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Mannheimiosis in Swiss albino mice

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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 pneumonic 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- biotype A (ferments arabinose) and biotype 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 glucosidal* [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 ºC under aerobic condition and another set of plates were incubated at 37 ºC 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 ºC 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 ºC - 15 min, 40 cycles of 94 ºC for 30s, 62 ºC for 30s and 72 ºC for 30s; final extension at 72 ºC for 10min. 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 H_2S 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 reveled 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.

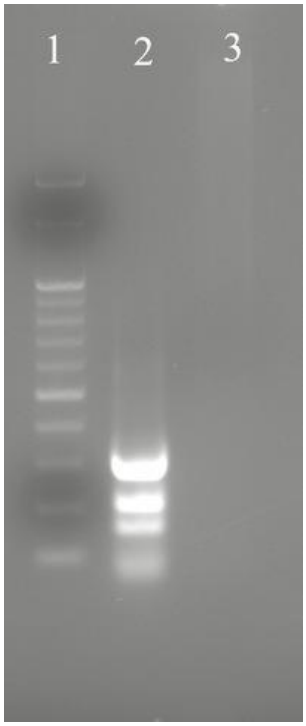

Fig 1: Multiplex PCR showing four bands of 90bp, 170bp, 206bp and 304bp for the DNA extracted from *M. glucosida* isolate from mice

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

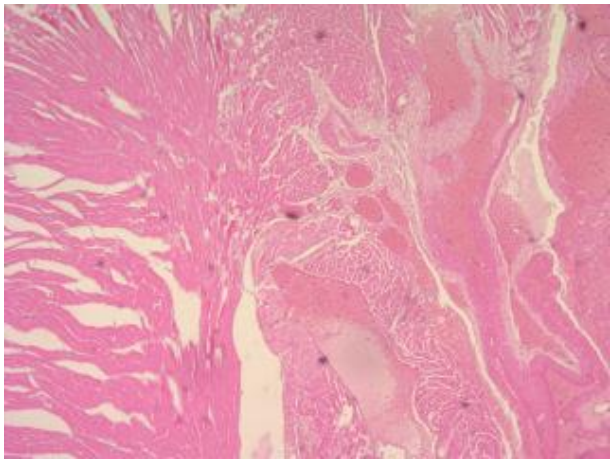

Fig 2: Mouse – Heart Haemorrhage in the myocardium. H&E 100X

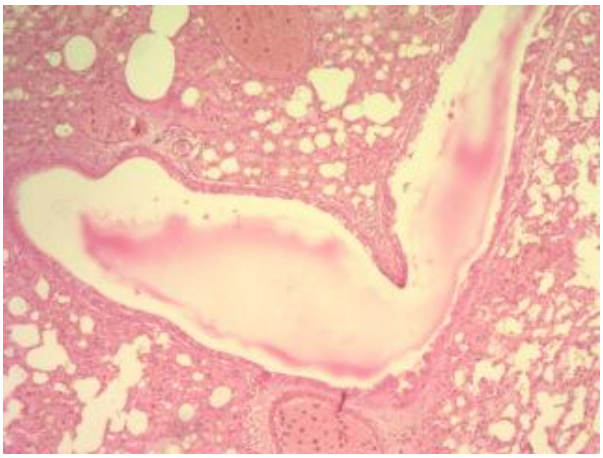

Fig 3: Mouse – Eosinophilic exudate in lumen of bronchioles. H&E 100X

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

Primer set	Sequence	Product size
Lkt	F: 5'GCAGGAGGTGATTATTAAAGTGG3' R:5'CAGCAGTTATTGTCATACCTGAAC3'	206bp
Lkt2	F:5'CTC CTTTAGAAAAGCTGGAAAAC3' R:5'TTTTGCCAAGTGGTGTATTGC3,	170bp
HP	F:5'CGAGCAAGCACAATTACATTATGG3' R:5'CACCGTCAAATTCCTGTGGATAAC3'	90bp
16S	F:5'GCTAACTCCGTGCCAGCAG3' R:5'CGTGGACTACCAGGGTATCTAATC3'	304bp

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

1. https://ca-biomed.org/csbr/pdf/fs-whynecessary.pdf, 18 April, 2019.
2. Adamu JY, Ameh JA. Pasteurellosis to mannheimiosis: Taxonomic changes. Nigerian Veterinary Journal. 2007; 28(1):75-7.
3. Chen HI, Hulten K, Clarridge JE. Taxonomic subgroups of Pasteurella multocida correlate with clinical presentation. Journal of Clinical Microbiology. 2002; 40:3438-3411.
4. Loubinox J, Lozneiwski A, Lion C, Garin D, Weber M, Le Faou AE. Value of enterobacterial repetitive intergenic consensus PCR for study of *Pasteurella multocida* strains isolated from mouths of dogs. Journal of Clinical Microbiology. 1999; 37:2488-2492.
5. Smith GR. The Characteristics of two types of *Pasteurella hemolytica* associated with different pathological conditions in sheep. The Journal of Pathology and Bacteriology. 1961; 81:431-440.
6. Angen O, Mutters R, Caugant DA, Olsen JE, Bisgaard M. Taxonomic relationships of the *Pasteurella haemolytica* complex as evaluated by DNA–DNA hybridizations and16srRNA sequencing with proposal of *Mannheimia haemolytica gen.nov., comb.nov., Mannheimia granulomatis comb.nov., Mannheimia glucosida sp.nov., Mannheimia ruminalis sp.nov.and Mannheimia varigena sp.nov.* International Journal of Systemic Bacteriology. 1999; 49:67-86.
7. Angen O, Quirie M, Donachie W, Bisgaard M. Investigations on the species specificity of *Mannheimia (Pasteurella) haemolytica* serotyping. Veterinary Microbiology.1999a; 65(4):283-2908.
8. Sneath PHA, Stevens M. *Actinobacillus rossii sp. nov., Actinobacillus seminis sp. nov., nom. Rev., Pasteurella bettii sp. nov., Pasteurella lymphangitidis sp. nov., Pasteurella mairi sp.* Nov and *Pasteurella trehalosi sp. Nov.* International journal of systemic bacteriology. 1990; 40:148-153.
9. Poulsen LL, Reinert TM, Sand RL, Bisgaard M, Christensen H, Olsen JE *et al*. Occurrence of haemolytic *Mannheimia spp.* in apparently healthy sheep in Norway. Acta Veterinaria Scandinavia. 2006; 48: 19.
10. Odugbo MO, Odama LE, Umoh JU, Makinde AA. Serotypes of *Pasteurella hemolytica* from pneumonic lungs of sheep in northern Nigeria. Small Ruminant Research. 2003; 48:239-243.
11. Omaleki L, Barber SR, Allen JL, Browning GF. *Mannheimia* Species Associated with Ovine mastitis. Journal of Clinical Microbiology. 2010; 48(9):3419 - 3422.
12. Omaleki L, Browning GF, Allen JL, Barber SR. Molecular epidemiology of *Mannheimia haemolytica* and *Mannheimia glucosida* associated with ovine mastitis. Journal of Veterinary Diagnostic Investigation. 2012; 24(4):730-734.
13. Boot R and Bisgaard M. Reclassification of 30 pasteurellaceae strains isolated from rodents. 1995; Laboratory Animals. 29; 314-319.
14. Christensen H, Bojesen AM, Bisgaard M. *Mannheimia caviae* sp. nov., isolated from epidemic conjunctivitis and otitis media in guinea pigs. International Journal of Systematic Evolutionary Microbiology. 2011; 61:1699-704.
15. Lau JSY, Omaleki L, Turni C, Barber SR, Browning GF, Francis MJ *et al*. Human wound infection with *Mannheimia glucosida* following lamb bite. Journal of Clinical Microbiology. 2015; 53:3374-3376.
16. Ward GE, Moffat R, Olfert E. Abortion in mice associated with *Pasteurella pneumotropica*. Journal of clinical Microbiology. 1978; 8:177-180.
17. Quinn PJ, Markey BK, Leonard FC, Hartigan P, Fanning S, Fitz Patrick ES. Veterinary Microbiology and Microbial diseases. 2nd edition. Wiley -Blackwell, UK 2011.Section II, Chapter 27:300-308.
18. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 1966; 36:493-496.
19. Alexander WT, Cook SR, Yanke LJ, Booker CW, Morley PS, Read RR *et al*. A multiplex polymerase chain reaction assay for the identification of *Mannheimia haemolytica, Mannheimia glucosida* and *Mannheimia ruminalis*. Veterinary Microbiology. 2008; 130:165-175.
20. Hawari AD, Hassawi DS, Sweiss M. Isolation and identification of *Mannheimia haemolytica* and *Pasteurella multocida* in sheep and goats using biochemical tests and Random Amplified Polymorphic

DNA (RAPD) Analysis. Journal of Biological Sciences. 2008; 8(7):1251-1254.
21. Wessman GE, Geraldine H. Characterization of *Pasteurella hemolytica* isolated from the respiratory tract of cattle. Canadian Journal of Comparative medicine. 1968; 32:498-504.
22. Tabatabaei M, Abdollahi F. Isolation and identification of *Mannheimia haemolytica* by culture and polymerase chain reaction from sheep's pulmonary samples in Shiraz, Iran, Veterinary World. 2018; 11(5):636-641.
23. Affandi AS, Abdullah JFF, Saharee AA, Sabri J. Clinical response and pathological changes associated with Pasteurella multocida type B:2 infection through oral route inoculation in mice. Proceedings of the 7th Seminar of Veterinary Sciences, Feb. 2-Mar. 2, University Putra Malaysia, 2012.
24. Faez FJ, Affandi SA, Osman AY, Adamu L, Zamri-Saad M, Haron AW *et al*. Clinico-pathological features in mice following oral exposure to *Pasteurella multocid*a type B:2. IOSR Journal of Agriculture and Veterinary Science. 2013;3: 35-39. DOI: 10.9790/2380 0343539
25. Khaleel MM, Abdullah FFJ, Adamu L, Abba Y, Haron AW, Zamri-Saad M *et al*. Histopathological changes in mice infected with river water contaminated by Pasteurella Multocida type B:2. American Journal of Animal and Veterinary Sciences, 2014; 9(2):71-76.
26. Tefera G, Smola J. *Pasteurella haemolytica* complex of *Pasteurella sensu stricto* as new genus Mannheimia: Changes in taxonomy. Veterinární medicína, 2001; 46(4):119-124.
27. Kehrenberg C, Schulze-Tanzil G, Martel J-L, Chaslus-Dancla E, Schwarz S. Antimicrobial resistance in *Pasteurella* and *Mannheimia*: epidemiology and genetic basis. Vet Res. 2001; 32:323-339.
28. Ott LC, Stromberg ZR, Redweik GAJ, Wannemuehler MJ, Mellata M. Mouse genetic background affects transfer of an antibiotic resistance plasmid in the gastrointestinal tract. 2020. M Sphere 5:e00847-19