A Maggot Mystery in Emergence of Ignatzschineria Bacteraemia in a Febrile White Yorkshire Pig Short running Title: Ignatzschineria spp. bacteremia associated with maggot infested Pig

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Abstract

We report the incidence of bacteraemia associated with *Ignatzschineria* spp. for the first time from animal clinical case presumably, as a post complication of maggot wound in a White Yorkshire pig. We described a clinical history of a febrile adult white Yorkshire pig and the isolation of *Ignatzschineria* spp. from blood sample. The isolate was characterized phenotypically and further identified by 16S rRNA sequence analysis. Its occurrence may be misdiagnosed in veterinary hospitals especially in low-resource settings, often leading to the underreporting of such emerging infections, since the diagnostic facilities are still in very primitive phase in developing countries. More information on speciation is needed with much about the epidemiology and pathogenesis of this emerging pathogen in order to explore its role in the lives of animals and humans. Novel pathogens continue to emerge in human, domestic animal, wildlife and plant populations, yet the population dynamics of this kind of biological invasion remain poorly understood. This rapid communication may redirect the scientific community working for animal and human health worldwide to unveil such rare emerging infections.

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Abstract

We report the incidence of bacteraemia associated with*Ignatzschineria* spp. for the first time from animal clinical case presumably, as a post complication of maggot wound in a White Yorkshire pig. We described a clinical history of a febrile adult white Yorkshire pig and the isolation of *Ignatzschineria* spp. from blood sample. The isolate was characterized phenotypically and further identified by 16S rRNA sequence analysis. Its occurrence may be misdiagnosed in veterinary hospitals especially in low-resource settings, often leading to the underreporting of such emerging infections, since the diagnostic facilities are still in very primitive phase in developing countries. More information on speciation is needed with much about the epidemiology and pathogenesis of this emerging pathogen in order to explore its role in the lives of animals and humans. Novel pathogens continue to emerge in human, domestic animal, wildlife and plant populations, yet the population dynamics of this kind of biological invasion remain poorly understood. This rapid communication may redirect the scientific community working for animal and human health worldwide to unveil such rare emerging infections.

Keywords: Bacteraemia, Maggot infestation, *Ignatzschineria* spp., Myiasis in animals, Open wound, Emerging pathogen.

Introduction

Myiasis is an infestation of animals and humans by various dipteran larvae, it was defined by Zumpt (1965) as "the infestation of live vertebrate animals with dipterous larvae, which at least for a certain period, feed on host's dead or living tissues, liquid body substances, or ingested food". Such invasions may result in mild to severe illness or even death. Flies of several families are obligatory parasites on domestic animals, the most important family causing wound myiasis is*Calliphoridae* and included genera of flies are *Calliphora, Lucilia, Chrysomyia* and *Cochliomyia* (Nadrah et al., 2021) but flies belonging to family *Calliphoridae* and *Sarcophagidae*mostly cause traumatic wound and subsequent myiasis (Roy and Dasgupta, 1982) which is one of the commonest and widespread clinical problems in veterinary practice in India (Chhabra & Pathak, 2009). Exposed lesions attract and stimulate females to oviposit (Francesconi & Lupi, 2012). Because flies and their larvae are the hosts to several bacterial species, there are possibilities of secondary bacterial infections of the wound and also bacteraemia sets in if invaded to the bloodstream. The bacteria which are most frequently associated with myiasis-induced bacteraemia are *Wohlfahrtiimonas* spp. and *Ignatzschineria*spp. (Ahmad et al., 2022; Lysaght et al., 2018). Both the genera are well known inhabitants of the salivary glands of larvae of many fly species (Barker et al., 2014). The presence of *Ignatzschineria*spp. has been reported from diverse environmental samples and animal excreta (Juteau et al., 2004; De Luna et al., 2009). Here we describe the very first incidence of myiasis with subsequent*Ignatzschineria* spp. bacteraemia from animal clinical case, presumably as a post complication of maggot wound in an adult male of White Yorkshire pigs.

Materials and Methods

In September 2021, a febrile male pig aging about 2 years of breed White Yorkshire was presented with a history of pyrexia, cachexia and partial anorexia from village Nardaha of Chhattisgarh state, India (Latitude: 21.2918203^{O}N Longitude: 81.4313321^{O}E). On physical examination it was found that pig was wounded at the base of pinna and as per the history the animal was suffered with maggot infested wound and treated with a subcutaneous dose of Ivermectin @ 200 mcg/kilogram of body weight. The animal belonged to a herd of about 250 animals, inclusive of piglets and adult animals which were kept under semi-intensive production system. Peripheral blood smear was prepared from febrile pig, air dried and blood samples were collected aseptically. The collected samples were transported to the laboratory on ice and processed for isolation of the pathogens.

Sheep Blood agar (SBA) and MacConkey agar (MLA) were used as primary culture media for preliminary isolation of organisms according to methods described by Quinn et al. (2013). Briefly, blood samples were

streaked on SBA and MLA plates and incubated at 37°C for 24 hours. Single colony of was picked up from primary culture and re-streaked on fresh SBA plate and incubated at 37°C for 24 hours to obtain pure culture. The organism was characterized by Gram's reaction, biochemical reactions and further identified by 16S rRNA sequence analysis. The isolate was subjected to antibiotic sensitivity testing by determination of minimum inhibitory concentration (MIC). The EZY MIC Strips were obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India. The isolates were tested against most commonly used antibiotics and the results were interpreted as per CLSI 2020 guidelines with breakpoints for other

non-Enterobacteriaceae applied. The antibiotics used for testing were amoxycillin clavulanic acid, oxytetracycline, tetracycline, doxycycline, kanamycin, gentamicin, vancomycin, rifampicin, cotrimoxazole, ciprofloxacin, ofloxacin, pefloxacin, levofloxacin, cefotaxime, cefotaxime-clavulanic acid, ceftriaxone, streptomycin and colistin.

Results and Discussions

Bacteriological cultures revealed as dominant growth of non-haemolytic, smooth, greyish- white colonies of about 1 mm diameter size on sheep blood agar (Fig. 1) and non-lactose-fermenting colonies on MacConkey agar at 37@C for 24 hours of incubation. Phenotypically, the organism was Gram-negative bacilli (Fig. 2), positive for oxidase, catalase, nitrate reduction and phenyl alanine deamination while negative for urease, H2S production and did not utilize citrate, lysine, and ornithine. The isolate could not able to ferment adonitol, arabinose, cellobiose, glucose, dulcitol, galactose, inositol, inulin, lactose, mannitol, mannose, raffinose, rhamnose, salicin, sorbitol and sucrose and trehalose. Antibiotic Sensitivity Testing showed resistance towards tetracycline group of antibiotics while showed susceptibility to all other tested antimicrobials. According to the test results the animals were treated with Enrofloxacin @ 5mg/Kg body weight per day for 5 days, the animal was afebrile from the 2nd day onward. The 16S rRNA gene was amplified by PCR, and the amplicon underwent Sanger sequencing (GCC Biotech Pvt. Ltd. Kolkata, India). The nucleotide sequence data were analysed by BLAST (https://blast.ncbi.nlm.nih.gov), which yielded 98.5% sequence homology to both *Ignatzschineria*spp., assembled the genome sequences and submitted to GenBank under accession numbers OP021690 and OP021691.This sequence was analyzed on the nucleotide BLAST (BLASTn) suite of tools available on the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Default parameters were employed to search the highly similar sequences among 16S rRNA genes in the database.

Phylogenetic analysis

Two types of distance trees were generated after NCBI BLAST using neighbour-joining and fast minimum evolution methods. Both the trees were visualized on the iTOL server (http://itol.embl.de) for better grouping of all the sequences. . A total of 100 sequences in the NCBI database were found to be similar to the queried 16S rRNA gene. Among these, 70 sequences were having a query cover of 100% and the remaining 30 sequences were having a query cover of more than 90%. When comparing the percentage identity of a queried sequence with similar sequences in NCBI, 72 sequences were having more than 97% identity; while, only 28 were having less than 95% identity. For evolutionary analysis, the available genome sequences of *Ignatzschineria* were retrieved from the NCBI database and constructed phylogenetic tree employing neighbour-joining method (Fig. 3) and fast minimum evolution methods (Fig.4) with bootstrap analysis using 1,000 replicates.

Wounds infected with maggots are the commonest incidences in animals (Sinha, 2012) however, there is no evidence of occurrence of*Ignatzschineria* spp. bacteraemia reported from animal clinical cases so far. Whereas there are several earlier reports of occurrence of*Ignatzschineria* spp. bacteraemia from human patients with poor hygiene (Gupta et al., 2011; Baker at al., 2014; Cipolla et al., 2018). There are reported human case of *Ignatzschineria* spp. bacteremia wherein most of the described cases of *Ignatzschineria* spp. infections are associated with myiasis (Do et al., 2021; Heddema et al., 2016; Le Brun et al., 2015). Similar to the previous reports, our clinical isolate *Ignatzschineria* spp. was found to be Gram-negative, non-sporulating, non-haemolytic, nonmotile, rod-shaped bacteria (Nadrah et al., 2021; DiFranza et al., 2021). Although,*Ignatzschineria* spp. are generally difficult to be isolated and identified in routine bacteriological

procedures and MALDI TOF – MS therefore, they are identified by 16S rRNA gene amplification and sequencing (Do et al., 2021; Gupta et al., 2011). Similar to previous reports of identification of *Ignatzschineria* spp., the isolate in our case was identified by 16S rRNA sequence analysis. It is strongly indicated by previous reports that *Ignatzschineria* spp. transmitted by flies are more commonly associated with human wound myiasis. Bacteria carried by maggots can spread into the bloodstream of the infested host, causing systemic infections as documented in previous case reports (Nadrah et al., 2021; Gupta et al., 2011; Snyder et a., 2020). There is a well-documented relation between*Ignatzschineria* spp. infection and maggot infestation thus, our hypothesis that the bacteraemia caused by *Ignatzschineria* spp. in maggot infested pig is justifiable. Moreover, the bacterium the pig became febrile only after receiving a dose of ivermectin treatment that might have caused death of larvae and systemic release of the inhabiting*Ignatzschineria* spp.

In conclusion, we reported the isolation of *Ignatzschineria* spp. probably for the first time from an animal clinical case of bacteraemia presumably, as a post complication of maggot wound in an adult White Yorkshire pig. The emergence of novel pathogens is one of the greatest challenges to global health security. The detection of this pathogen may be misdiagnosed in veterinary hospitals especially in low-resource settings, often leading to the underreporting of such emerging infections, since the diagnostic facilities are still in very primitive phase in developing countries. More information on virulence and epidemiology is needed of this emerging pathogen in order to explore its role in the lives of animals and humans. Novel pathogens continue to emerge in human, domestic animal, wildlife and plant populations landscapes, yet the population dynamics of this kind of biological invasion remain poorly understood. Hence, we must be prepared to recognize the signs, identify the threat to reduce the spread of infections and health consequences before they harm the health of animals and people throughout the world.

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Ethics statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the authors confirm that no ethical approval was required as this work was carried out with collected clinical samples for diagnosis. No animal experimentation was conducted in the present study.

Conflict of interest

The authors declare no conflict of interest.

Data availability statement

The nucleotide data that generated in the present study and support the findings of this study are available in NCBI (https://www. ncbi.nlm.nih.gov)

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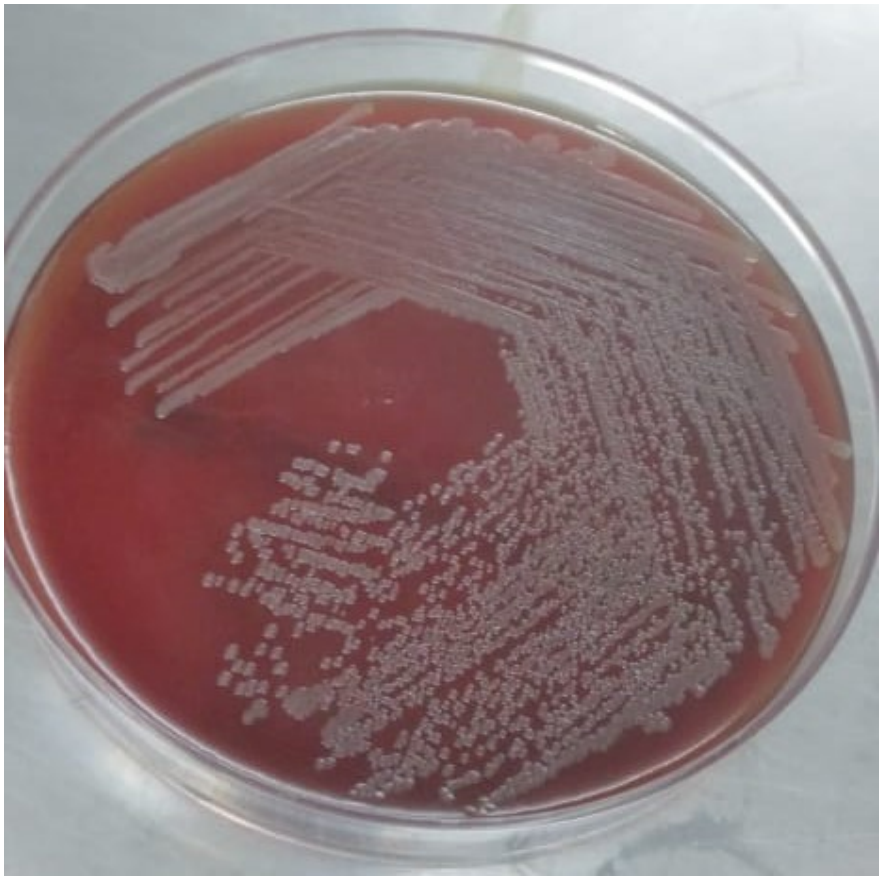
Caption for figures Fig. 1 Photograph showing blood oozing wound at the base of pinna of a White Yorkshire Pig Fig.2 Smooth, greyish white, non-hemolyic colonies on 5% sheep blood agar plate Fig. 3 a. and b.Phylogenetic tree employing neighbour-joining method and. Phylogenetic tree employing minimum

evolution method based on 16S rRNA gene sequence of *Ignatzschineria* spp. Isolate 761-PWBD04 from bacteraemic pig (in coloured block) with bootstrap analysis using 1,000 replicates. Fig.4.

Figure: 1:

Figure.2.



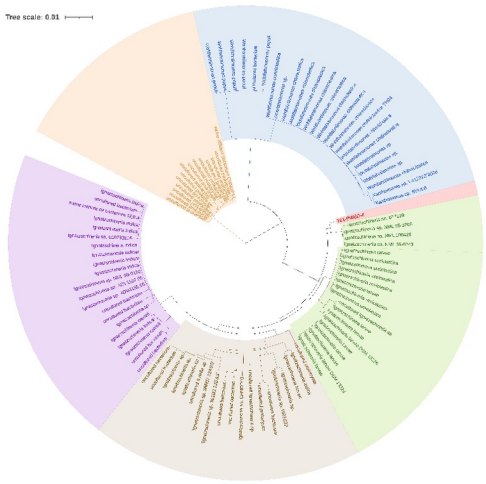


Figure 3a.

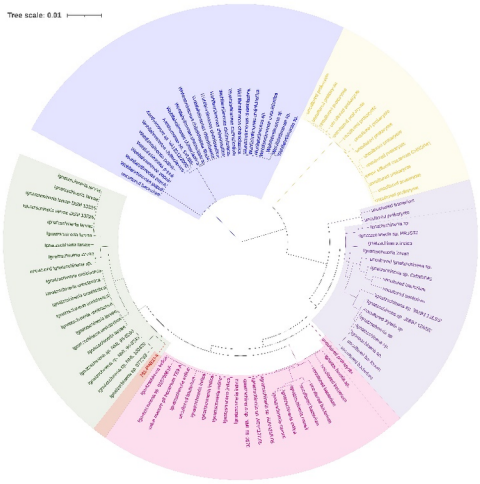


Figure. 3b.