stock associated with *Aeromonas hydrophila* biotype 1. J. General Appl. Microb., 41 (6) 517- 521.

El-Khashab, E. F. (2001): Patrhogenicity of *Aeromonas hydrophila* infection in chicks. Beni-Suef. Vet. Med. J. XI (2) 737- 749.

Garcia, M.E.; Domenech, A.; Dominguez, L.; Ramiro, F. and Fernandez Garayzabal, J.F. (1992): *Aeromonas hydrophila* conjunctivitis in pet parrot (amazona versicolor). Pet bird medicine: case report. Avian dis., 36, 1110-1111.

Gerlach, H. and Bitzer, K. (1971): Infection with *Aeromonas hydrophila* in young Turkeys.Dtsch. tierarzh-Wochenschr., 78,593-608.

Glunder, G. (1988): Occurrence of *Aeromonas hydrophila* in birds. J. Vet. Med. B, 35: 331- 337.

Hird, W.; Diesch, S. L. M.; Ckinnel, R. G.; Gorham E.; Martin, F.B.; Kurtz, S.W. and Dubrovlng, E. (1981): *Aeromonas hydrophila* in wild- caught frogs and tappoles (rana-Pipiens) in minnsota. Lab. Anim. Sci.31, 166-169.

Janda, J. M. and Duffey, S. (1988): Mesophilic Aeromonads in human disease: current Taxonomy, Laboratory identification and infectious disease spectrum. Rev. Inf. Dis.10: 980.

Kreig, N.R. and Holt, J.G. (1984): Bergey's manual of systemic Bacteriology. 8th Ed.Williams and Wilkins, London.

Merino, S; Rubires, X.; Knochel, S. and Tomas, J. M. (1995): Emerging Pathogens: *Aeromonas* spp. Int. Food Microb. 28:157.

Miyazaki, T. and Jo (1985): A histopathological study on motile Aeromonad disease in ayu. Fish Pathol. 20: 55: 09.

Miyazaki, T. and Kaige, N. (1985): A histopathological study on motile Aeromonad disease in crucian carp. Fish Pathol. 20: 55.

Paniagua, C.; Arguello-villares, J. L.; Arias, M. A. and Herreros, H. (1998): *Aeromonas* hydrophila associated with a severe outbreak of infection in farmed rabbits. Zentralblatt Fur Hygiene und Umwell Med, 201, 423- 436.

Rosskoff, W. J. and Woerpel, R W. (1996): Diseases of cage and aviary birds, 3rd edition, Williams and Wilkins, awaverly com.

Setta, A. M. H. (2004): Studies on *Aeromonas hydrophila* in chickens. MVSc , Thesis (Diseases of birds an rabbits), Facult. Vet. Med., Cairo Univ.

Shane, S.M. and Gifford, D.H. (1985): Prevalence and Pathogenicity of *Aeromonas hydrophila*, Fish pathol.21:181-185.

Shane, S. M. and Gifford, D. M. (1984): Prevalence and pathogenicity of A. hydrophila. Av. Dis., 29: 681-689.

Turnbull, P.C.B.; Lee, J.V.; Miliotis, M.D.; VanDeWalle, S.; Kooenhof, H.J; Jeffery, L. and Bryant, T.N. (1984): Entero toxin production in relation to Taxonomic grouping and source of isolation of *Aeromonas* species. J. Clin. Microbial. 19: 175-180.

Zeinab-Gera M. S. A. (2007): Studies in *Aeromonas hydrophila* in chicken. PhD, Thesis (Bacteriology, Immunology, Mycology) Depart. Of Microbiol. Facult. of Vet. Med., Cairo Univ.

دراسات على إمراضية ميكروبات الإيروموناس لسلالة الدجاج الفيومى المحلى

تمت دراسة إمراضية معزولات محلية ممثلة لميكروبات الإيروموناس لعدد ١٠٠ كتكوت من النوع الفيومى المحلي. عند عمر ١٢ وم قسمت الكتاكيت عشوائياً إلى ٤ مجموعات متساوية ؛ كل منها ٢٥ كتكوت وذلك بعد تجميع مسحات زرق لإثبات خلوها من عدوى ميكروبات الإيروموناس. تم حقن الكتاكيت تحت الجلد بواقع ٠.٥ مللى /كتكوت تحتوى على ٩ X١٠° وحدة مكونة للمستعمرة البكتيرية. حقنت المجموعة الأولى ميكروب الإيروموناس هيدروفيلا ؛ المجموعة الثانية ميكروب الإيروموناس كافيا والمجموعة الثالثة ميكروب الإيروموناس سوبيرأ بينما تركت المجموعة الرابعة كمجموعة ضابطة بدون حقن. أظهرت النتائج وجود أعراض مرضية طفيفة فى صورة إسهال خلال فترة الملاحظة. بينما أظهر التشريح المرضى احتقان عام فى الجثث. الحالات التى أظهرت نقص شديد فى الأوزان كانت الافات المرضية متركزة فى الامعاء حيث كانت ممتلئة بسائل مائى وغازات. نتائج إعادة عزل الميكروب اظهرت ان نسب عزل ميكروب الإيروموناس هيدروفيلا و ميكروب الإيروموناس كافيا ١٠٠ % من كل الأعضاء أما ميكروب الإيروموناس سوبيرأ فكانت نسب العزل ١٠٠ و ١٠٠ و ٨٠ % من الكبد والامعاء و القلب ؛ على التوالى. بينما كانت المسحات الأسبوعية كلها ايجابية. بالفحص النسيجى المرضى لكبد الدجاج المعدى تبين وجود تنكرز تجمعى مع تخلل الخلايا البيضاء وحيدة النواة والتى اختلفت حدتها بين المجموعات حيث كانت أقلها فى ميكروب الإيروموناس هيدروفيلا (مجموعة ١) عن ميكروب الإيروموناس سافيا (مجموعة ٢) وان الاكثر شدة فى المجموعة الثالثة (مجموعة ٣) المعدية بالإيروموناس سوبيرأ والتى شهدت تحللا شديدا فى خليا الكبد. أما التغيرات فى أنسجة الأمعاء والمتمثل فى تنكرز الخلايا الطلائية و الغدد مع تخلل الخلايا الليمفاوية فكانت أشدها فى مجموعة ٣ ثم مجموعة ٢ بينما كانت مجموعة ١ الأقل. تحت ظروف التجربة كانت معزولات ميكروب الإيروموناس الحقلية من الدجاج المصاب و المستخدمة لدراسة ضراوتها بالحقن تحت الجلد فى كتاكيت عمر ١٢ يوم كانت طفيفة الضراوة محدثة إسهال تبادلى طويل المدى.