



Omphalitis in ducklings with *Staphylococcus aureus* infection

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ABSTRACT

Outbreak of Omphalitis was reported in the week old ducklings at a research duck farm of Regional centre. The clinical signs were swollen abdomen contains cheesy caseous content into yolk sac, oedema, redness, few cases of inflammation at abdomen, septicaemia, showed little interest in food and water resulting gradual severely dehydrated. The isolation of *Staphylococcus aureus* from Omphalitis of 20 ducklings. Of the 20 Omphalitis cases 13 were khakis and 7 were of white pekings. The isolation and identification of the isolates were accomplished by cultural, microscopic and biochemical characterization. The antibiogram showed that the isolates were highly sensitive to Agithromycin, Ciprofloxacin, Cloxacillin, Enrofloxacin, Gentamicin and Floxidin. They were moderately sensitive to Chloramphenicol, Ofloxacin, Furazolidon and Doxicillin but were resistant to Neomycin, Kanamycin and Sulphamethizole, Cefixime/clavulanic acid.

Keywords: Duckling, infection, Omphalitis, *Staphylococcus aureus*, Isolation

Omphalitis is also known as “Mushy Chicks” is an inflammatory condition of navel (umbilicus) involvement in birds largely due to some common infection of *E.coli*, *Streptococci* spp., *Proteus* spp., *Enterococci* spp, *Bacillus* are common. It may be both single, or mixed infection for Omphalitis. Both ascending and descending infections cause the condition. The condition prevails both in chicken, ducks and other domestic birds during early neonatal and embryonic life. The number of infections in eggs is not very high (0.5-6%) but the embryonic death occurs at late incubation period to neonatal week old ducklings. Excess humidity and contaminated incubator also aggravate the condition (Shringi *et al.*, 2014). As the disease is infectious, yolk being the rich sources of nutrients help in multiplication of bacteria and histological disturbances at naval hamper absorption of yolk. The clinical signs of the condition are swollen abdomen, oedematous



naval area, redness, inflammation with occasional abscession at naval. Distended abdomen with engorged blood vessels under go lysis and wetness leading to “Mushy duckling disease similarly it is called “Mushi chick disease” in chicken. Other associated symptoms are dehydration, visceral gout, emaciation, diarrhoea with vent pasting, increased yolk and mild inflammatory changes in wall of yolk sac. In duck eggs, soil contamination with wet litter may take up the infection during incubation causing Omphalitis. Very few reports in duck omphalitis have been reported. However, some report on duckling Omphalitis available (Shrama and Kausik, 1986; Mishra, 1989). The causes of infections are wide but common infection of Omphalitis are *Escherichia coli*, *Proteus mirabilis*, *Staphylococcus aureus*, *Salmonella* spp. and *Streptococci* spp (Sharma, *et.al.*, 1985; Chaudhury *et.al.*, 1993). Source of infections may be horizontal infection at hatchery, fecal contamination of eggs and vertically through egg infection at oviducts. An attempt was made to identify the cause of Omphalitis in younger ducklings.

Table 1. Biochemical characterization of *Staphylococcus aureus*

Indole test	Ammonia production	Methylene red test	Voges Proskauer	Methylene blue reduc	Nitrate reduction	Gelatin liquefaction	Coagulase test
-	+	+	+	+	±	+	+

= Negative, + = Positive, ± = both negative and positive

MATERIALS AND METHODS

Ducklings

The Regional Research Centre, Central Avian Research Institute, Bhubaneswar maintains four different breeds of duck namely Pati, the desi variety, Khaki Campbell, white pekin and Muscovy, locally called Moti. We are maintaining more than 4000 ducks for experimental production and research purpose. The eggs are hatched for ducklings for research as well as for field supply. Some ducklings are found weak, under developed, swollen umbilicus contains cheesy caseous content into yolk sac. Inflammatory changes with small abscesses at naval and distended abdomen that restricted movement of the ducklings. Embryonic and post hatch death within week with characteristic lesions were observed.

Clinical cases of Omphalitis

Some ducklings of newly hatched flocks showing swollen abdomen, oedema, redness, few cases of inflammatory changes, septicaemia, the ducklings showed little interest in food and water resulting gradual severely dehydrated. Depression, drooping of the head, and huddling near the heat source usually are the only signs. The ducklings consequently died with week post hatch with inflamed naval unabsorbed yolk. Opening of carcass showed accumulated pus of golden colour. A number 20 ducklings which were suffering from Omphalitis mostly of Khaki and white pekin were taken into consideration for bacterial isolation. Post mortem findings of the dead ducklings with Omphalitis characterized by enlarged abdomen, yellowish golden colour pus accumulated in the abdominal cavity and necrosis of the tissue.

Table 2: Antibacterial sensitivity against isolates of *Streptococcus aureus*

Antimicrobial	Concentration (Mcg)	Inhibition Zone (mm)	Sensitivity
Agithromycin	15	30	H
Cefixime/clavulanic	30	0	R
Chloramphenicol	30	20	M
Ciprofloxacin	30	30	H
Cloxacillin	30	30	H
Doxicillin	30	18	M
Enrofloxacin	10	36	H
Floxidin	20	26	H
Furazolidon	50	18	M
Gentamicin	30	30	H
Kanamycin	30	0	R
Ofloxacin	5	20	M
Neomycin	30	14	R
Sulphamethizole	300	0	R

Isolation of bacteria

The abscess and tissue swabs were collected aseptically with sterile swabs from the naval and internal organs from 20 ducklings of one to seven day old. Bacterial cultivation of suspected swabs were accomplished in Nutrient broth, Peptone water, Mannitol agar and Nutrient agar. Primary bacteria from Nutrient agar were cultivated into blood agar. Microscopic examination of colonies and bacterial smear with Gram's stain were made regularly. Biochemical characterization of the isolated were made for H₂S production, coagulase reaction, production of ammonia, nitrate reduction test, methylene blue reduction test,, gelatine liquefaction, vogue



proskauer and methyl red test. Isolation and identification made as per standard method (Bennett and Lancette, 2001) with few modification.

Antibacterial sensitivity

After preliminary and confirmative identification of bacteria responsible for Omphalitis, antibacterial sensitivity tests were conducted as per standard methods in Mueller-Hinton media (Bauer *et al.*, 1966). A number of 14 different antibiotic discs with different concentration were used to find the range of sensitivity. The antibiotics with minimum inhibitory concentration in micrgram of drug used were Agithromycin (15), Cefexime/Clavulinic acid (5/10), Chloramphenicol(30), Ciprofloxacin (30), Cloxacillin(30), Doxicillin (30), Enrofloxacin (10), Floxdin (20), Furazolidon (50), Gentamicin (30), Kanamycin (30), Neomycin (30), Ofloxacin (5) and Sulphomethizole (300). The Muller Hinton agar was prepared and air dried over night under 4°C. Nutrient broth culture of 18 hour was poured onto the agar plate and again air dried. The antibacterial discs were placed on the media with sterile forceps for slow release of the drug into media. The plates were incubated at 37°C for over 24 hours. The zone of inhibition if any was measured.

The degree of sensitivity with a particular antibiotic was determined as per the sensitivity table supplied with antibiotic disc by Hi-Media Laboratories Private limited, Mumbai.

RESULTS AND DISCUSSION

Of the 20 duckling sample taken for bacterial isolation and identification, 13 were from Khaki Campbell ducklings and seven were from White Pekin ducklings. Cultivation of bacteria of the samples were performed in nutrient broth, peptone water and nutrient agar. Almost all the broth and agar media contained pure cultures. Few isolates were found to be mild mixed infection, this may be to contamination. Further cultivation for primary bacteria from Nutrient agar to blood agar. All the primary media show well growth of colonies. Blood agar was used for abundant growth of bacteria other than expected ones as the blood agar is enriched media for growth. Nutrient and peptone water showed opaque culture with mild powdery sedimentation. In Nutrient agar growth of characteristic yellowish, smooth, rounded, glistening, butter like opaque colonies revealed. In mannitol agar, the bacterial colonies were smooth, rounded, opaque and golden yellow colour with butyric consistency. Mannitol agar contains high NaCl that prevent growth of other bacteria than *Streptococcus*, moreocer, fermentation of mannitol produce golden colour pigment triterpenoid carotenoid carboxylic acid that is the indication of *Staphylococcus aureus*. Microscopically, the bacteria were Gram positive cocci, non sporing, non motile, accumulated in clusters and some scattered paired bacteria. Biochemical characterization showed that the bacteria were positive for

nitrate reduction, production of hydrogen sulphite, liquefaction of gelatine, highly positive in coagulation test, methylene blue reduction, Voges- Proskauer, Methylene blue reduction and production of ammonia. The isolates were negative to indole (Table 1). Cultural, microscopic observation and biochemical tests revealed that the isolates were staphylococcus species. The most frequent staphylococcus infection of veterinary importance is *Staphylococcus aureus*, *Staphylococcus pyogenes* var *albus* and *Staphylococcus pyogenes*. Except staphylococcus aureus none can reduce mannitol to form golden coloured pigment. Therefore, the isolates of bacteria were *Staphylococcus aureus*. Staphylococcus is naturally inhabited on soil, mammalian and avian body surfaces. Some of staphylococcus is commensal and others are pathogenic for human and animals. Isolation and identification of the organism needs detailed cultural, microscopical, biochemical tests for characterization. These cultural, microscopic and biochemical characterizations are the primary tools for isolation and primary and confirmative species identification. For isolation and identification of bacterial species, cultural and biochemical characterizations are the tools for most of the time. Identification of *Staphylococcus aureus* by cultural, microscopical observation and biochemical tests reported in chicks and ducklings (Sunday *et al.*, 2010; Chakraborty *et al.*, 2011).

The antibiogram of different antibacterials revealed that the isolates of *Streptococcus aureus* were highly sensitive to Agithromycin, Ciprofloxacin, Cloxacillin, Enrofloxacin, Gentamicin and Floxidin. Whereas, Chloramphenicol, Ofloxacin, Furazolidon, Doxycillin showed moderately sensitive while Neomycin, Kanamycin, Sulphamethizole, Cefixime/clavulanic acid found to be less sensitive or resistant (Table-2). *Staphylococcus aureus* is ubiquitous bacteria causes several systemic and localized infections. Frequent use of antibiotics for treatment of animals and human infections develops resistance. For effective treatment of any staphylococcal infection needs antibiogram. Several workers reported sensitivity and resistance with different antibiotics (Jeffrey, *et al.*, 1993; Lin *et al.*, 2009)

Summary

Ducklings Omphalitis was reported in the newly hatched ducklings at a research duck farm. Clinical signs of Omphalitis were swollen abdomen contains caseous content in yolk sac, oedema, redness, inflammatory abdomen, septicaemia, anorexia, resulting gradual severe dehydration. The causative bacteria isolated was *Staphylococcus aureus* from Omphalitis of 20 ducklings. Ducklings of Khakies and White Pekins were affected. The isolation and identification of the isolates were accomplished by cultural, microscopic and biochemical characterization. The isolates were highly sensitive to Agithromycin, Ciprofloxacin, Cloxacillin, Enrofloxacin, Gentamicin and Floxidin. They were moderately sensitive to Chloramphenicol, Ofloxacin, Furazolidon and Doxycillin but were resistant to Neomycin, Kanamycin and Sulphamethizole, Cefixime/clavulanic acid.



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