THE INCIDENCE OF SALMONELLA PULLORUM IN WILD PHEASANTS IN SOUTHERN MICHIGAN

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W. J. Stafseth
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RALPH C. BELDING

A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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AN ABSTRACT

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The objective of this study was to determine the incidence of Salmonella pullorum in wild pheasants in southern Michigan. The presence of pheasants in and around poultry yards and ranges and the importance of pullorum disease in artificially reared pheasants was considered as sufficient justification for undertaking this study.

This work included adult and immature birds as well as pheasant eggs from abandoned nests. Specimens from eight counties were examined and cultured bacteriologically for salmonellae.

Tissues and culture media recommended by the North Central States Poultry Disease Conference were used in this study.

Out of a total of sixty-five wild pheasants subjected to bacteriological culture, five were found to harbor S. pullorum. This represents 7.69 per cent of the birds cultured. The organism was recovered from four males and from one female.

No lesions characteristic of pullorum disease were detected in any of the infected birds. All five were apparently healthy and in good flesh. All the infected birds were found during February and March, at the time when the activity of the gonads was increasing. The first gross indication of testicular enlargement was detected on February 27. Ovarian development was first noted one week later.

Four of the five cultures of S. pullorum isolated produced acid from dextrose and mannitol. The fifth culture produced both acid and gas from these substances. As shown in Table I, this was the organism which had a preponderance of the variant (XII_2) antigen. The other four isolates were standard strains, and agglutinated only with XII_3 antiserum.

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INTRODUCTION

The pheasant (Phasianus colchicus torquatus) is frequently seen feeding in close proximity to both chickens and turkeys in Michigan. Most of our wild pheasants are found in the southern part of the state. This area also has the greatest concentration of domestic fowl.

The importance of salmonella infections in pheasants reared in captivity has long been recognized. However, the state game farm, which has a breeding stock of between one and two thousand adult pheasants, has been free from infection in recent years. This farm is maintained by the Michigan Department of Conservation in Ingham County near Mason. All birds on the farm are blood tested annually for pullorum disease under the supervision of the Michigan State Poultry Improvement Association using polyvalent antigen and the whole blood agglutination test. During the last two years no reactors have been detected in these birds. In the four previous years all reactors were submitted to the poultry diagnostic laboratory at Michigan State College. Salmonella pullorum was not recovered from any of these birds. Very few references to the disease in wild pheasants can be found in the available literature.

The writer, with the cooperation of the Michigan Department of Conservation, has attempted to determine whether or not pullorum disease is present in wild pheasants in Michigan. This study included adult and immature birds as well as pheasant eggs from abandoned nests. Specimens from eight counties were examined and cultured bacteriologically for the presence of salmonella types.

REVIEW OF LITERATURE

Since Rettger (1900) first described the etiological agent of pullorum disease, a great deal of work has been done on the problem of this infection in domestic fowl. Hewitt (1928) reported finding pullorum disease in domestic turkey poults. Since that time the infection has been found in most species of domestic birds as well as in a host of wild species including upland game birds. Shillinger and Morley (1937) stated that, of birds reared on game farms, the pheasant appeared to be most susceptible to infection with Salmonella pullorum. Hendrickson and Hilbert (1931) also reported on pullorum disease in pheasants hatched and reared in confinement.

Only one report of the isolation of S. pullorum from a wild pheasant has been found in the available literature. Cass and Williams (1947) reported the recovery of S. pullorum from a single adult male bird in Minnesota. This pheasant apparently died as a result of injury; no lesions indicative of pullorum disease were found.

No reference to attempts to isolate S. pullorum from pheasant eggs could be found. Egg transmission of this organism has been firmly established for domestic fowl. Mallmann and Moore (1936) reported a 25.08 per cent incidence of infection in 393 infertile eggs

and 241 eggs containing dead germs and chicks dead in the shell, using materials and methods comparable to those used in this survey. These eggs were laid by reactor chickens.

The pheasant is also susceptible to infection with other salmonellae. Edwards, Bruner, and Moran (1948) reported the following isolations from pheasants: Salmonella derby, Salmonella give, Salmonella kentucky, Salmonella meleagridis, Salmonella oranienburg, Salmonella san diego, and Salmonella typhimurium, as well as Salmonella pullorum.

MATERIALS AND METHODS

The pheasants used in this work were collected under a scientific collector's permit issued by the Game Division of the Michigan Department of Conservation. A total of sixty-five birds were collected from Cass, Clinton, and Ingham counties. Thirty-three were males, including one which was about ten days old. Thirty-two were females, including three which were between one and two weeks of age.

Thirty-six eggs were collected by conservation officers in Ingham, Clinton, Hillsdale, Macomb, Saginaw, Shiawassee, and Washtenaw counties, and were cultured bacteriologically. These eggs were from abandoned nests, or were those left after the remainder had hatched. Some of these specimens were in an advanced stage of decomposition, while others were relatively free from bacterial contamination.

Birds were autopsied as soon as possible after they were collected. Portions of the heart and pericardial sac, lung, liver, and drained gall bladder, spleen, pancreas, kidney, and gonad with the gonad base were streaked on SS and Bismuth Sulfite agar plates (Difco) and were then placed in 125 ml Erlenmeyer flasks containing

50 ml of Selenite F broth (B.B.L.). A portion of the ilium in the region of the yolk sac stalk and a section of the lower intestine including both cecal tonsils were placed in a second flask of Selenite F broth. In a few instances it was possible to include a blood agar plate containing 5 per cent avian blood. This was done when the birds were not mutilated by shotgun pellets passing through the viscera.

Agar plates and broth cultures were incubated at 37° C for 24 hours. A loopful of the broth culture was streaked onto both Bismuth Sulfite and Brilliant Green agar plates (Difco) which were incubated at 37° C for 24 hours. Colonies resembling those produced by members of the salmonella group were picked from the solid media and inoculated into lactose motility medium tubes. This medium is a modification by Darby (unpublished) of the Difco product and contains 1.8 per cent motility medium (Difco), 0.2 per cent beef extract, 1.0 per cent lactose, and 1.0 per cent Andrade indicator. After 24 hours' incubation at 37° C, the cultures were examined for growth, motility, and indication of lactose fermentation. Cultures from tubes which showed growth of organisms without lactose fermentation were transferred to lactose, maltose, sucrose, mannitol, and dextrose fermentation tubes. Inoculations were also made into a Kligler Iron agar slant (Difco) and indol medium.

Organisms which produced reactions characteristic of S. pullorum were stained by the Gram technic. Slide agglutinations were conducted using known pullorum positive chicken serum. S. pullorum cultures were also typed for the presence of XII_2 and XII_3 antigens using specifically adsorbed sera. The XII_2 specific antiserum was prepared by adsorbing Salmonella reading antiserum (IV, XII_1, XII_2) with S. pullorum (IX, XII_1, XII_3). The XII_3 specific antiserum was prepared by adsorbing Salmonella paratyphi A, var. durazzo antiserum (II, XII_1, XII_3) with S. reading.

The eggs used in this work were painted with colloidal iodine, air dried, and opened aseptically. The entire contents were added to flasks containing 50 ml of Selenite F medium. After incubation at 37° C for 24 hours, the broth was streaked onto Bismuth Sulfite and Brilliant Green agar plates.

RESULTS

Out of a total of sixty-five wild pheasants subjected to bacteriological culture, five were found to harbor S. pullorum. This represents 7.69 per cent of the birds cultured. The organism was recovered from four males and from one female.

No lesions characteristic of pullorum disease were detected in any of the infected birds. All five were apparently healthy and in good flesh. All the infected birds were found during February and March, at the time when the activity of the gonads was increasing. The first gross indication of testicular enlargement was detected on February 27. Ovarian development was first noted one week later.

Four of the five cultures of S. pullorum isolated produced acid from dextrose and mannitol. The fifth culture produced both acid and gas from these substances. As shown in Table I, this was the organism which had a preponderance of the variant (XII_2) antigen. The other four isolates were standard strains, and agglutinated only with XII_3 antiserum.

TABLE I

BIOCHEMICAL AND SEROLOGICAL CHARACTERISTICS OF SALMONELLA PULLORUM ISOLATED FROM PHEASANTS

	Culture Number				
	16	17	19	20	39
Date (1954)	2/19	2/19	2/23	2/27	3/30
County	Ingham	Ingham	Cass	Clinton	Clinton
Motility	-	-	-	-	-
Dextrose	A	A	A	A	AG
Lactose	-	-	-	-	-
Maltose	-	-	-	-	-
Mannitol	A	A	A	A	AG
Sucrose	-	-	-	-	-
Indol	-	-	-	-	-
Hydrogen Sulfide	S	S	+	+	+
Serological Type	Std.	Std.	Std.	Std.	Var.

+ = positive; - = negative; A = acid produced; AG = acid and gas produced; S = small amount; Std. = Standard; Var. = Variant.

Bacteria other than S. pullorum isolated from the pheasants in this study included micrococci, coliform types, paracolon organisms, and members of the proteus group.

Dr. L. D. Fay, Game Pathologist of the Michigan Department of Conservation, tested one of the four regular strains isolated for pathogenicity in day-old pheasant chicks. Under artificial brooding conditions, this organism killed twenty-four out of twenty-five chicks within nine days after they had received the culture by mouth.

No pathogenic bacteria were recovered from any of the eggs cultured.

DISCUSSION

The results of this study indicate that wild pheasants are a potential source of pullorum disease in domestic fowl on range. In chickens and turkeys S. pullorum is commonly isolated from the heart and pericardial sac, liver, lung, pancreas, kidney, and gonads, as well as from the intestinal tract. These intestinal carriers are considered to be a major source of the spread of salmonellae from one adult bird to another. Due to the passage of shot through the viscera, it was impossible to determine the sites of infection in the pheasants cultured. Since four of the five birds yielded S. pullorum from the intestines as well as from the other organs cultured, it seems logical to assume that some of these birds were intestinal carriers. Such birds could very easily carry the organism to areas previously free from salmonellae, and could account for "unexplained" outbreaks of pullorum disease in clean flocks of domestic birds.

From the point of view of workers in game management, domestic fowl represent a reservoir of infection for pheasants. The Conservation Department has kept pullorum disease out of the birds on the state game farm for at least the last six years, and yet the infection is present in the wild pheasants in this state. It appears

logical to assume that the presence of the disease in wild pheasants is due to an environmental infection rather than representing spread through birds released by the game farm.

Cass and Williams (1947) cited work done by Bennett, which is apparently out of print, in which he artificially infected four pheasants with S. pullorum. One of the infected females which showed ovarian lesions on necropsy produced no eggs in the period of two months following subcutaneous injection. A control female laid thirty-seven eggs during the same period. It is probable that egg transmission of the organism occurs in pheasants as it does in domestic fowl. Considering these facts in conjunction with the proved pathogenicity of S. pullorum for pheasant chicks and the known presence of the infection in our wild pheasants, it is interesting to speculate on the possibility of this disease as a factor contributing to the fluctuation in pheasant population which recently occurred in this state.

It is worthy of note that four of the five strains isolated from pheasants produced only acid from dextrose and mannitol. While this deviation from the normal is encountered occasionally, the typical culture of S. pullorum produces both acid and gas. Two of these atypical isolates were recovered from pheasants collected less than one mile from the college poultry flocks. During this same season

a similar strain of S. pullorum was isolated from one of the college birds.

An interesting feature of this study was the failure to recover any salmonella types other than S. pullorum in view of the report by Edwards of seven paratyphoid types from pheasants. The media and technics used in this work have proved satisfactory for the isolation of paratyphoid organisms from domestic fowl, and are those recommended by the North Central States Poultry Disease Conference.

CONCLUSIONS

A survey was made to determine the incidence of Salmonella pullorum in wild pheasants in southern Michigan. Sixty-five pheasants and thirty-six eggs were cultured. Five isolations of S. pullorum were made from the pheasants. The organism was not recovered from any of the eggs examined.

When the isolates were typed serologically, one variant type was found. Four of the cultures were atypical in that they produced only acid from dextrose and mannitol. No salmonella types other than S. pullorum were recovered from these specimens.

It is apparent from the results of this survey that a significant number of wild pheasants in Michigan harbor S. pullorum.

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