SHORT COMMUNICATION

Cultural Isolation and Identification of Possible Causative Agents of Swollen Head Syndrome in Broilers

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Abstract

The present study was conducted for isolation and identification of possible causative agents from broiler birds affected with swollen head syndrome in and around Anand, Gujarat. During six months of study period, a total 40 swab samples were collected from 20 different commercial broiler farms affected with swollen head syndrome. All the twenty broiler flocks under the study were found positive for the presence of *Escherichia coli*. During the study, overall total of 83 different bacterial isolates were obtained from 20 affected broiler flocks. The isolates included *Escherichia coli* (34.94%), *Staphylococcus aureus* (32.53%), *Proteus* spp. (6.02%), *Corynebacterium* spp. (7.23%) and Gram negative rods (19.28%). *Escherichia coli* was found to be the highest prevalent pathogen in swollen head syndrome during the study.

Keywords: Anand, Broiler birds, Isolation and identification, Swollen head syndrome.

Ind J Vet Sci and Biotech (2020): 10.21887/ijvsbt.16.(2,3,&4).19

Introduction

Swollen head syndrome (SHS) is usually multifactorial disease where the initial lesions are caused by *Avian metapneumovirus (aMPV)*, while the clinical signs are outcome of bacterial complications and the severity of disease rely on environmental factors. The swollen head syndrome (SHS) is one of the localized form of colibacillosis that has been described in most intense poultry-producing areas and *E. coli* present as a secondary pathogen (Abdelmoez *et al.,* 2019). The information on SHS in broiler farms of Gujarat is meagre. Hence the present study was planned to isolate and identify the possible causative agents of swollen head syndrome in broiler flocks in and around Anand.

Materials and Methods

The study was conducted in twenty broiler flocks affected with swollen head syndrome in and around Anand, Gujarat. A total 40 swab samples of subcutaneous edema and sinuses exudates were collected under aseptic conditions with sterile swabs for further processing and microbiological analysis. Collected samples were inoculated on MacConkey agar and blood agar media. The inoculated media plates were incubated aerobically at 37°C and inspected for growth after 24 hours of incubation. The colonies were then re-inoculated on specific selective media. After incubation, bacterial colonies were investigated on the basis of colony characterization, staining, cultural and biochemical characters as per the Bergey's manual of Determinative Bacteriology.

The smears of all isolates were stained by Gram's staining method and morphology of bacteria was observed under microscope. Gram negative pink coloured bacilli were recorded as characteristics of *Escherichia coli*. For confirmation of *E. coli* isolates IMViC pattern (Indole test, Methyl Red (MR) test, Voges- Proskauer (VP) test and Citrate test) was carried out. The data was analyzed using descriptive statistics.

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How to cite this article: Momin, S.G., Ghodasara, D.J., Mathakiya, R.A., Dave, C.J., Jani, P.B., Kabariya, D.V., & Pradhnya Choudhari1 (2020). Cultural Isolation and Identification of Possible Causative Agents of Swollen Head Syndrome in Broilers. Ind J Vet Sci and Biotech, 16(2,3,&4): 86-88.

Source of support: Nil

Conflict of interest: None.

Submitted: 18/07/2020 **Accepted:** 28/11/2020 **Published:** 25/12/2020

Results and Discussion

Different bacterial species associated with swollen head syndrome were *Escherichia coli, Staphylococcus aureus, Proteus* spp., *Corynebacterium* spp. and gram negative rods.

The frequency of isolated bacterial strains is summarized in Table 1. A total of 83 bacterial strain belonging to different genera isolated were *Escherichia coli* 29 (34.94%), *Staphylococcus aureus* 27 (32.53%), Gram negative rods 16 (19.28%), *Corynebacterium* spp. 6 (7.23%) and *Proteus* spp. 5 (6.02%). Results suggest that *E. coli* is the highest prevalent

Table 1: Bacteria isolated from swab samples obtained from broiler birds affected with swollen head syndrome

Sr. No.	*Bacteria*	*No. of isolates*	*Percentage*
1	Escherichia coli	29	34.94
2	Staphylococcus aureus	27	32.53
3	*Proteus* spp.	05	06.02
4	*Corynebacterium* spp.	06	07.23
5	Gram negative rods	16	19.28
Total	–	83	100.00

Table 2: Cultural characteristics of bacterial isolates from subcutaneous edema and sinuses exudates of broilers suffering from swollen head syndrome

Bacterial Isolates	*Cultural characteristics on*			
	Blood agar	*MacConkey agar*	*EMB agar*	*Mannitol salt agar*
Escherichia coli	Greyish white colour colony without haemolysis	Lactose fermenting pink colony	Greenish metallic sheen	-
Staphylococcus aureus	Yellowish grey and mucoid colour colony without haemolysis	-	-	Yellowish colonies with mannitol fermentation and pink colonies without mannitol fermentation
Proteus spp.	Swarming growth	-	-	-
Corynebacterium spp.	Small white colony	-	-	-
Gram negative rods	White opaque colony	Lactose non fermenting pale colonies	-	-

pathogen responsible for causing swollen head syndrome in broilers.

Escherichia coli was isolated from all twenty flocks, while *Staphylococcus aureus* was found in 16 flocks. The other bacteria, Gram negative rods, *Proteus* and *Corynebacterium* were found in 11, 4 and 4 flocks, respectively. Out of 20 flocks, 19 flocks were found with mixed infection. Only one flock was recorded with pure *E. coli* isolates. According to sample wise distribution of isolated bacteria, mixed infection was found in 33 samples out of 40 samples. *Escherichia coli, Staphylococcus aureus,* Gram negative rods and *Proteus* were found singly in 2, 2, 2 and 1 samples, respectively. Out of 40 samples, 27 samples showed mixed infection with *Escherichia coli* and 6 samples showed mixed infection other than *Escherichia coli.*

Escherichia coli as a secondary bacterial infection has been reported for the development of swollen head syndrome by many workers. Georgiades *et al.* (2001) observed that the most common isolated bacteria from SHS were *Escherichia coli* followed by *Staphylococcus aureus.* Radwan *et al.* (2018) and Abdelmoez *et al.* (2019) observed mixed bacterial infection in swollen head affected flocks *viz., Escherichia coli, Streptococcus dysgalactiae, Pseudomonas aeruginosa* and *Proteus mirabilis*. However in present study, *Escherichia coli* was most commonly isolated bacteria followed by *Staphylococcus aureus,* whereas *Streptococcus* was not found during present study.

In present study, total five different isolates were found. Cultural characteristics of these isolates were recorded based on colonial morphology on different and specific media. The details of cultural characteristics and specific media are given in Table 2. On blood agar greyish white colour colony was considered as *E. coli*. Yellowish and whitish colony without haemolysis was considered as *Staphylococcus aureus*. Swarming growth was considered as *Proteus* spp., small white colour colony *Corynebacterium* spp., and white opaque colony was considered as Gram negative rods.

E. coli gave greenish metalic sheen on specific media like Eosin Methylene Blue (EMB) media, and on MacConkey (MAC) media it gave lactose fermenting pink colour colony. On specific media, Mannitol Salt Agar (MSA), *Staphylococcus aureus* gave yellowish colonies with mannitol fermentation and pinkish colonies without mannitol fermentation**.**

The IMViC pattern of *Escherichia coli* and *Proteus* found were (+,+,-,-) and (-,+,-,+), respectively. Amaechi (2002) and Shringi *et al.* (2014) observed pink coloured bacilli and IMViC pattern for *Escherichia coli* as was recorded during the present study.

Based on isolation and identification of bacteria from swab samples of subcutaneous oedema and sinus exudates of various broiler farms, it was concluded that in swollen head syndrome the most common prevalent pathogen was *E. coli.* The other bacteria identified were *Staphylococcus aureus*, *Proteus*, *Corynebacterium* and Gram negative rods.

Acknowledgement

Authors Thank the Dean of Veterinary College, and authorities of Anand Agricultural University, Anand for the fund and facilities provided for this research work.

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