

Report on anaerogenic *Salmonella enteritidis* bioserotype Pullorum in chickens in the vicinity of Manaus, Amazonas, Brazil

JOSEPH RICHARD CAIN (*), CHERYL ANN McCARTHY (**), JURANDIR CHAVES DE VASCONCELOS (*), MARIA DE FÁTIMA DA SILVA PINHEIRO (*) E MARY ANNE ASCENZI (***)

ABSTRACT

Bacteriological and aviculturological evidence is presented which demonstrates the presence of anaerogenic *Salmonella enteritidis* bioserotype Pullorum in chickens in the vicinity of Manaus, Amazonas, Brazil. This bacterium is the etiological agent of pullorum disease (bacillary white diarrhea) in chickens and salmonellosis in humans.

INTRODUCTION

Salmonella gallinarum was named by Klein in 1889 (Breed et al, 1957). In 1899, Rettger first isolated the bacillus subsequently called *Salmonella pullorum*, (Bier, 1970). The literature through the 1960s generally considers these species as distinct etiological agents, respectively, of avian typhoid (Klein's disease) and pullorum disease (bacillary white diarrhea). In addition, avian salmonellosis was for a long time confused and combined with avian cholera (Marques, 1965), as a study of early literature makes obvious.

Kauffman (1954) proposed that non-motile strains serologically belonging to *Salmonella* Group D (1, 9, 12) are best designated by the name *Salmonella gallinarum-pullorum*, as numerous biochemical types of such strains occur, which make an exact distinction between *S. gallinarum* and *S. pullorum* impossible. Nevertheless, he further suggested that such cultures which are anaerogenic and which promptly ferment dulcitol and maltose may be classified as *S. gallinarum*; whereas *S. pullorum*, usually aerogenic, is dulcitol negative, grows sparsely on artificial media, and is usually maltose negative.

Ewing (1972) affirms that the bioserotypes Pullorum and Gallinarum (of the species he nominates *S. enteritidis*), cannot be distinguished by serological methods alone; however, he provides 13 biochemical tests which can be used to differentiate these bioserotypes.

Corrêa (1970) took the position that *S. gallinarum* and *S. pullorum*, antigenically identical, cannot longer be considered as distinct species, since they are united with one another by numerous intermediate types. He proposed that the disease (s) produced be called avian salmonellosis with one set of manifestations in the chick and another in the adult bird.

In chicks, *S. enteritidis* bioserotype Pullorum is septicemic: it is found in the blood and in most organs. Effects include high mortality, retardation, and possibly the "carrier" condition in survivors.

In man the disease is manifested by an infection producing acute gastroenteritis of varying intensity, duration, and spread. The organism is transmitted to humans through contact with infected animals or offal, and by way of infected eggs and meat (chicken) which have been insufficiently cooked before consumption.

The disease can be controlled. Data from the Center for Disease Control (1973, 1974a, 1974b) indicate that total reported cases in the United States of America from non-human sources of *S. pullorum* for the years 1971 through 1973 were 45, while total reported cases from human sources for the years 1966 through 1973 were 23.

Control of avian salmonellosis involves elimination of all infected fowl. Treatment

(*) — Instituto Nacional de Pesquisas da Amazônia

(**) — Associação de Crédito e Assistência Rural do Amazonas

(***) — Empresa Brasileira de Pesquisas Agropecuárias

with antibiotics and sulfa drugs can reduce mortality, but many of the survivors become carriers (Merck, 1967) which spread the disease by laying infected eggs, polluting the environment with contaminated feces, and possibly ending up as infective (inadequately cooked) meat.

A careful review of the Amazonian literature from earliest years (Instituto Brasileiro de Bibliografia e Documentação, ed., 1963 and 1972) failed to uncover any previous reports of avian salmonellosis in this region, although it has been reported from other Brazilian areas. Commercial poultry production in this region is recent. Since 1970, although there has been nothing published, cases of fowl pox, Newcastle's disease, Marek's disease, lymphoid leukosis, chronic respiratory disease, coccidiosis, and infectious coriza, among others, have been observed by producers and agencies working with chickens.

MATERIALS AND METHODS

In the June bacteriological analyses, sample chickens were taken from 2- and 3-week-old lots. These were sacrificed and necropsied. Lungs, blood, intestinal contents, urates, and feces were collected aseptically. These materials were immediately brought to the bacteriology laboratory and directly streaked onto plates of *Salmonella-Shigella* agar (SS), brilliant green agar (BG), and MacConkey agar (M). Suspect colonies were picked at 24 h and 48 h onto triple sugar iron agar (TSI). Cultures on TSI, suspect for *Salmonella*, were reisolated and characterized biochemically and serologically.

Sample chicks in the July bacteriological analyses were from a 5-week-old lot. These were sacrificed and necropsied. Lungs, blood, intestinal contents, urates, and feces were collected aseptically. These were directly streaked onto plates of SS, BG, and M, as above. These same materials were inoculated into selenite-cystine (SC) and tetrathionate broth base + I₂KI + brilliant green (TBB+), both for enrichment, with subsequent streaking onto SS, BG, and M, isolation onto TSI, re-isolation if suspect, then biochemical and serological characterization.

Also in the July analyses, collections were taken of rations, drinking water, and bedding. For preenrichment, these materials were inoculated into lactose broth. Subsequently, enrichment passage was into SC and TBB+, then the plates of SS, BG, and M, with picking onto TSI, re-isolation if suspect, and biochemical and serological characterization.

Media were prepared and tests conducted according to specifications found in the Bacteriological Analytical Manuals (1969, 1973) in conjunction with the Official Methods of Analysis of the Association of Official Analytical Chemists (1970), wherever applicable.

The coccidiosis test was done according to standard procedures.

The necropsies were done by a team of two of the authors. A selection of newly dead and very sick birds was made for laboratory examination. The live birds were sacrificed by dislocating the neck without breaking the skin or rupturing the trachea or esophagus. General autopsy procedures were followed on all birds. Only collections from newly sacrificed birds were used for the bacteriological analyses.

RESULTS

Bacteriological

The 9 cultures isolated in June from lungs, intestinal contents, and feces of 2 of the chicks (aged 2 and 3 weeks) and the 4 cultures isolated in July from feces and blood of 1 of the chicks (aged 5 weeks) were biochemically and serologically *Salmonella enteritidis* biotype Pullorum.

Results of the tests were:

adonitol	—
arabinose	A
arginine	—
cellobiose	—
citrate (Kauffmann)	—
citrate (Simmons)	—
dulcitol	—
glucose	A
glycerol	—
indole	—
inositol	—

KCN	—
lactose	—
lysine iron agar	+, with H ₂ S (1 day)
malonate	—
maltose	—
mannitol	A
mucate	—
MR	+
ornithine	+
raffinose	A
rhamnose	A
saccharose	—
salicin	—
sorbitol	A
tartrate (Jordan)	—
d-tartrate (Kauffmann)	—
i-tartrate (Kauffmann)	—
l-tartrate (Kauffmann)	+
trehalose	A
urea	—
VP	—
xylose	A
gram stain	negative, non-sporulating bacillus.
motility	—
serology	positive in <i>Salmonella</i> somatic polyvalent, positive in somatic group D, and positive in 9-12 ₃ antisera.
TSI agar	alk slant/acid butt/no gas /with H ₂ S (after 2-3 d)/slow growing on the agar.

Key: + = positive

— = negative (carbohydrate media were observed for 10 d)

A = acid (within 24 h), except for trehalose which required 3 d for all cultures to go acid.

Aviculturological

The symptomology in June included the following:
 no coughing, sneezing, or rattling (no evident respiratory symptoms);
 no exudate from the eyes, nose, or mouth;
 no excessive nervousness;

retarded growth in all broilers, with retardation most notable in the oldest flock (8 weeks old);
 non-uniform growth among chicks of the same age and variety;
 high mortality;
 twisted neck ("star gazing");
 white, pasty diarrhea;
 emaciation; and
 slow feather development.

The following abnormalities were found to be most common among all birds (21) examined in June:

heart pale, with white growths;
 liver of variable color — light to dark — with most being dark, hemorrhages ranging in size from pin point to massive;
 spleen enlarged, hemorrhaged, with greyish-white growths;
 intestines discolored and, in several cases, with a thick, sticky, white fluid inside;
 when gizzard showed lesions, these were usually small hemorrhages;
 yolk sacs were usually not absorbed;
 kidneys were hard and filled with urates, as were the ureters; and
 lungs congested, necrotic, with white-grey tumorlike growths, hemorrhagic, with pus.

In July the symptomology included the following:

high mortality;
 retarded growth;
 non-uniform growth among chicks of the same age and variety;
 white to brown-white diarrhea;
 excessive noise when at rest; and
 emaciation.

The following lesions were found in the July sample of birds:

heart pale, with white growths;
 liver dark, with yellowish plaques giving a "freezer-burn" appearance;
 spleen enlarged, hemorrhagic, with greyish-white growths;
 small intestine friable, with gross, diffuse tumorlike growths and blood tinged contents;
 gizzard hemorrhagic;

yolk sac unabsorbed or yolk stalk present;
walls of cecum swollen;
kidneys pale;
adrenal glands enlarged; and
lungs friable and necrotic.

DISCUSSION

The senior author, who has written of food microbiology in Brazil (Cain, 1973a; Cain, 1973b) has for months been curious about the incidence of food-borne salmonellosis in the human population of Manaus. While no data yet have been published which can confirm "guesses", it would seem that many complain of intestinal disorders approximating salmonellosis following the not uncommon practice of eating insufficiently cooked chicken. The same may be true of inadequately cooked eggs.

Thus, when the opportunity arose to study bacteriologically two cases of avian disease of presumed salmonella origin, this author was interested.

The symptomology and postmortem findings were indicative of both *pullorum* disease and avian typhoid. The authors struggled with the problem of distinguishing clearly these two diseases, since various authoritative publications (Biedma & Sequeira, 1945; Bier, 1970; Brunini, 1954; Chemist's Veterinary Handbook, 1955; Farris, 1950; Marques, 1965; Merck, 1967; and Salsbury, 1962) describe them as quite distinct, with different etiological agents: *S. pullorum* and *S. gallinarum*, respectively.

The bacteriological work resulted in the isolation and characterization of a total of 13 cultures, all identical according to the indicated biochemical, serological, and other tests.

We were impressed with Kauffmann's (1954) view that there is only one serological species, namely, *S. gallinarum-pullorum*, although biochemical distinctions might suffice to allow the use of one or another of the separate species denominations. However, this really didn't help resolve our problem of deciding, on clinical and bacteriological grounds, which disease we were onto. We were aware that written descriptions occasionally indicated that pullorum disease pri-

marily afflicts chicks while avian typhoid is mostly found in adult birds.

We uncovered Corrêa's (1970) treatment of the disease(s). Corrêa holds that *S. gallinarum* and *S. pullorum* can no longer be considered distinct species, but rather as varieties of one species (*S. gallinarum-pullorum*), united one with the other by numerous intermediate types. And, he indicates that this single species causes both diseases, pullorum and avian typhoid, which diseases appear to be distinct only because manifestations are variable in accordance with the age of the ave affected.

While we are unable to resolve the question of whether or not the same organism (strain) is capable of being the etiological agent for both pullorum and avian typhoid, as suggested by Corrêa, we conclude that our 13 cultures are *Salmonella enteritidis* bioserotype Pullorum, in accordance with the characterization given by Ewing (1972). Confirmation of our identifications has been made by the Enterobacteriology Branch, Center for Disease Control, Atlanta, Georgia 30333, U.S.A..

ACKNOWLEDGEMENTS

Special thanks are due to Alma McWhorter-Murlin of the Enteric Section, Enterobacteriology Branch, Bureau of Laboratories, Center for Disease Control, Atlanta, Georgia, USA, for the biochemical and serological confirmation of the cultures.

In addition, the authors acknowledge the assistance of G.S. Jacobsen of the Wisconsin Animal Health Laboratories, Madison, Wisconsin, USA; Betty C. Hobbs and Peter C. B. Turnbull of the Food Hygiene Laboratory, London, England; L. LeMinor of the Institut Pasteur, Paris, France; M. J. Fanelli and Darrell Behymer of the Department of Epidemiology and Preventive Medicine, School of Veterinary Science, University of California at Davis, USA; Thomas Styer, U.S. Peace Corps, Belo Horizonte, M.G., Brazil; and Br. Romard Barthel, St. Edward's University, Austin, Texas, USA.

The International Center for Medical Research in Cali, Colombia, was generous with media and sera.

SUMÁRIO

No período de junho a julho de 1974, houve um surto de doença em 2 (duas) granjas distintamente localizadas, com um índice de mortalidade em média de 30%. Sintomas e necrópsias indicaram Salmonelose em ambos os casos. Testes de Coccidiose realizados apresentaram resultado negativo. Pintainhos de faixa etária 2-5 semanas foram sacrificados e necropsiados, obtendo-se amostras de pulmões, sangue, conteúdo intestinal, uratos e fezes. Subseqüentemente foram utilizadas coletas de rações, água de beber e detritos da cama aviária. Das análises bacteriológicas feitas em todo o material coletado, foi demonstrado a presença de *Salmonella enteritidis* biosorotipo Pullorum em pulmões, sangue, conteúdo intestinal e fezes. Esta bactéria é o agente etiológico da doença em pintainhos, denominada Pulorose ou Diarréia bacilar branca. Foi constatado que os lotes de pintainhos infectados vieram da mesma chocadeira. Diagnose seguinte, tratamento e controles foram medidas aplicadas com resultados satisfatórios. Só recentemente, é que estão sendo instaladas granjas de larga escala nesta região e estão se expandindo. Então, as tentativas para controle desta doença devem ser vigorosas, por razões sanitárias e econômicas, em virtude da fatalidade em galinhas e sérias infecções que podem ocasionar no homem.

LITERATURE CITED

- ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS
1970 — **Official Methods of Analysis of the A.O.A.C.** 11. ed. Washington.
- BACTERIOLOGICAL ANALYTICAL MANUAL
1969 — **BAM**, U.S. Department of Health, Education and Welfare, Public Health Service, Food and Drug Administration, Division of Microbiology. 2. ed. Washington.
1973 — **BAM**, U.S. Department of Health, Education and Welfare, Public Health Service, Food and Drug Administration, Division of Microbiology. 3. ed. Washington.
- BIEDMA, P.C. & SEQUEIRA, O. DE
1945 — **Cartilha Avícola Brasileira**. 7. ed. São Paulo. Chácaras e Quintais.
- BIER, OTTO
1970 — **Bacteriologia e Imunologia**. 15. ed. São Paulo. Edições Melhoramentos.
- BREED, R. S. ET ALII
1957 — **Bergey's Manual of Determinative Bacteriology**. 7. ed. Baltimore. Williams and Wilkins.
- BRUNINI, JOÃO
1954 — **Manual de Avicultura**. 2. ed. São Paulo. Edições UCB.
- CAIN, J. R. ET ALII
1973a — A report on the incidence of bacterial contamination of foods in the state of Minas Gerais, Brazil. In: **Proceedings of the Fourth International Conference on Global Aspects of Applied Microbiology**. São Paulo.
1973b — Inadequacy of microbiological standards for foods in relation to the Brazilian reality. In: **Proceedings of the Fourth International Conferences on Global Aspects of Applied Microbiology**. São Paulo.
- CENTER FOR DISEASE CONTROL
1973 — **Salmonella Surveillance Annual Summary 1972 issued November 1973**. Atlanta. Bureau of Epidemiology.
1974a — **Salmonella Surveillance January-September 1973 issued February 1974**. Atlanta. Bureau of Epidemiology.
1974b — **Salmonella Surveillance October-December 1973 issued June 1974**. Atlanta. Bureau of Epidemiology.
- CHEMIST & DRUGGIST
1955 — **The Chemist's Veterinary Handbook**. London, England.
- CORRÊA, OUTUBRINO
1970 — **Doenças Infeciosas dos Animais Domésticos. 1. Doenças Causadas por Bactérias**. São Paulo. Livraria Freitas Bastos.
- EWING, W. H.
1972 — **Isolation and Identification of Salmonella and Shigella**. U.S.H.E.W.P.H.S. Atlanta. Center for Disease Control, Enterobacteriology Branch.
- FARRIS, EDMOND J., ED.
1950 — **The Care and Breeding of Laboratory Animals**. New York. John Wiley & Sons, Inc.
- INPA & IBBD
1963 — **Amazônia — Bibliografia**. 1614/1962. Rio de Janeiro. v. 1.
1972 — **Amazônia — Bibliografia**. 1601/1970. Rio de Janeiro. v. 2.
- KAUFFMANN, F.
1954 — **Enterobacteriaceae**. 2. ed. Copenhagen. Ejnard Munksgaard Publisher.
- MARQUES, MÁRIO
1965 — **Galinhas e Ovos**. 13. ed. Lisboa. A.M. Teixeira e Cia. Ltda.
- MERCK
1967 — **The Merck Poultry Serviceman's Manual**. New York. Merck, Sharp & Dohme, International.
- SALSBURY
1962 — **Salsbury's Manual of Poultry Diseases**. Iowa. Salsbury's Laboratories.