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An Unusual Occurrence of Colisepticemia in Budgerigars (Melopsittacus undulatus)

P.M. Priya¹, Deepthi S. Pillai¹, P. Rameshkumar² and P. Senthamilselvan²
¹Department of Veterinary Microbiology, ²Department of Veterinary Pathology,
College of Veterinary and Animal Sciences, Pookot, Wayanad DT, Kerala, India, 673576

Abstract: A rare case of colisepticemia in budgerigar was reported from Kerala. Overcrowding coincident with increased noise and low light levels appeared to be the predisposing factors.

Key words: Coliseptisemia, E. coli, budgerigar, Kerala, India

Introduction

Colisepticemia is considered one of the leading causes of economic loss in the poultry industry worldwide. (Gross, 1991). Budgerigars are granivorous pet birds and their diet consisting exclusively of seeds has an inhibitory effect on *E. coli* colonization in intestines. Hence, the family *Enterobacteriaceae*, *E. coli* in particular, does not belongs to the intestinal flora of these birds. This was indicated by the fact that the feces of only 9 % of healthy budgerigars were positive for enterobacteria. (Glunder, 2002). Transcending through the literature, only few evidence of its occurrence in budgerigar was reported. Therefore the identification of *E. coli* from the budgerigar prompted the authors to place on record an unusual case.

Materials and Methods

Two dead budgerigars of 6 months old were brought to the Department of Pathology, Pookot Veterinary College, Kerala for conducting postmortem examination. History revealed the birds had clinical signs of listlessness, disinclined for feed and water, diarrhea and pasty vent. The gross lesions observed were fibrinous pericarditis, necrotic foci on liver, congested spleen and hemorrhagic enteritis. Heart blood was tested for Salmonella pullorum antibodies by slide agglutination test and the blood smear prepared from both the birds were stained by Leishman's stain. Heart blood, pieces of liver, spleen, brain and intestine were collected aseptically, processed and inoculated into the allantoic cavity of 9day-old embryonated chicken eggs following standard procedure to identify viral aetiology. All the inoculated eggs were incubated at 37°C and candled daily. The tissues were separately cultured on Typtone soya agar and incubated at 37°C for bacterial growth. The pure cultures obtained were identified as per the method described by Quinn et al. (1994).

Chicken embryo lethality test and mice pathogenicity test were done as recommended by Giovanardi *et al.* (2005) to prove the pathogenicity of the isolate. For chicken embryo lethality test, overnight incubated broth culture of

the isolate was adjusted to a concentration of $1x10^4$ cfu/ml and 0.1ml inoculum each was injected into the allantoic cavity of four 9-day-old embryonated chicken eggs. One egg was kept as control. Eggs were incubated at 37° C and were candled daily to identify dead embryos. For mice pathogenicity test, two mice were subcutaneously inoculated with 0.1 - 0.2 ml each of BHI broth culture containing $1x10^8$ cfu/ml and monitored every 6 hrs interval for mortality. One mouse was kept as control.

A sensitivity test of 7 antimicrobial agents frequently used in local poultry farms was carried out by standard disc diffusion method as per Bauer *et al.* (1966). A 4-hours-old Brain Heart Infusion (BHI) broth culture of the organism was swabbed onto the surface of Muller-Hinton agar (Himedia). The following antibiotics and amounts per discs were used: amoxycillin (10µg), ampicillin (10µg), enrofloxacin (10µg), oxytetracycline (30µg), gentamicin (10µg), co-trimoxazole (25µg; sulpha 23.75 / trimethoprim 1.25µg) and chloramphenicol (10µg). The discs were placed on the medium and were incubated at 37°C for 24 hrs. The inhibition zones were measured and the results were interpreted using the HiAntibiotic zone scale and Zone size interpretative chart.

Results

On attempt for virus isolation, no death of embryos / haemagglutination of chicken red cells were noticed and the harvested allantoic fluid was passaged for a minimum of three times before declaring as negative for Newcastle disease.

The blood samples were negative for *Salmonella pullorum* antibodies. Microscopical examination of blood smear did not reveal bipolar organisms but numerous short rods were seen. Next day, on the agar plate the colonies formed were creamy, mucoid, glistening, opaque and circular with entire edges. Mucoid, pink lactose fermenting colonies were seen on Mac Conkey agar. On eosin methylene blue agar, the colonies formed had blackish centers with metallic sheen which is of value for identification. (Merchant and Packer,

2002). The isolate was Gram-negative short rods, non-motile and capsulated. It was catalase positive, oxidase negative, reduced nitrate and + + - - on IMViC test. It produced acid and gas from glucose, lactose, fructose, galactose, maltose, mannitol, arabinose, rhamnose, trehalose and xylose, but did not ferment dextrin, starch, cellulobiose, adonitol and inositol.

On lethality test, all the inoculated embryos were found to be dead on 30-48 hrs post-inoculation (PI) and the organism was reisolated from the embryo. Also both the inoculated mice were died on 30 hr PI. On post-mortem examination, hepatitis and pericarditis were noticed and the organism was reisolated from the heart blood, liver and spleen.

The antibiotic sensitivity test showed the isolate was resistant to ampicillin, amoxycillin, co-trimoxazole and oxytetracycline but sensitive to enrofloxacin, gentamicin and chloramphenicol. Based on the results, two more ailing birds in the flock were treated with enrofloxacin and no further mortality was observed.

Discussion

The laboratory identification and pathogenicity tests were in accordance with Ngeleka *et al.* (1996) and Giovanardi *et al.* (2005) for characterizing avian *E. coli* isolates. These findings proved that the death could be due to colisepticemia. This forms the first confirmed report of colisepticemia in budgerigar from Kerala.

High level of resistance to ampicillin, co-trimoxazole and tetracycline were reported earlier by Ngeleka *et al.* (1996) and Giovanardi *et al.* (2005).

Survey of the cage units at the farmer's premises revealed that the birds were kept at high stocking density coincident with increased noise and low light levels. Hence the stress due to overcrowding may enhance the chance of colonization of the gut with *E. coli*. On the other hand it seems nearly impossible to colonize the intestine with *E. coli* or *Klebsiella spp*. (Glunder, 2002). Isolation of pathogenic strains of *E. coli* from budgerigar has a major public health concern as there is every possibility of transmission of colibacillosis from these

birds to man (Fowler and Miller, 1999) resulting in mild to acute illness.

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