Some Observations on Pseudomonas Infection in Poultry

by L. Niilo[1]

Pseudomonas aeruginosa has at times been considered a significant factor in diseases of poultry. Essex *et al* (1) described an outbreak of a disease involving this organism in some 400 chicks which resulted in a 75 percent mortality. Stafseth (2) and Stafseth *et al* (3) reported a similar outbreak in a flock of 19,000 turkeys in which the morbidity was 50.0 percent, although the mortality was surprisingly low. Another outbreak of Pseudomonas infection, involving 87-day old turkey poults with a mortality rate of approximately 80.0 percent, has been reported by Chute (4).

OBSERVATIONS

Cases

On numerous occasions *Pseudomonas aeruginosa* has been isolated at our laboratory from disease conditions in Southern Alberta poultry flocks. During the past year, four significant outbreaks of Pseudomonas infection have been recorded. These involved chickens, turkeys and ducks. A representative number of birds from each affected flock were examined (Table I). Isolation of the organism from 60-100 percent of the birds submitted and the failure to find other pathogens appeared to confirm that the condition noted was caused by *P. aeruginosa.*

Clinical Picture

The affected birds presented a general picture of depression, diarrhea and often severe keratitis. *Post mortem* examination revealed enteritis in almost all birds, varying from a catarrhal to a hemorrhagic type. The catarrhal type of enteritis predominated. A very congested carcass was seen in young chicks indicating the acuteness of the disease.

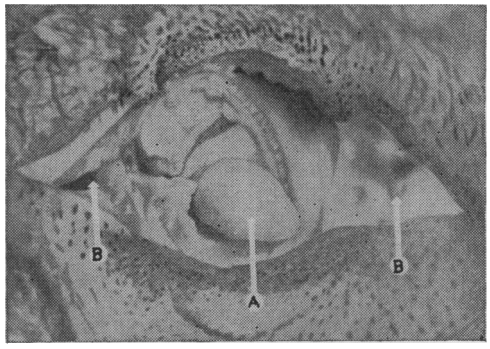
Keratitis, perhaps the most outstanding feature, was present in about 50 percent of the older birds examined. This lesion was not present in young birds. The extent of keratitis varied from clouding of the cornea to complete corneal necrosis and exudative keratoconjunctivitis with accompanying blindness. Often an eye presented a picture showing a large yellowish-white necrotic mass protruding from the cornea (Fig. 1) which, by gentle traction, could be pulled off, leaving a circumscribed deep cavity (Fig. 2). No noticeable changes were observed in the interior of the eyeball, although the eyeball itself appeared to be somewhat atrophied.

TABLE I

Four Outbreaks of **P. aeruginosa** Infection in Southern Alberta Poultry Flocks

Outbreak	Month	Species	Age	Number in Flock	Percent Morbidity (approx.)	Percent Mortality (approx.)	Number of birds Examined	Number of Isolations
1	Jul.	Chicks	2 wks.	2200	5	30	28	20
2	Oct.	Turkeys	7 mos.	4500	25	10	8	8
3	Jan.	Ducks	11 mos.	300	20	20	12	8
4	Feb.	Turkeys	8 mos.	800	15	10	5	3

1. Animal Pathology Laboratories, Health of Animals Division, Canada Department of Agriculture, Prairie Area Branch Laboratory, Lethbridge, Alberta



Close-up View of a Turkey Eye.
A — necrotic mass protruding from cornea;
B — incisions made at lateral and medial canthi to allow reflection of the eyelids.

Advanced cases of keratitis were bilateral.

LABORATORY METHODS

Bacteriology

Portions of intestine, liver, spleen, heart or heart blood, and in cases of keratitis, eyes, were collected aseptically for bacteriological cultures. These specimens were macerated and cultured onto blood agar medium as well as being inoculated into tetrathionate and selenite broths (Difco). All media were incubated aerobically at 37°C. The blood agar plates were examined the next day for growth and colonies were picked for identification on differential media. After 18-24 hours of incubation a loopful of growth from the broths was streaked onto McConkey's and nutrient agar plates and incubated at 37°C for another 24 hours.

Identification of non-lactose fermenters, which were picked from the McConkey's plates, was carried out on basis of colony pigmentation, cytochrome oxidase test (5), indol production, nitrate reduction, gelatin liquefaction, carbohydrate fermentation and hemolysin production. If pigment production was profuse on the nutrient agar plates subsequent steps in identification were omitted. Occasional pigmented (lactose-positive) colonies on McConkey's medium were interpreted as *E. coli* and/or coliforms without further biochemical tests.

Antibiotic Sensitivity

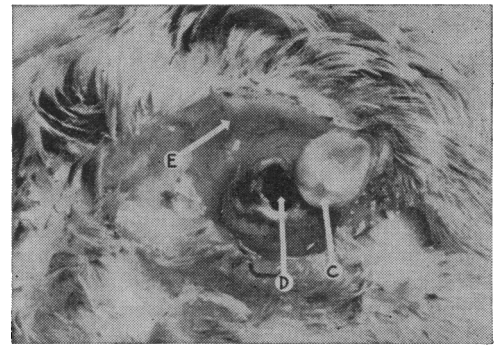
Antibiotic sensitivity tests using the disc method (Difco) were conducted on all strains. These tests were repeated several months later to detect if any change in sensitivity due to frequent subculturing had occurred.

Transmission Experiment

An attempt was made to transmit the disease by inoculating fresh cultures by various routes into one-month-old chicks. A total of 16 chicks were inoculated by the subcutaneous, intramuscular, intravenous, intraperitoneal routes and by conjunctival instillation; oral administration via the drinking water was also attempted. Dosage varied from 0.1 ml to 1.0 ml of 18 hrs. broth cultures. Unaffected birds were repeatedly inoculated at ten-day intervals and kept under observation for a total period of two months.

RESULTS

The greatest number of isolations were made from the intestinal tract, followed by those from the liver, blood,



Close-up View of a Duck Eye.
C — necrotic mass pulled off;
D — deep cavity left after removal of the necrotic mass;
E — palpebral conjunctiva (incised and reflected).

and other organs. All affected eyes yielded *P. aeruginosa* in pure or almost pure culture. Other organisms found on cultures, especially those from the intestine, were mostly of the coliforms group or occasionally, some Bacillus spp. All strains appeared to be homogeneous by cultural characteristics and biochemical reactions which were typical for this organism (6). Moderate hemolysis on blood-agar was exhibited and pyocyanin production was noticeable on the nutrient agar plates. Cytochrome oxidase test (5) gave a rapid reaction.

The results of antibiotic sensitivity tests (Table II) on individual isolations within a flock showed no appreciable variation; however some differences were noted between the strains from specific outbreaks. Whether or not this variation has resulted from possible previous medication with antibiotics or medicated feeds could not be ascertained. The results of tests repeated at a later date did not vary from the original findings.

Sulfathiazole and neomycin were recommended for treatment after the diagnosis was established. Following the use of these products the disease was gradually brought under control.

The transmission experiments were very discouraging. When large doses (more than 0.8 ml) were used, the birds died in less than 24 hours. By employing sublethal doses inoculated by the intravenous route, only two birds developed the disease within 12 days, one with accompanying unilateral keratitis. *P. aeruginosa* was recovered from both chickens. Transmission by oral administration was not successful regardless of the dosage.

DISCUSSION

It is apparent that infection with *P. aeruginosa* in a particular flock of poultry may not be sporadic, but can reach significant proportions. Certain outstanding points can be deduced from the outbreaks reported:

1. Outbreaks occur predominantly in flocks of young or growing birds.
2. Turkeys may be more susceptible to this infection, but chickens and ducks may also be involved.
3. Experimental transmission of the disease is difficult (1, 2, 3, 4, 7).
4. Keratitis or keratoconjunctivitis, if present, is an outstanding and, perhaps, a clinically diagnostic symptom.

TABLE II

Antibiotic Sensitivity Tests on Four Strains of **P. aeruginosa**

Antibiotic	Strain 1 (Chicks)	Strain 2 (Turkeys)	Strain 3 (Ducks)	Strain 4 (Turkeys
Penicillin	R	R	R	R
Streptomycin	R	MS	MS	S
Chloromycetin	R	R	R	R
Aureomycin	R	R	R	R
Terramycin	R	R	R	R
Tetracycline	R	R	R	R
Furacin	R	R	R	R
Neomycin	MS	S	S	S
Polymyxin-B	S	S	S	S
Sulfathiazole	S	S	MS	R
Sulfadiazine	MS	R	MS	R
Sulfamerazine	MS	R	R	R

R = resistant; S = sensitive; MS = moderately sensitive.

5. There is a reason to believe that a predisposing cause or causes precipitate an outbreak.

Since *P. aeruginosa* is considered a soil and waterborne organism with universal distribution, it is safe to assume that it frequently can gain access to poultry flocks. Failure to infect healthy birds successfully, may indicate that it is not only the exposure to the organism or its pathogenicity that determines a natural infection. There probably are predisposing causes; one of these, age, has been mentioned by other workers (1). Nutritional status of a flock may be involved. It has been postulated that vitamin A deficiency plays a role in this infection (3, 4). Although there is no concrete evidence to support this, it is felt that a vitamin A deficiency may have been involved in outbreaks reported.

As already mentioned, keratitis is a unique feature in Pseudomonas infection, but it does not appear in all cases. From our observations we noted that only in chronic cases involving older birds in which the disease has been present for a week or more, do the eye lesions develop. More acute cases will end in death before keratitis develops. Eye lesions have also been noted in other infections and have been described in infections of turkeys in Alberta due to Arizona paracolon (8). A differentiating point seems to be that in Arizona paracolon infection, the eye lesions appear inside the eyeball, either in the form of pus in the chambers or destruction of the retinal layers, while the cornea and conjunctiva remain unaffected (8). In Pseudomonas infection the inner structures appear relatively unaffected and keratitis is the main feature. Cultural isolation of the organism, however, is still necessary for final diagnosis.

P. aeruginosa is resistant to most antibiotics; however, the results of our antibiotic sensitivity test indicate that sulfathiazole, neomycin, streptomycin and, possibly, polymyxin-B or some combination may be effective treatment. An apparent effectiveness of sulfamerazine in controlling a natural outbreak in turkeys has once been reported (9).

SUMMARY

1. Four outbreaks of infection in chickens, turkeys and ducks in Southern Alberta caused by *P. aeruginosa* have been described.
2. Observations on antibiotic sensitivity of *P. aeruginosa* and on its pathogenicity to experimental birds have been presented.
3. Eye lesions as they occur in Pseudomonas infection and in Arizona paracolon infection have been compared.
4. It is postulated that some predisposing factor is always present prior to a flock infection involving this organism.

RESUME

L'auteur décrit quatre épidémies d'une infection à Pseudomonas chez des poulets, des dindes et des canards du sud de l'Alberta. L'âge des oiseaux variait de deux semaines à onze mois et le taux de mortalité, de 10 à 30 pour cent. Le principal symptôme était de la kératite. Les souches de *Pseudomonas aeruginosa* qu'il a isolées se sont avérées résistantes à la majorité des antibiotiques; cependant elles ont manifesté une sensibilité variable à la streptomycine, la néomycine, la polymixine-B et au sulfathiazole. La transmission expérimentale de l'infection a été difficile à réaliser. Les causes prédisposantes de la maladie dans les gros troupeaux seraient vraisemblablement d'origine nutritive.

ACKNOWLEDGMENTS

The author wishes to acknowledge the technical assistance of Mr. Kent Duncan and Mr. D. D. Carpenter. The author also wishes to thank Dr. R. J. Avery and Mr. J. L. Byrne for their criticism and assistance in preparing the manuscript.

REFERENCES

1. **ESSEX, H. E., McKENNEY, F. D., and MANN, F. C.** Pseudomonas pyocyanea a significant factor in a disease of chickens. J A.V.M.A. **77**: 174-184, 1930.
2. **STAFSETH. H. J.** Pseudomonas infection in turkeys. Poultry Sci. **18**: 412, 1939.
3. **STAFSETH, H. J., MACK, W., and RYFF. J. F.** Pseudomonas infection in turkeys. Poultry Sci. **19**: 126-130, 1940
4. **CHUTE, H. L.** An outbreak of Pseudomonas infection in poults. Canad. J. Comp. Med. **13**: 112-115, 1949.
5. **GABY, W. L. and HADLEY, C.** Practical laboratory test for the identification of **Pseudomonas aeruginosa.** J Bact. **74**: 356-358, 1957.
6. **BERGEY'S MANUAL OF DETERMINATIVE BACTERIOLOGY.** Williams and Wilkins Co., Baltimore, 7th Ed. p. 99, 1957.
7. **PETERSON, E. H.** Pseudomonas infection in turkeys. J.A.V.M.A **107**: 79, 1945.
8. **BIGLAND, C. H. and QUON, A. B.** Infections of poultry with Arizona paracolon in Alberta. Canad. J. Comp. Med. **22**: 308-312, 1958
9. **JONES, J. C. and ANDERSON, G. W.** Sulfamerazine in the treatment of a Pseudomonas infection of turkey poults. J.A.V.M.A. **113**: 458-459, 1948.

The following is the substance of two papers read to Swiss Veterinary Societies in 1957 by Dr. H. Glattli. The translation was made by Dr. A Hess of Kitchener, Ontario Dr. Hess feels that, while the number of cases treated is yet too small to prove that milk fever is caused by an allergen, nevertheless Glattli's theory is an interesting one and deserves far more attention than it has yet received in either Europe or America.

Is Milk Fever an Allergic Disease?

Glattli believes that allergy may play a part in the causation of milk fever. His observation that calcium gluconate is used in the treatment of allergies in humans led him to investigate a possible relationship between allergy and milk fever in cattle. He treated a number of cases of milk fever with 40 - 50 cc. of antihistamine* combined with a relatively small dose (30 cc.) of calcium gluconate, and got better results than he had done with the intravenous injection of 500 cc. of calcium gluconate solution.

Next Glattli endeavoured to produce puerperal paresis experimentally. He selected a cow which was in late lactation and was giving very little milk. This individual had never had milk fever, and was presumed to be non-sensitive to the postulated allergens. It was essential to choose an animal that could be sensitized during the course of the experiment.

Colostrum was obtained from a cow down with milk fever and 20 cc. injected subcutaneously into the recipient. There was no reaction. Eight days later a large area on each shoulder of the experimental animal was shaved. A portion of skin within each shaven area was scarified with the point of a sharp scalpel. Glattli then rubbed colostrum which supposedly contained allergen into the right side, and colostrum presumed to be allergen-free into the left side. Within half an hour the right shoulder swelled and became very red; the scarifications opened and serous liquid poured from the openings. There was no reaction on the left side. Later Glattli injected 2 - 3 cc. of colostrum intravenously into the same cow, which quickly developed symptoms of milk fever.

Glattli felt that the result of the skin test was very gratifying. In his opinion it proved that puerperal paralysis is an allergosis, that the allergen is present in the colostrum of cows suffering from this disease, and that there is no allergen present in the colostrum of primipara. Fortunately sensitive cows are comparatively rare, or milk fever would be more common than it is.

In Glattli's opinion intravenous calcium gluconate does not restore the blood-calcium level; it acts instead by neutralizing the allergen or, and he considers this to be even more likely, it neutralized the histamine which is liberated by the antigen-antibody reaction. Air insufflation either prevents the diffusion of the allergen in the alveolas of the udder, or else the oxygen of the air, insufflated under pressure, neutralizes the allergen.

* Antistin (Ciba)