

# PULLORUM DISEASE

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## 1. Introduction

- a. PD is worldwide in distribution. *It is still common in Mexico, Central and South America, Africa, and parts of Asia.*
- b. Chickens and turkeys are the primary host species
- c. *Salmonella pullorum* has adapted to chickens to the point where it is less able to produce severe clinical disease in adult birds. (Avian Pathology 30:221-231, 2001)

## 2. Etiology

- a. *Salmonella pullorum*
- b. Somatic (O antigens) serogroup D<sub>1</sub> by the Kauffmann- White procedure
- c. Survives for years in a favorable environment
- d. Only the ornithine decarboxylase test definitely separates it from *Salmonella gallinarum*
- e. Somatic antigens - 1, 9, 12<sub>1</sub>, 12<sub>2</sub>, and 12<sub>3</sub>
- f. *S. pullorum* exists in three antigenic forms:  
Standard form - 1, 9, 12<sub>1</sub>, 12<sub>3</sub>  
Intermediate form - 1, 9, 12<sub>1</sub>, 12<sub>2</sub>, 12<sub>3</sub>  
Variant form - 1, 9, 12<sub>1</sub>, 12<sub>2</sub>  
Standard and variant strains are used in commercial testing antigens.

*Salmonella pullorum* are motile with swarming (moving across the surface of a medium) and swimming (movement through liquid medium) modes of motility. SP produce flagella while in birds and the flagella initiate an antibody response. (AJVR 60:1322-1327, 1999; Avian Dis. 42:807-811, 1998) For nearly 100 years, this organism was classified as nonmotile.

## 3. Natural Host

- a. Chickens are the natural host
- b. Turkeys are believed to originally have been infected by chickens and are now an important host also.
- c. Infections in chickens and turkeys are usually lifelong
- d. Lighter breeds (Leghorns) have fewer reactors in a flock than heavy

breeds such as Rhode Island Red and New Hampshire.

- e. Females have a greater percentage of reactors than males, *possibly due to localized infection of ovarian follicles.*
- f. Unusual hosts - ducks, guinea fowl, pheasants (Vet. Record 144:283-287, 1999), quail, sparrows, canaries, bullfinches, parrots, and peafowl. Wild turkeys have yielded positive tests in a few instances. (Avian Dis 42:393-396, 1998)
- g. Human infections have occasionally been reported

#### 4. **Transmission**

- a. Infected hatching eggs - up to 1/3 of the eggs are infected *because of contamination of the ovum after ovulation or localization in the ova before ovulation*
  - i. Maternal antibody prevents embryonic mortality in infected eggs
- b. Feces - fecal contamination of feed, water, and litter. Chicks infected at 4 days of age can harbor infection until they come into lay and then produce *S. pullorum*-contaminated eggs and infected progeny. (Avian Pathology 30:221-231, 2001)
  - i. During hatching or brooding from infected to uninfected chicks
  - ii. Fecal contamination of footwear, hands, clothing, crates, feedsacks, etc.
  - iii. Attendants, feed dealers, bird buyers, and visitors who move between farms and between bird houses
- c. Cannibalism
- d. Egg eating

#### 5. **Mortality**

- a. Peak mortality during the 2nd or 3rd week of life
- b. Resistance increases rapidly during the first 5 - 10 days of age with increased blood lymphocytes and body temperature
- c. From 0% up to 100%
- d. Mortality is higher in birds stressed by shipping

#### 6. **Clinical Signs**

- a. Somnolence (sleepiness) & huddle together

- b. Inappetence (anorexia)
- c. Drooping wings
- d. Diarrhea and dehydration
- e. Chalk-white excreta around vent
- f. Labored breathing
- g. \*Retarded growth in survivors
- h. \*Lameness due to swollen joints
- i. Adults
  - i. Adults usually appear to be clinically normal.
  - ii. Reduced egg production, fertility, and hatchability. Hatchability in the 1990 outbreak was 55%.

\*may be associated with use of antibiotics early in life

## 7. **Gross Lesions**

- a. Liver - \*white nodules, enlarged, dark
- b. Swollen spleen, kidneys
- c. Retained yolk sac - creamy or cheesy consistency
- d. Ceca - \*white nodules in the wall, yellow creamy or cheesy material inside
- e. Lungs - \*white nodules, yellow-gray pneumonia
- f. Heart - \*white nodules in muscle, white material covering the surface (pericarditis)
- g. Swollen hock and/or wing joints - sticky, straw-colored fluid in joints
- h. Kidneys - congested with urates in ureters
- i. Adults - Misshapen, discolored ova, peritonitis, pericarditis, white foci or nodules in infected male testes

\*Nodules can be confused with tumors, such as those caused by Marek's disease.

## 8. **Immunity**

- a. Infected at 4 days of age
  - i. Detectable agglutinating antibodies at 20 - 40 days of age
  - ii. Maximum antibody production at 100 days of age
- b. Infected at maturity
  - i. Agglutinating antibodies at 3 - 10 days of age

9. **Diagnosis**

- a. Isolation and identification. Antibiotic therapy can reduce the number of organisms in tissues to undetectable levels.
- b. Most body tissues contain the organism - liver, spleen, ceca, yolk sac, and joint fluid

10. **Treatment** - No drug or combination of drugs will eliminate infection from a flock. Survivors have a high percentage of carriers at maturity.

- a. Destroy survivors
- b. Clean and disinfect quarters
- c. Replace with clean stock

11. **Prevention of Vertical Transmission** (hen to chick via eggs)

- a. **Eliminate carriers** by serological testing. Because of cross-reactivity with other bacteria, antibody tests provide only presumptive identification.
  - i. Tube agglutination test - all poultry
  - ii. Microagglutination test - all poultry
  - iii. Enzyme-linked immunosorbent assay (ELISA) - all poultry
  - iv. Rapid serum test - all poultry (including turkeys)
  - v. Stained-antigen whole blood test - all poultry except turkeys
- b. **Nonpullorum reactors**
  - i. Other bacteria possess antigens closely related to *Salmonella pullorum*. The 12<sub>2</sub> is frequently the factor responsible for cross reactions. In a 1993 study conducted in Georgia, *S. pullorum* was isolated from the tissues of less than 6% of tube agglutination reactors.
  - ii. *Staphylococcus epidermidis*, *Micrococcus sp.*, *Aerobacter sp.*, *Proteus sp.*, *Escherichia coli*, *Arizona*, *Providencia*, *Citrobacter*, other salmonella (especially Group D, such as *Salmonella enteritidis*)
- c. **Retesting**. Each bird must be identified and a pullorum testing

program developed to retest the flock until no reactors are found.

- i. Done at 2- to 4-week intervals until two consecutive negative tests of the entire flock at not less than a 21-day interval between tests.
- ii. Two or three retests are often sufficient to eliminate all infected birds.
- iii. Retesting may not eliminate the disease. Environmental contamination may re-infect a flock after removal of reactors.
- d. **Heavy breeds** such as Hamps, Rocks, or Reds should be retested at frequent intervals.
- e. **Minimum Age at Testing.** (NPIP 145.14 Blood Testing)
  - i. Chickens - more than 4 months of age
  - ii. Turkeys - more than 12 weeks of age
  - iii. Gamebirds - more than 4 months of age or sexual maturity, whichever comes first
  - iv. Emu, rhea, cassowary - at least 12 months of age or upon reaching sexual maturity
- f. **Minimum Number Tested.** (NPIP 145.14 Blood Testing)
  - i. 30 birds per house, with at least 1 bird taken from each pen and unit in the house
  - ii. Ratio of male and female birds tested must be the same as the ratio of male to female birds in the flock
  - iii. In houses containing fewer than 30 birds, all birds in the house must be tested.
- g. **Reporting Testing Results.** All tests for pullorum-typhoid shall be reported to the Official State Agency within 10 days following completion of such tests. (NPIP 145.14 Blood Testing)
- h. **Positive Reactors.** Reactors shall be submitted to an authorized laboratory for bacteriological examination within 10 days from the date of reading the official blood test. If there are more than 4 reactors in a flock, a minimum of 4 reactors shall be submitted to the authorized laboratory. (NPIP 145.14 Blood Testing)

## 12. Control

### a. Hatchery

- i. Only eggs from PD-free flocks used in hatcheries
- ii. Hatching eggs should be disinfected.
- iii. Breakage of unhatched eggs in the incubator room should be avoided.
- iv. Equipment in the hatchery should be cleaned and disinfected between hatches with an approved disinfectant.
- v. Chick boxes should not be reused unless properly sterilized between use.

### b. Farm

- i. Purchase chicks and poults from NPIP sources free of pullorum disease.
- ii. No mixing of PD-free stock with poultry from other farms.
- iii. Place chicks and poults in an environment that can be cleaned and sanitized (no dirt floors) to eliminate residual salmonella from previous flocks
- iv. Male birds should be added to the supply flock as early as possible, preferably as chicks, and from a U. S. Pullorum Clean source.
- v. The replacement flock should be raised on clean ground away from the barnyard to avoid picking up pullorum disease from a contaminated yard.
- vi. The laying house should be cleaned and disinfected before the pullets are housed.

## 13. Biosecurity Program.

- a. Mechanical Carriers. *Salmonella pullorum* can be carried on human clothing, crates, and equipment. Prospective customers should not be allowed in the brooder room.
- b. Proper Dead Bird Disposal. Dead birds may be a source of infection for flockmates.
- c. Insect Control. Salmonella may survive in insects even after cleaning and disinfection has occurred.

- d. Rodent Control. *Salmonella* may survive in rodents even after cleaning and disinfection has occurred.
- e. Noncontaminated Water. Chlorination may be necessary in some cases.
- f. Noncontaminated Feed. Chicks and poults should receive pelletized, crumbled feed. Feed ingredients must be free of salmonella.
- g. Birdproof Houses. Free-flying birds rarely carry *Salmonella pullorum*.

## Pullorum Disease Review Questions

10. Does chilling, overheating, crowding, inadequate feeding, or poor feed cause pullorum disease?

No, poor brooding, etc., may increase the loss but will not cause pullorum disease. Pullorum disease is caused by infectious bacteria called *Salmonella pullorum*.

11. Do infected adult birds show any evidence or symptoms of the disease?

No, usually they appear to be perfectly healthy looking birds.

12. What lesions are seen in the adult bird?

The ovary may be dark in color which is often referred to as a blighted ovary. Yellowish exudate may be present in the heart sac.

13. Can you be absolutely sure of your diagnosis of pullorum disease by symptoms and lesions found in baby chicks? Adult birds?

No, the only reliable diagnosis of pullorum disease is based on the isolation and identification of *S. pullorum* by laboratory methods. If unusual death loss is encountered in a brood of chicks or poults, laboratory tests should be made to confirm the field diagnosis. Sick chicks should not be brought to the hatchery for diagnosis.

14. Can other fowl such as ducks, geese, pigeons, pheasants, or guinea fowl contract and disseminate pullorum disease?

Yes, other fowl may disseminate pullorum disease and thus hatcheries under NPIP are required to test all fowl that may be used as hatchery supply flocks.

## Pullorum Disease Testing

15. How can adult carriers of the disease be detected?

There are three recognized official tests for pullorum disease in chickens: (1) Rapid Whole Blood Test, (2) Serum Plate Test, and (3) Tube Test. There are only two recognized tests in turkeys: (1) Tube Test, and (2) Serum Plate Test. The rapid whole blood test is used in chickens and the tube test in turkeys.

16. What is the basis of application of these tests?

A bird that survives the infection and retains *S. pullorum* in its tissues develops substances in the blood called **antibodies**. These antibodies can be detected by using an antigen which is applied by one of the three methods mentioned above.

17. What is an **antigen**?

Pullorum antigen is made of dead *S. pullorum* organisms killed by adding a chemical and are then suspended in a salt solution. When the rapid whole blood test is used, a blue or red dye is added to stain the bacteria, thus making the test easier to interpret. With the serum plate or tube test, no dye is added to the antigen.

18. What happens when the blood of a pullorum carrier bird containing **antibodies** is mixed with the rapid whole blood crystal violet pullorum **antigen**?

Within an interval of 15 seconds to 2 minutes, blue clumps are seen in the mixture; this is the result of the specific antibodies combining with killed bacteria known as the antigen, and aggregation or clumping occurs. This reaction is known as agglutination.

19. Can pullorum antigen spread the disease?

No, the antigen is killed organisms and if spilled in a poultry house will not spread pullorum disease.

20. How should the pullorum antigen be stored when not being used?

The antigen should be kept in the refrigerator (45° F) when not being used. Out-dated antigen should not be used.

21. Pullorum testing agents should not work in the hatchery unless they change their clothing.

22. Pullorum testing equipment must be cleaned and disinfected between farms.

# **Pullorum Stained Antigen**

**K Polyvalent**

**List No. 0777**

**500 Tests (25 ml)**

**Read in full, follow directions carefully.**

## **For pullorum-typhoid testing**

Fort Dodge Animal Health, Inc., Pullorum Stained Antigen, K Polyvalent, is used to detect infection caused by both standard and variant strains of *Salmonella pullorum*, *Salmonella gallinarum*, and certain other specific members of the Salmonella group in chickens.

Store in the dark at not over 45°F (17°C). Do not freeze. Shake gently before use.

## **Equipment necessary for testing**

Antigen, testing plate, thermometer, bleeding needle, blood loop (standardized), small glass of water for rinsing loop, disinfectant, a pail of water, soft cloths or a chamois skin, and a device to hold birds individually (or in groups of 10 or less) while waiting for the reaction to develop, are necessary equipment for testing.

## **Handling the birds**

Handle the birds carefully. Use chutes or catching coops to help catch the birds, do not chase or excite them. Birds should be held on tables or in coops until test is completed.

## **Testing**

- a. Test in groups of 6 or more birds – conveniently as many as there are squares across the testing plate. Test surface should be between 70° and 80°F (21° and 26°C). Mix antigen and place a drop (0.05 cc) on a testing square.
- b. Pluck feathers from elbow region on underside of wing. Puncture blue vein with bleeding needle, and lift a fresh non-clotting drop with blood loop (0.02 cc).
- c. Transfer blood to testing square, stirring into antigen with loop, making a smear 1 inch in diameter. Rotate test plate in a circular motion to thoroughly mix the antigen and blood and to facilitate agglutination. Read test (see explanation below). Release bird if no reaction occurs. Retest partial or suspicious agglutinations. Isolate birds showing positive reactions.
- d. Between birds: Rinse loop in clean water and dry by touching it to a piece of clean blotting paper.

**Reading the tests:** Positive reactions are indicated by a clumping of the antigen in well-developed, blue-colored clusters surrounded by clear spaces – easily seen against a white background. The greater the agglutination ability of the blood, the more rapid the clumping and the larger the clumps. A lesser reaction shows small, but clearly visible clumps surrounded by spaces only partially clear. A fine, barely visible granulation sometimes occurs and there may be a fine marginal flocculation just before the smear dries. These should be regarded as negative. Reactions which occur after 2 minutes should not be considered positive.

Biological reactions may vary between clear-cut positive and negative. A reactor should show clumping as at left. A right, a typical negative test.

When interpreting reactions in this test, several factors, such as flock history, should be considered. The judgement of a professional flock manager should always be exercised before birds are condemned.

### **Follow up**

In flocks showing positive reactors or suspicious agglutinations, it is desirable to examine representative birds bacteriologically to determine the presence or absence of *Salmonella pullorum*.

### **Pointers on testing**

- a. Perform the test out of direct sunlight in a dust-free area.
- b. Maintain 70° to 80°F (21° to 26°C) temperature on testing surface. A lower temperature may affect accuracy of results.
- c. Correct proportions are important: a loopful (0.02 cc) of blood to 1 drop (0.05) of antigen.
- d. On completion of testing, remove reacting birds from premises and burn litter. Then disinfect house and equipment with a suitable disinfectant, such as Fort Dodge Health, Inc., Germex.

### **Blood testing problems**

Faulty technique, improper interpretation, unsuitable equipment, or defective antigen may cause erroneous results in the usually reliable agglutination test.

Occasionally, large numbers of positive reactors of a previously tested flock may be the result of one or more of the following:

- a. Contaminated premises may infect other birds.
- b. Vaccination with a vaccine containing *Salmonella gallinarum*, or certain other

strains of Salmonella may agglutinate the antigen. Do not test flocks for pullorum or typhoid within a 60-day period after vaccinating for fowl typhoid.

- c. All positive reactors may not have been removed in previous tests.

### **Things to avoid**

Deteriorated antigen may give false readings. Before testing, check a drop of antigen, without blood, on the plate for spontaneous agglutination. Excessive evaporation, high temperatures, or incorrectly interpreting late powder or marginal flocculation as positive reactions may also lead to false readings. Delay in reading tests causes errors; tests should not be read after 2 minutes. Testers should use care, not speed, for the number of birds tested is less important than maximum accuracy.

### **Cleansing the testing surface**

Clean plate with clear water. Hot water may coagulate blood, making it difficult to remove. Soaps, disinfectants, or cleaning compounds may leave a residue which may affect subsequent tests. Grease on plate may prevent blood antigen mixture from spreading properly. It may be removed with soap, after which plate must be thoroughly rinsed. After cleaning, polish plate with clean cloth, leaving no blood or lint.