ABSTRACT

A study was undertaken to determine the prevalence of various serotypes of *Pasteurella multocida* recovered from avian sources during 2011 to 2014. Agar gel precipitation test (AGPT) was employed to identify the somatic (O) group serotypes. A total of 120 suspected poultry samples were investigated for possible recovery of *Pasteurella multocida*. From field samples 31 isolates were recovered from which 08 different *Pasteurella multocida* serotypes were confirmed by AGPT. *Pasteurella multocida* serotypes and the frequency of its isolation was as follows: Somatic (O) type T-1 (64.51%), T-3 (12.90%), T-4 (3.22%), T-10 (3.22%), T-14 (3.22%), T-1xT-3 (6.45%), T-3xT-4 (3.22%) and T-16 (3.22%)

Keywords: Avian, Fowl cholera, *Pasteurella multocida*, Agar gel precipitation test, Maharashtra

INTRODUCTION

Fowl cholera, caused by *Pasteurella multocida*, occur sporadically or enzootically in most countries of the world wherever intensive poultry production occurs, and is known as a bacterial disease of major economic importance due to its high mortality (Rimler and Glisson, 1997). *Pasteurella multocida* is gram negative, capsulated, bipolar non-motile rod shaped bacteria. It affects the birds of all ages; however, chickens less than 16 weeks of age are generally quite resistant. Death losses from Fowl Cholera in chickens usually occur in laying flocks (Arshad *et al.*, 2003) This disease remains a significant obstacle
to sustainable poultry production in most parts of tropical Asia and Africa (Dashe et al., 2013). According to recent reports Fowl cholera is also occurred in broilers at sub-acute level (Srinivasan et al., 2011). In India, there are several strains of *Pasteurella multocida* are identified which differ in virulence hence there are variations in severity of the disease, lesion and mortality (Chauhan and Roy, 2007). Generally, four specific capsular serotypes (A, B, D and E) are recognized by the PHA (Passive Haemagglutination) test and 16 specific somatic serotypes T1 to T16 by the AGPT (Agar Gel-diffusion Precipitation test) described by Heddleston et al., (1972). Certain serotypes of *Pasteurella multocida* usually Heddleston serotype T-1 causes avian cholera regularly (Mooer et al., 1993).

In India, occurrence of fowl cholera has been regularly reported from the states of Assam (Lalrinthunga and Burah, 1993), Andhra Pradesh (Prasad et al., 1997), Karnataka (Kumar and Sreemanarayana, 1978), Maharashtra (Kulkami et al.,1990), Orissa (Panda et al.,1981), Punjab (Dhillon et al., 1989), Tamilnadu (Srinivasan et al., 2011) and West Bengal (Chakraborty et al., 1989).

The present investigation was designed to reveal the recovery rate of different *Pasteurella multocida* somatic (O) serotypes infecting the avian species during the last three years (2011 - 2014) by Agar Gel Precipitation test (AGPT) as described by Heddleston et al., 1972.

**MATERIALS AND METHODS**

A total of 120 samples were collected from dead and infected poultry birds in various areas of Maharashtra state at different time interval for the period between 2011 to 2014 and were investigated bacteriologically with special reference to search for *Pasteurella multocida*, an etiological agent of fowl cholera in chickens.

Casein Sucrose Yeast (CSY) agar supplemented with 5 – 7 % defibrinated sheep blood was used for isolation of organism. MacConkey’s Agar was used as negative control as *Pasteurella multocida* fails to grow on it.

Liver heart blood and bone marrow were separately streaked directly on both Casein Sucrose Yeast (CSY) agar and Mac Conkey’s agar and incubated at 37°C for 24 –48 hours in aerobic condition. Colonies having the appearance of *Pasteurella multocida* on CSY agar and fails to grow on MacConkey’s agar were carefully selected from each plate and were subsequently identified phenotypically and biochemically by using various tests suggested by Dashe et al., (2013).

Isolates having biochemical properties were taken up for further serological studies. Serotyping of isolates was conducted according to the scheme of Heddleston (1972) using T-1 to T-16 hyper immune sera. Sera employed for serotyping were obtained from Oyster Biologicals Pvt. Ltd, Oman (MH).
RESULTS AND DISCUSSION

The Table 1 and Fig. 1 is showing the total number of different isolates and frequency percentage of each serotype as related to total number of isolates isolated in and around Maharashtra state. It could be seen that 08 different Pasteurella multocida (O) serotypes were recovered from all birds. It is clear that Pasteurella multocida somatic (O) type T-1 is the most widespread of all serotypes.

Table 1: The serotyping of Pasteurella multocida isolates on the basis of somatic antigen.

<table>
<thead>
<tr>
<th>Pasteurella multocida somatic serotypes</th>
<th>Occurrence from 31 field isolates</th>
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<tbody>
<tr>
<td></td>
<td>Frequency</td>
</tr>
<tr>
<td>T-1</td>
<td>20</td>
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<tr>
<td>T-3</td>
<td>04</td>
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<tr>
<td>T-4</td>
<td>01</td>
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<tr>
<td>T-10</td>
<td>01</td>
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<td>T-14</td>
<td>01</td>
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<tr>
<td>T-1 x T-3</td>
<td>02</td>
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<tr>
<td>T-3 x T-4</td>
<td>01</td>
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<tr>
<td>T-16</td>
<td>01</td>
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</tbody>
</table>

Figure 1. Prevalence of somatic types of Pasteurella multocida in Maharashtra

Poor hygienic conditions, density of farms can definitely contributed to the substantial increase in both number and types of recovered Pasteurella. It is felt that
Pasteurella multocida initial introduction in to a poultry house may be due to rats, mice, cats and dog as well as many species of wild animals as it is, however, a common inhabitant of the oral cavity of many animals (Chauhan and Roy, 2007; Turni et al., 2014). The high percent recovery of Pasteurella multocida displayed in this work could be explained by the fact that the majority of the poultry farms, which were reared by native individuals under poor hygienic conditions. From the results, it could be concluded that the serotype T-1 of Pasteurella multocida contributes to 64.51% of all isolates whereas T-3 comprises only 12.90% and T-1xT-3 forms 6.45%. Remaining other Pasteurella include different forms level in 12.88%.

The Pasteurella serotype T-1xT-3 has characteristics of serotype 1 and serotype 3. Those most commonly found in poultry are 1, 3 and 4 or combinations thereof, such as 1, 3 or 3, 4 (Glisson, 1997). This suggested that certain strains could possess a complex somatic antigenic structure and such strains could possibly also infect a wider host range. Such strains of complex antigenic structure could be of great epizootiologic significance, as they could play an important role in the transmission of disease from one host species to the other. The complexity of somatic antigenic structure was recognized (Namoika and Bruner, 1963, Namioka and Murata, 1964).

CONCLUSION
In order to understand the principles of fowl cholera vaccination, it is important to understand the relevance of the serotyping system for Pasteurella multocida. Since it is unreasonable to include all 16 Pasteurella multocida in a bacterin and since only a few serotypes commonly cause the disease in poultry. So from our work, it can be concluded that most prevalent T-1 and T-3 types can be used as a suitable candidate strains for vaccine production for said area.

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