



RESEARCH COMMUNICATION

Pasteurella gallinarum: Zimbabwean experience of a versatile pathogen

K. MOHAN¹, F. DZIVA¹ and D. CHITAURO²

ABSTRACT

MOHAN, K., DZIVA, F. & CHITAURO, D. 2000. *Pasteurella gallinarum*: Zimbabwean experience of a versatile pathogen. *Onderstepoort Journal of Veterinary Research*, 67:301–305

Pasteurella gallinarum-related outbreaks in chickens and African guinea fowls are described. Four outbreaks were recorded in chickens and one in guinea fowls. Periorbital swelling and keratoconjunctivitis were the consistently present clinical signs in all the diseased birds. In several, swollen hocks and wattles were also discerned. Birds which succumbed to the infection showed petechiation in the internal organs and evidence of airsacculitis. *Pasteurella gallinarum* was isolated from the lesions and also from conjunctival swabs of the apparently healthy in-contact birds. There was no evidence of concurrent infection with *Haemophilus*, *Mycoplasma* or *Chlamydia*. Quinolone therapy when resorted to on one of the farms resolved the clinical signs. Phenotypes of 28 isolates were studied. The results compared well with the *Pasteurella gallinarum* isolates reported earlier from elsewhere. It was also found that results of xylose fermentation and ONPG test appear to be a variable character. There is no earlier report of *P. gallinarum* infection in guinea fowls.

Keywords: Chicken, guinea fowls, *Pasteurella gallinarum*, Zimbabwe

INTRODUCTION

Pasteurella gallinarum was first reported by Hall, Hedleston, Legenhausen & Hughes (1955) from cases of fowl cholera. They also reported that conjunctiva, nasopharynx and internal organs were frequently colonized sites in the order of frequency mentioned. *Pasteurella gallinarum* has subsequently been isolated from disease in chickens, ducks, geese and turkeys, and has been incriminated as the causative agent in a wide range of pathological lesions, from septicaemic to localized. The latter comprised sinusitis, airsacculitis, conjunctivitis, hepatitis, salpingitis, inflammation of wattle/comb, synovitis and/or pericarditis/endocarditis. Experimental infection in chickens has been reported to have induced endocarditis,

hepatic/splenic infarcts, nephritis and pneumonia. Despite these reports, *P. gallinarum* is considered to be of low virulence and naturally occurring cases have been reported to have been concurrently infected with coronavirus and/or *Mycoplasma synoviae* (Bisgaard & Dam 1981; Bock, Samberg & Mushin 1977; Droual, Walker, Shivaprasad, Jeffrey, Meteyer, Chen & Shapiro 1992; Droual, Shivaprasad, Meteyer, Shapiro & Walker 1992a; Mushin, Bock & Abrams 1977; Nigrelli, Pascucci, Cessi & Zavanella 1983; Ojo 1971; Tjahjowati, Orr, Chirino-Trejo & Mills 1995; Yadav, Sharma & Sethi 1977). We report four *Pasteurella gallinarum*-related outbreaks on three different properties in domestic fowls and one in African guinea fowls. Details of these outbreaks, disease manifestations and the phenotype of the isolates have been described.

Within one year, three outbreaks at 4–5 months' intervals were reported on property no. 1, a very large commercial poultry farm. Initially, the broiler-parent stock aged 28–68 weeks were involved but subsequently pullets and broilers were also affected. The

¹ Faculty of Veterinary Science, University of Zimbabwe, P.O. Box MP 167, Mount Pleasant, Harare, Zimbabwe

² Barrington Vetcare, Waterfalls, Harare, Zimbabwe

Accepted for publication 12 October 2000—Editor



FIG. 1. Onset of the disease: Periorbital swelling and keratoconjunctivitis set in



FIG. 3. Terminal case: Total loss of an eye resulting from keratoconjunctivitis



FIG. 2. Advanced case: Intense periorbital swelling; complete closure of the eye

flock size was estimated to be over 24 000 of which, in the first outbreak, the disease occurred in a flock of 500 birds and resulted in a morbidity rate which peaked at 60% and a mortality rate of between 1 and 2%. In the subsequent outbreaks, a flock of 2 000 birds was affected but the morbidity and mortality rates were lower. The entire flock was vaccinated against ten different viral diseases, including infectious bronchitis, laryngo-tracheitis and the three bacterial diseases, fowl coryza, chronic respiratory disease and infectious synovitis (*Mycoplasma synoviae*) but not against pasteurellosis. Onset of the disease under review was marked by sneezing and

coughing which progressed to a variety of other clinical manifestations. Most affected birds showed periorbital sinus-related swellings, keratoconjunctivitis (Fig. 1 and 2) and swollen hocks in this order of frequency. In addition, a number also manifested swollen wattles. In several birds a severe keratoconjunctivitis resulted in a total loss of the affected eye (Fig. 3). Nineteen live birds were received at intervals, 14 of which showed various manifestations of the disease and five were apparently healthy in-contacts. In addition, we also received conjunctival and choanal swabs from 25 apparently healthy in-contacts. On property no. 2, a communal farm, broilers aged 8–10 weeks were reported to be affected. Information on vaccination, total flock numbers and other details were not made available, but the farmer reported loosing over 50 birds during the course of the outbreak. The clinical manifestations reported were similar to those observed on property no. 1, except that a few of the affected birds also suffered from torticollis. One freshly dead bird for post mortem investigation was received. The outbreak on property no. 3, a communal farm, was reported in African guinea fowls. The flock size comprised 150 adult and layers of which 10 suffered from the disease and two died. The birds had not been vaccinated against any viral or bacterial infection. Two sick birds were received for investigation.

Fowl coryza and/or mycoplasmosis on all the three properties was suspected by the attending veterinarians.

Specimens collected from the sick birds sampled, comprised aspirates from swollen sinuses, hocks, wattles and conjunctival swabs. Repeat samples were collected from the sinuses and hocks. Heart

TABLE 1 Phenotypic details of *P. gallinarum*

Property	1 ^b	2 ^b	3 ^b
1 Gram-stained cell morphology	Pasteurella-like	Pasteurella-like	Pasteurella-like
2 Growth in air/CO ₂ /anaerobic	+	+ ^a	+
3 Haemolysis on BA	-	-	-
4 Growth on MacConkey	-	-	-
5 Catalase and oxidase	+	+	+
6 Urease, citrate utilization, motility and Aesculin hydrolysis	-	-	-
7 O/F	F	F	F
8 Nitrate and Nitrite reduction	+	+	+
9 Acid in PW sugars: Arabinose, dulcitol, inositol, lactose, mannitol, salicin and sorbitol	-	-	-
Glucose, maltose, mannose, sucrose and trehalose	+	+	+
10 Xylose	+	-	-
11 ONPG	-	-	+
12 H ₂ S lead acetate	+	+	+
13 Indole	-	-	-
14 Ornithine, arginine, lysine utilization	-	-	-

^a Extremely capnophilic

^b Indicates isolates from property 1, 2 and 3

TABLE 2 Phenotype of *P. haemolytica*

Property	
1 Morphology	Pasteurella-like
2 Growth in air, CO ₂ , anaerobic and on MA	+
3 Catalase and oxidase	+
4 Motility, indole, aesculin hydrolysis, citrate utilization and urease	-
5 Haemolysis on BA	+
6 O/F	F
7 H ₂ S with lead acetate paper	+
8 Acid in PW sugars: Arabinose, dulcitol, inositol, lactose, maltose mannitol and salicin	-
Glucose, sorbitol, sucrose, trehalose and xylose	+
9 ONPG	+
10 Arginine, ornithine and lysine utilization	-

anal swabs were collected from apparently healthy birds received for investigation. All the specimens were cultured for *Haemophilus*, *Actinobacillus* and *Pasteurella* spp. while the joint and sinus aspirates and air sac swabs were also cultured for *Mycoplasma* spp. Gram-stained smears of the joint aspirates were examined and also investigated for *Chlamydia* employing Modified Ziehl-Neelsen (MZN) staining and Fluorescent Antibody Technique, (FAT: conjugate from DAKO, Denmark). For bacterial culture, 5% sheep blood agar (BA), chocolate agar (CA), MacConkey agar (MA) and brain heart infusion (BHI) broth (all from Oxoid, UK) were employed. All the plates except MA were incubated under elevated atmospheric CO₂ levels (candle jar) at 37 °C. Bacterial isolates were characterized following the methods described by Barrow & Feltham (1993). The methods employed to culture and identify *Mycoplasma* were those that have been described elsewhere (Mohan, Obwolo & Hill 1992). Control measures instituted on Property No.1 included treatment with a quinolone preparation placed in drinking water whereas on property no. 3, a tylosine preparation was recommended. No information on measures adopted to control the infection on property no. 2 was obtained.

blood, pericardial exudate and liver specimens were collected on post mortem examination when air sac swabs were also made. Only conjunctival and cho-

One isolate originating from a bird on property no. 1 and the solitary isolate of *Pasteurella. haemolytica* were forwarded to KVL, Denmark for confirmation.

Six of the 14 diseased birds received from the commercial farm died within 3–4 days after arrival but the remaining eight were sacrificed at intervals for post mortem examination. None of the apparently healthy in-contacts developed clinical manifestation but they were also sacrificed at intervals. On post mortem examination, petechiation of internal organs, airsacculitis and synovitis were the most marked pathological lesions observed in all those which had shown evidence of clinical disease but no gross lesions were noticed in any of the healthy in-contacts. Gross lesions observed in the bird from the communal farm were similar to those from the commercial flock. From all the birds which died, *P. gallinarum* was consistently isolated in pure culture from the aspirates of the periorbital and synovial swellings and swollen wattles.

A mixed growth of *P. gallinarum* with either a *Staphylococcus* sp. and/or *Escherichia coli* was, however, obtained from conjunctival swabs, except in one case from which *P. haemolytica* was isolated. *Pasteurella gallinarum* was cultured from the heart blood of only those which succumbed to the infection, but the livers and air sacs were found to be colonized in all the diseased birds. The conjunctival swabs of 25 healthy in-contacts yielded *Pasteurella gallinarum* but the results of isolation from the choana were equivocal owing to the overgrowth by *Proteus* sp. Conjunctival swabs and sinus aspirates from both the diseased guinea fowls yielded *P. gallinarum*, the latter in pure culture. The quinolone therapy administered on property no. 1 resolved the clinical signs in most of the sick birds; but those which did not respond to this therapy were culled.

The phenotypic properties of 28 strains, 25 originating from the commercial farm, one from a chicken on a communal farm and two from the guinea fowls were studied. The strains from all three properties were similar except in their reaction in xylose fermentation and ONPG test. The results of the tests in respect to representative strains of *P. gallinarum* are detailed in Table 1, and those of the *P. haemolytica* isolate in Table 2.

Investigations for *Mycoplasma* yielded negative results; and negative results were also obtained in smears stained FAT and MZN for *Chlamydia*.

Despite the wide range of pathological lesions attributable to *P. gallinarum* infection (Bisgaard & Dam 1981; Droual *et al.* 1992; Nigrelli *et al.* 1983) the cases that were encountered, all consistently showed two common clinical manifestations, viz. periorbital swelling and keratoconjunctivitis, and in lesser frequency, swollen hocks and wattles. The presence of these lesions appears to have lead the clinicians to tentatively diagnose either fowl coryza or mycoplasmosis. Gross lesions consistently present on necropsy of the birds which succumbed were widespread

petechiation and airsacculitis, suggestive of septicaemia. The outbreaks on all the three properties could be attributed to *P. gallinarum* infection since concurrent infection with *Haemophilus*, *Mycoplasma* or *Chlamydia* were excluded and it is considered that the solitary isolate of *P. haemolytica* was incidental.

Although we did not investigate for a possible virus cause, the vaccination records on property no. 1 which state that the birds were inoculated against important viral diseases including infectious bronchitis and laryngo-tracheitis are suggestive of the absence of corona and herpesvirus etiology. A possible viral etiology of the disease is also unlikely if the results of quinolone therapy on property no. 1 are taken into consideration. It is noteworthy that Droual *et al.* (1992a) have reported concurrent infection with *P. gallinarum*, coronavirus and *M. synoviae*. Our results of the isolation of *P. gallinarum* from apparently healthy in-contacts corroborate the findings of Hall *et al.* (1955) that the conjunctiva is the most frequently colonized site. The factors which contributed in translating the *P. gallinarum* pathogenicity in the affected flocks from one of a benign state of healthy co-existence as obtained in the in-contact birds to one of severe pathogenicity in those manifesting clinical disease could not be ascertained. This particular aspect and the wide-ranging lesions reported by others justify that *P. gallinarum*, be described as a 'versatile' pathogen. Naturally occurring serial passages of *P. multocida* (*sensu stricto*) from a carrier to non-carrier animals and stress of overcrowding are the two well known factors which trigger shipping fever outbreaks in cattle. Phenotypic details of our isolates compared very well with those of earlier reports. Different pattern of reactions in xylose fermentation and ONPG test is not unusual. The reactions in these two tests have been reported to be variable (Droual *et al.* 1992; Hall *et al.* 1955). There is only one published report of a *P. gallinarum*-related outbreak in domestic fowls from Africa (Ojo 1971) but the disease caused by this organism in guinea fowl has so far not been reported.

ACKNOWLEDGEMENTS

This work was funded by the UZ Research Board Grant No. 3465. We thank Prof. M. Bisgaard, Royal Veterinary and Agriculture University, Denmark for confirming identification of the isolates and Mrs A. Pawandiwa for technical assistance. A part of this work was presented in the HAP99 Conference at Mabula, South Africa.

REFERENCES

- BARROW, G.I. & FELTHAM, R.K.A. 1993. Cowan and Steel's *Manual for the Identification of Medical Bacteria*. Cambridge, Cambridge University Press.

- BISGAARD, M. & DAM, A. 1981. Salpingitis in poultry. *Nordisk Veterinaermedicin*, 33:81–89.
- BOCK, R.R., SAMBERG, Y. & MUSHIN, R. 1977. An outbreak of disease in poultry associated with *Pasteurella gallinarum*. *Revue Veterinaire*, 34:99–103.
- DROUAL, R., WALKER, R.L., SHIVAPRASAD, H.L., JEFFREY, J.S., METEYER, C.U., CHEN, R.P. & SHAPIRO, D.P. 1992. An atypical strain of *Pasteurella gallinarum* pathogenic, phenotypic and genotypic characteristics. *Avian Diseases*, 36:693–699.
- DROUAL, R., SHIVAPRASAD, H.L., METEYER, C.U., SHAPIRO, D.P. & WALKER, R.L. 1992a. Severe mortality in broiler chickens associated with *Mycoplasma synoviae* and *Pasteurella gallinarum*. *Avian Diseases*, 36:803–807.
- HALL, W.J., HEDDLESTON, K.L., LEGENCHAUSEN, D.H. & HUGHES, R.W. 1955. Studies on Pasteurellosis: A new species of *Pasteurella* encountered in chronic fowl cholera. *American Journal of Veterinary Research*, 16:598–604.
- MOHAN, K., OBWOLO, M.J. & HILL, F.W.G. 1992. *Mycoplasma ovipneumoniae* infection in Zimbabwean goats and sheep. *Journal of Comparative Pathology*, 107:73–79.
- MUSHIN, R., BOCK, R.R. & ABRAMS, M. 1977. Studies on *Pasteurella gallinarum*. *Avian Pathology*, 6:415–423.
- NIGRELLI, D., PASCUCCI, S., CESSI, D. & ZAVANELLA, M. 1983. Report of nine foci of *Pasteurella gallinarum* infections. Characteristics of the strains isolated. *Clinica-Veterinaria*, 106:156–160.
- OJO, M.O. 1971. Studies on *Pasteurella* spp. isolated from cases of keratoconjunctivitis of poultry in the western state of Nigeria. *Bulletin of Epizootic Diseases in Africa*, 19:357–363.
- TJAHJOWATI, G., ORR, J.P., CHIRIRO-TREJO, M. & MILLS, J.H.L. 1995. Experimental reproduction of endocarditis with *Pasteurella gallinarum* in mature Leghorn chickens. *Avian Diseases*, 39:489–498.
- YADAV, M.P., SHARMA, V.D. & SETHI, M.S. 1977. An outbreak of fowl cholera due to *Pasteurella gallinarum* in India. *Avian Diseases*, 21:323–325.