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Multi Drug Resistant Pseudomonas Aeruginosa: A Secondary Invader and Cause of Mortality in Foot-and-Mouth Disease Outbreak

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Multi Drug Resistant *Pseudomonas Aeruginosa:* A Secondary Invader and Cause of Mortality in Foot-and-Mouth Disease Outbreak

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I. Introduction

Foot-and-mouth disease (FMD) is a highly contagious and devastating viral disease of the cloven-hoofed animals including cattle and buffaloes; and considered as a serious threat to the economy of the livestock industry all over the world the world (Verma *et al.,* 2008; Rodriguez and Gay, 2011; Verma *et al.,* 2012; Ding *et al.,* 2013; Xu *et al.,* 2013; Chakraborty et al., 2014). The disease is clinically characterized by pyrexia, vesicles on the mouth, muzzle, tongue, teats, inter digital space of feet and other hairless parts of skin (Teifke *et al.,* 2012). The morbidity rate is high almost reaching to 100% but mortality rate is low approximately 10% in adult animals but may reach upto 50% in young animals (claves) due to myocarditis (Gulbahar *et al.,* 2007; Raies *et al.,* 2009; Verma *et al.,* 2010a,b, 2012; Chakraborty *et al.,* 2014). The immune-suppression due to FMDV usually leads to development of secondary bacterial infection in the affected ignored animals. The bacteria of genus *Pseudomonas* are ubiquitous in nature and are well known to cause nosocomial infections. Drug resistance and severe toxaemia due to *Pseudomonas* infections are supposed to produce high case fatality rate in immune-suppressed host (Blanc *et al.,* 1998; Geyik, *et al.,* 2003; Hamud-Socoro, 2004). There are many factors which are responsible for *P. aeruginosa* infection in cattle and buffaloes, particularly exposure of open wounds with contaminated soils and water. Under stress, weak and debilitated animals suffering from skin injuries may lead to adherence of *Pseudomonas* with the skin surface and may contaminate the wound and form abscess. Drug resistance of the organism supports bacterial survival in wounds and further entry in circulation leading to involvement of multiple system viz., respiratory and urinary tracts. In advanced stages of the infection severe toxaemia leads to mortality. Hence, the aim of the present study was to reveal the cause of mortality in cattle and buffaloes following to an outbreak of FMD disease in Chandauli district of Uttar Pradesh state, India.

II. Materials and Methods

a) Study area, animal and management

The incidence occurred in village Daina, district Chandauli, Uttar Pradesh, India. At the time of incidence the population of dairy animals (cattle and buffaloes) in the village was approximately 1200. The animals were kept individually or in groups. The animal rearing practices included stall feeding of wheat/paddy straw, concentrate, and mineral mixture with *ad lib* water

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supply. Buffaloes of the village were having access to local water body situated outside the village.

b) History, clinical examination and data collection

During the visit, animals were thoroughly examined by animal health researchers of Department of Veterinary Epidemiology and Preventive Medicine; and Department of Veterinary Clinical Medicine, Uttar Pradesh Pandit Deen Dayal Upadhayaya Pashu Chikitsa Vigyan Vishwavidyalaya Evum Go-Anusandhan Sansthan (DUVASU), Mathura, India. The health and basic record books of the herd, compiled by veterinary and animal care staff, were also examined and analysed for occurrence of the disease, morbidity and mortality etc. All the animals were having the history of vaccination with *Pasteurella multocida* biotype A vaccine (Biological Product Section, Badshabagh, Lucknow). However, FMD vaccination was lacking in the village. The area was having the history of flood or water logging nearly two months prior to outbreak. The hygiene and sanitation conditions in the villages were unsatisfactory. Majority of the animals were suffering with pyrexia, vesicular lesions in teats and foot (Figure 2 and 3) and respiratory distress. The morbidity and mortality rate was quite high with the death of large number of animals. The clinical signs and history were suggestive of Foot-and-Mouth Disease.

Figure 2 : Vesicular lesion on teats

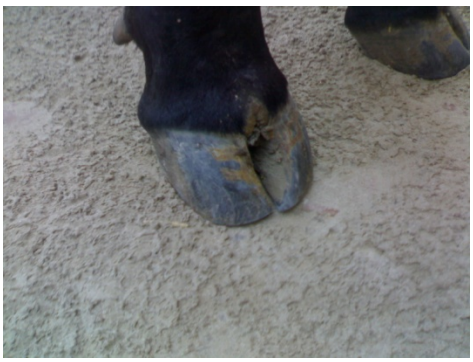

Figure 3 : Vesicular lesions on feet

c) Sample collection

About 15 blood samples from sick animals were collected aseptically from juglar vein and transported on ice to the laboratory. Sera from all the blood samples were separated and stored at 20°C, while clots were processed for the microbiological identification.

d) Laboratory Examination

All the 15 sera samples were analyzed for the presence of antibodies against FMD virus type O, A and Asia-1 using liquid phase blocking ELISA (LPB-ELISA) as per the method described by Hamblin *et al.*, 1986. All the 15 blood clots were processed for bacteriological examination by incubating the samples at 37°C onto Nutrient Agar, MacConkey Agar and 5% Sheep Blood Agar and examined after 24-48h as per the method described by Cruickshank *et al.,* 1975. The causative agent was isolated and characterized with conventional microbiological and biochemical methods.

e) Antibiogram

Pseudomonas aeruginosa isolates were assessed for their antimicrobial susceptibility testing against commonly used antibacterial drugs by disc-diffusion method (Bauer *et al.,* 1996) following the NCCLS guidelines (NCCLS, 2002).

III. Results and Discussion

In the present study, clinical signs of the animals were indicative of Foot-and-Mouth Disease. On retrospective study, the sera samples showed high titre against FMDV serotype 'O' suggesting the infection of serotype 'O' of FMD virus. However, there is as such no report of high mortality due to FMD in cattle and

particularly in buffaloes. Serotype 'O' is predominantly causes FMD in cattle but in this outbreak high titre against FMDV serotype 'O' in buffaloes also suggest some antigenic alteration or host adaptability of pre-existing serotype 'O' virus in India. There are previous studies reporting the high prevalence of FMDV serotype 'O' in Uttar Pradesh state, India (Verma *et al.,* 2008). The involvement of buffaloes in such outbreaks with higher rate of mortality than cattle is of major concern as buffaloes were suggested to show clinical signs less commonly as compared to cattle (Chakraborty *et al.,* 2014). Moreover, India is having the largest population of buffaloes, accounting for nearly 57% of the world buffalo population, and buffaloes are considered as back bone of rural economy (Kumar, 2005).

Monoculture *Pseudomonas aeruginosa* was isolated from blood. The isolated bacteria were found to be motile, produced characteristics colonies in nutrient agar along with pigmentation, showed β-hemolysis on blood agar (Figure 4) and grew on MacConkey agar.

Figure 4 : β-hemolysis on blood agar

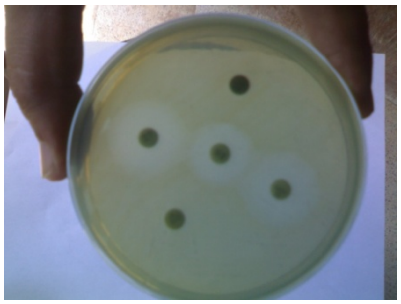

Figure 5 : Antibiogram on Nutrient agar

On Gram's staining, the bacteria appeared Gram-negative, pink colour, medium size bacilli. Biochemical examination revealed battery of reactions characteristic to *Pseudomonas aeruginosa*. Based on the zone of inhibition, observations of drug sensitivity tests revealed resistance against amoxicillin+clavulanic acid, enrofloxacin, ofloxacin, levofloxacin, intermediate sensitivity to penicillin, gentamicin and tylosin, and sensitivity to amikacin, ceftriaxone+tazobactum and cefotaxim (Figure 5). The present findings were comparable to the studies conducted by previous researchers (Sun *et al.,* 2011; Ohnishi *et al.,* 2011; Mudau *et al.,* 2013). On the basis of present findings inference can be drawn that it was an outbreak of FMD, followed by secondary bacterial infection of *Pseudomonas aeruginosa*, which might have entered in the circulation through the lesions in foot, tongue and teats. Moreover, immunosuppression due to FMD further led to colonization of *Pseudomonas aeruginosa* in critical body organs, such as the lungs, heart and kidney leading to severe mortality. On the basis of results of antibiotic sensitivity testing, the authors suggested amikacin, ceftriaxone + tazobactum and cefotaxim for the treatment of affected animals and after treatment with these drugs the mortality among animals was controlled in the village.

IV. Conclusion

It can be concluded that occurrence of FMD leads to immunosuppression making affected animals more susceptible and prone to nosocomial infections viz. *Pseudomonas aeruginosa.* The drug sensitivity pattern revealed that isolates were resistant to many of commonly used broadspectrum antibiotics which might be the cause of failure of treatment. However, antibacterial like amikacin, ceftriaxone + tazobactum and cefotaxim appeared to be drugs of choice for the treatment of *Pseudomonas* infection as the recommendation of these drugs controlled the mortality of animals in the village. Hence, the control of secondary invaders should be considered on priority to avoid the mortality in the outbreak situations.

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