Mannheimiosis in Swiss albino mice

S Jaisree, S Hemalatha, R Srinivasan and Parimal Roy

Abstract
A Swiss albino mouse was submitted for necropsy from a lab animal unit in Chennai, Tamil Nadu. Congestion of lungs and petechiae were observed during necropsy. Swabs, smears and organs were collected for laboratory investigation. The smears revealed bipolar organism. Upon cultivation pinpoint pink colonies were observed in Mac Conkey agar and are weakly haemolytic in blood agar. The morphological, cultural and bio-chemical characters were suggestive of Mannheimia glucosida (M. glucosida). The organism was further confirmed by multiplex PCR. Histopathological examination revealed pneumatic changes in lungs, haemorrhage in the myocardium and fatty changes in the liver and kidney suggestive of pneumonia and toxicity. This study could be the first report of M. glucosida in mice.

Keywords: Mannheimia glucosida, mice, multiplex PCR

Introduction
In bio-medical research, 17-23 million animals are used in research every year. Among these 17-23 million, 95% of animals used were laboratory mice and rats [1]. Laboratory mice are the most convenient and commonly used animal tool in bio-medical research since they share more than 98% DNA with humans. Introduction of infectious agent in mice colonies have adverse effects on research. Pasteurella species are commensals in the upper respiratory tract of sheep, goat, wild and domestic animals and become pathogenic under stress condition [2, 3, 4]. The taxonomy of Pasteurella sp. are subjected to reclassification and now Pasteurella sp. are divided into Pasteurella multocida and Mannheimia haemolytica complex. There are two biotypes in M. hemolytica complex- bio type A (ferments arabinose) and bio type T (ferments trehalose) [5]. Seventeen serotypes (13 A and 4 T) are present in M. haemolytica complex. Serotypes A1, A2, A5, A6, A7, A8, A9, A12, A13, A14, A16 and A17 represents M. haemolytica, A11 serotype is designated as Mannheimia glucosidai [6-8]. M. glucosida was previously isolated from ruminants, rabbits, hares, guinea pigs, ovine pneumonia and ovine mastitis [9-14]. Recently, Jillian et Mannheimia glucosida infection has been reported in human cases [15]. Ward et al. (1978) isolated P. pneumotropica in mice from abortion cases [16]. However, the incidence of M. glucosida in mice could not be observed in the available literature. Possibly this could be the first report of M. glucosida in mice. Hence, the present study aims to report the occurrence of M. glucosida in mice causing pneumonia and death.

Materials and methods
Swiss Albino mice from a laboratory animal medicine unit in Chennai was submitted for necropsy. Necropsy was performed and specimens of heart blood smear and heart blood swab and lung swab were taken for bacterial isolation. Pieces of lung, liver, spleen, heart and kidney were collected in 10% formalin for histopathological examination.

Bacterial culture and identification
Heart blood smear was stained with Leishman stain as per standard procedure. Heart blood swab and lung swabs were directly streaked onto blood agar and Mac conkey agar plates. One set of plates were incubated at 37 °C under aerobic condition and another set of plates were incubated at 37 °C under anaerobic condition. Bio- chemical tests were performed on pure culture [17]. Antibiotic sensitivity test (ABST) was performed as per the recommended procedure [18].

Polymerase chain reaction (PCR)
Multiplex PCR was performed to identify the involvement of Mannheimia sp. DNA was extracted by boiling method.
Ten pure colonies of bacteria were mixed with 100µl of TE buffer (pH 8.0) incubated at 100 °C for 10 minutes and then snap cooled. The lysate was centrifuged at 13,000rpm for 15 minutes. The supernatant was taken as a template DNA. Primer sequences described in Table.1 were used for the PCR reaction [19]. A 25µl PCR mixture was prepared by adding 12.5 µl of red dye PCR master mix 2X (Ampliqon®), 10 picomoles of each primer, 2.5µl of DEPC water and 2 µl of DNA. PCR reaction was carried out with the following condition. 95 °C - 15 min, 40 cycles of 94 °C for 30s, 62 °C for 30s and 72 °C for 30s; final extension at 72 °C for 10 min. 20µl of PCR product was electrophoresed on 2% (w/v) agarose gel and visualized under gel doc system (Bio Rad).

Results and Discussion
In necropsy, the predominant lesion observed was congestion and consolidation of lungs. Heart blood smear stained with Leishman’s stain showed bipolar organism which is characteristic of the members of Pasteurellaceae. In blood agar the colonies are weakly hemolytic and grey in colour. In Mac conkey agar pin point pink coloured colonies were obtained. The colonies are indole –ve, urease –ve, citrate –ve, MR, VP –ve, ornitine decarboxylase –ve, no H₂S production on TSI, lysine decarboxylase –ve, ONPG –ve, oxidase +ve, catalase +ve, βglucosidase +ve, ferments lactose, arabinose, sorbitol, and glucose. Cultural and bio-chemical characters confirmed the presence of Mannheimia sp. The above findings corroborate with the earlier findings of Quinn et al. (2011), Alexander et al. (2008), Hawari et al. (2008), Poulsen et al. (2006) and Wessman and Geraldine (1968) [17, 20-22].

In order to identify the subspecies of Mannheimia, multiplex PCR was performed. In multiplex PCR four bands with a MW of 90bp, 170bp, 206bp and 304bp were obtained (fig.1) specific to HP protein, LKT2, LKT and 16s RNA respectively which is highly specific to M. glucosida and exactly correlates with the findings of Alexander et al. (2008) who optimized multiplex PCR for the differentiation Mannheimia sp. in cattle [18].

Histopathological examination of heart showed extensive haemorrhages in the myocardium and focal necrosis of myocardial fibre (fig.2). The lesions in lung include diffuse capillary congestion, mild interstitial and alveolar oedema, degeneration of alveolar wall, accumulation of exudate in the lumen of bronchioles (fig.3). Congestion and fatty changes were noticed in liver and kidney. Subcapsular haemorrhage and multifocal areas of haemosiderin pigmentation in red pulp were noticed in spleen. Histopathological lesions of lung and heart were suggestive of pneumonia and septicaemia. The lesions in liver, kidney and spleen indicate the possible involvement of toxin. M. glucosida alone doesn’t result in death. The toxicity may be the predisposing factor for the flaring up of M. glucosida in mice which caused pneumonia and resulted in death. The above findings correlate with the earlier findings of histopathological changes in mice tissue due to septicaemia mediated by Pasteurella sp. [23-25].

ABST test results showed that the current isolate was sensitive to oxytetracycline, Sulphadiazine, tylosin and gentamicin. Intermediately sensitive to ciprofloxacin and enrofloxacin and resistant to penicillin, erythromycin, streptomycin, ampicillin, cloxacillin, amoxicillin clavulanic acid. Earlier studies of antimicrobial resistance in other species reported that M. glucosida was resistant to tetracycline, gentamicin, kanamycin, streptomycin, sulphamethoxazole-trimethoprim, chloramphenicol [26-27]. In contrary to the earlier findings the current isolate is sensitive to tetracycline, sulphadiazine and gentamicin. The reason for such variation is, the absence of exposure to the above antibiotics since the mice colonies are maintained without any antibiotic as feed additive. Moreover, the recent findings revealed that the genetic background of mice influences the transfer of plasmid mediated antimicrobial resistance in the gastrointestinal tract [28].

The host spectrum for M. glucosida is increasing and recently M. glucosida was reported in human [10]. This is the first isolated case of M. glucosida in mice. Since the lab animal unit had been closed for renovation, we couldn’t find further cases of mortality in mice.

Lane 1 – 100bp MW marker
Lane 2- M. glucosida isolate from mice (4 bands of 90bp, 170bp, 206bp and 304bp)
Lane 3 – negative control

Fig 1: Multiplex PCR showing four bands of 90bp, 170bp, 206bp and 304bp for the DNA extracted from M. glucosida isolate from mice

Fig 2: Mouse – Heart Haemorrhage in the myocardium. H&E 100X
Considered in regular screening of health monitoring using GF.

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- Primers used for the identification of M. glucosida

Table 1: Primers used for the identification of M. glucosida

<table>
<thead>
<tr>
<th>Primer set</th>
<th>Sequence</th>
<th>Product size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lkt</td>
<td>F: 5’GCAGGAGTTGATTATTTAAGTGG3’  R: 5’CAGCGATTATTGTCATACCTGAAC3’</td>
<td>206bp</td>
</tr>
<tr>
<td>Lkt2</td>
<td>F: 5’CTCTTTAGAAAAGCTGAAAC3’  R: 5’TGTCAGGATTTGTTATTCG3,</td>
<td>170bp</td>
</tr>
<tr>
<td>HP</td>
<td>F: 5’GGCCGAAAGCACAATACATTATGG3’ R: 5’CACCCTCAAATTCTCGTGGAATAAC3’</td>
<td>90bp</td>
</tr>
<tr>
<td>16S</td>
<td>F: 5’GCTAACTCTGGCCACCGAG3’  R: 5’CGTGGAACCTACCAGGGGTATCTAATC3’</td>
<td>304bp</td>
</tr>
</tbody>
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Conclusions

Laboratory mice is one of the most popular animal models in bio-medical research. Even the introduction of single infectious agent may contaminate the animal facility and have devastating effect on research programs. Hence, M. glucosida may be considered in regular screening of health monitoring system in animal facility.

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References


