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Prevalence of Deg Nala disease in eastern India and its reproduction in buffaloes by feeding *Fusarium oxysporum* infested rice straw

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ABSTRACT

Objective: To undertake a study on prevalence of Deg Nala disease in eastern states of India and to reproduce the disease in buffaloes by the *Fusarium* spp., isolated from the affected region. **Methods:** During this investigation, a survey was conducted covering four states of eastern region to identify the Deg Nala cases as well as to isolate and characterize the causative agent(s). An experimental study was carried out to reproduce the disease in healthy male buffaloes (2–3 years age) by randomly dividing them into five groups (four in each group). Each individual group was fed with rice straw artificially infested with either of the two representative isolates of *Fusarium oxysporum (F. oxysporum)* (F01, F02) or representative reference strains of *Fusarium equiseti (F. equiseti)* (ITCCF-2470) and *Fusarium moniliforme (F. moniliforme)* (ITCCF-4821) for 30 days, whereas the control group was fed with normal rice straw only. **Results:** A total of 658 Deg Nala cases were recorded and 12 *Fusarium isolates* were identified from the mouldy rice straw collected from these affected areas. The characterization of the isolates revealed three species viz., *F. oxysporum*, *F. equiseti* and *F. moniliforme*, among which *F. oxysporum* was predominant. The disease was artificially reproduced in three buffaloes in F01 group and one in F02 group within 20–23 days by feeding F. oxysporum infested rice straw which resembled the clinical symptoms and gross lesions of natural Deg Nala cases. **Conclusions:** The field investigation and laboratory studies, including experimental production of Deg Nala disease suggest the possible involvement of mycotoxins. However, further investigations needs to be done to understand nature of the toxic factors involved in production of the Deg Nala disease.

1. Introduction

Deg Nala disease, named due its first occurrence in the areas bordering the course of Deg Nala (= Deg river), has been reported in the Indian subcontinent since 1930[1]. The disease is more prevalent in winter months mostly in the rice growing parts of India and has been reported in a number of states in India like Bihar, Gujarat, Haryana, Uttar Pradesh and West Bengal[2-7]. This disease is characterized by necrosis, followed by gangrene of the dependant parts of the body[7]. Because of this, the animals not only become weak and emaciated, but also at times more or less become crippled causing enormous economic losses due to decreased productivity and functional capacity in the form of reduced milk production and draught capacity. This disease is believed to be caused by mycotoxicoses resulting from ingestion of rice straw contaminated with *Fusarium* spp.[6-9]. Although this disease has been reproduced experimentally in buffaloes fed with mouldy rice straw by several workers[10-11], there is probably a single report in India on the reproduction of Deg Nala disease in buffalo calves fed rice straw artificially infested with *F. equiseti*[12]. This study describes experimentally reproduction of the disease in buffalo calves by *F. oxysporum*.

2. Materials and methods

2.1. Field survey

A preliminary survey was carried out in four states of eastern India viz., West Bengal, Bihar, Jharkhand and Orissa during winter months (November to February) to

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identify the prevalence of Deg Nala disease in buffaloes. The animals showing symptoms like oedematous swelling of the lower part of the leg resulting in lameness, and necrosis followed by gangrene of dependant parts of the body (tail and/ or legs) were treated as Deg Nala cases.

2.2. Isolation and identification of Fusarium spp.

Rice straw samples, collected from feeding troughs of both affected and unaffected herds, were examined for fungal infestation, if any and attempted for isolation of *Fusarium* spp. Scrapings from mould-infested portions of the straws were used for direct cultural examination on Sabouraud's dextrose agar (SDA) and SDA with cycloheximide and chloramphenicol (SDACC) (Hi Media, India). The samples (in duplicate) were kept under incubation for one week at 27 ℃ and 37 ℃[13]. The *Fusarium* isolates were subcultured on SDA, potato dextrose agar and carnation leaf agar (CLA) media for studying the characteristic features of macroconidia, microconidia, chlamydospore and conidiophores for detection of species[14]. The isolates were referred to the Department of Plant Pathology, Indian Agricultural Research Institute (IARI), New Delhi, India for confirmation.

2.3. Experimental design

To reproduce the disease, a total of 20 apparently healthy male buffaloes (non-descript breed) in the age group of 2-3 years were taken for experimental study. These animals were kept under observation for seven days. The buffaloes were randomly divided into five groups consisting of four buffaloes in each. Rice straws used for the experimental study were collected from locality where there was no report of such disease and were examined through culturing of representative samples for absence of any fungal infestations including *Fusarium* spp.

A total of four cultures of *Fusarium* spp. were used for this study of which two were representative isolates of *F. oxysporum* (FO-1, FO-2), confirmed by Department of Plant Pathology, IARI, New Delhi and the other two reference cultures, i.e. *F. equiseti* (ITCCF-2470) and *F. moniliforme* (ITCCF-4821), were received from the Department of Plant Pathology, IARI, New Delhi. All the cultures were maintained on SDA and subcultured at monthly intervals during the study period.

Each group of coarsely chopped rice straw was inoculated by spore suspension of above mentioned *Fusarium* culture isolates using peptone supplemented Czapek-Dox medium[15-16]. Only *Fusarium* infested rice straw were fed *ad libitum* to the respective group of experimental buffaloes for 30 days whereas the control group was fed with normal rice straw only.

2.4. Estimation of selenium

For estimation of selenium content, representative samples of hair, hoof, tail and skin of affected animals including soil and rice straw were collected from the affected as well as unaffected areas. Selenium content was estimated (in ppm level) by atomic absorption spectrophotometer (AAS) using Perkin Elmer Analyst 100 (Perkin Elmer, USA) at wavelength of 196 nm.

3. Results

Based on the observations during field survey, a total of 658 Deg Nala cases were identified in 42 villages of four states. Early symptoms in buffaloes include posterior weakness, disinclination to move and oedematous swelling around the fetlock joints of legs and tail, followed by pronouncement of wounds on the coronets, knee and hock regions. In advanced stage, sloughing off the hooves (Figure 1) took place exposing the sensitive laminae and the bones, thus resulting into lameness and loss of body weight. As the disease progressed, necrosis and gangrene developed on tail and legs (Figure 2). In most of the cases, these gangrenous lesions progressed towards root of the tail and in some cases necrosed portion sloughed off. The lower portion of the tail, particularly the tip, contracted into wrinkles.

Figure 1. Buffalo limbs showing sloughing off the hooves.

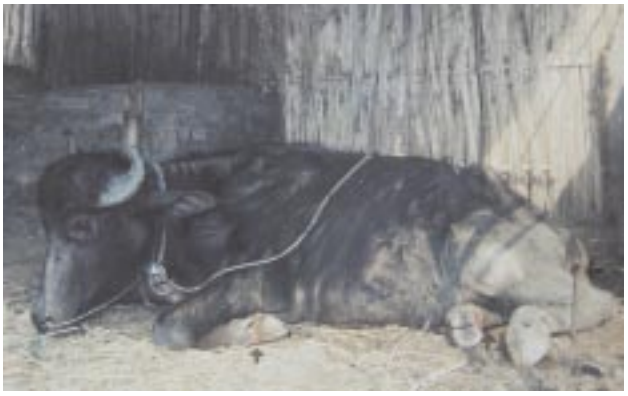

Figure 2. Advanced case of Deg Nala disease showing necrosis and gangrene on tail and legs of a buffalo.

On macroscopic examination of the rice straw samples from the troughs of both affected and unaffected herds, mould infestation was found only in feeding troughs of affected herds. The affected rice straws showed whitish and brownish

specks mostly in the young stems and leaves (Figure 3). Microscopic examination of these rice straw scrapings, having sickle-shaped macroconidia and 1-2 celled microconidia, resembling *Fusarium* species were considered for further course of studies. Upon culture and microscopical examination of these affected rice straws, a total of 12 *Fusarium* spp were isolated, apart from few isolates of other fungi like *Penicillium* (5), *Aspergillus* (4) and *Alternaria* (1). Characterization of Fusarium revealed three species namely, *F. oxysporum*, *F. equiseti* and *F. moniliforme*, among which *F. oxysporum* was predominant (six in number, F01 to F06).

Figure 3. Rice straws showing whitish and brownish specks in the young stems and leaves.

The selenium content in soil and straw samples collected from affected region ranged from 0.20-0.35 ppm and 0-0.60 ppm respectively whereas the content in different tissue materials like hair, hoof, tail and skin of the affected animals were 0.20-0.60, 0.60-0.80, 0.30-0.60 and 0-0.40 ppm, respectively.

The disease was artificially reproduced in three buffaloes in F01 group and one in F02 group within 20-23 days by feeding *F. oxysporum* infested rice straw (Figure 4). However, no such disease was reproduced either in two other experimental groups[*F. equiseti* (ITCCF-2470) and F. *moniliforme* (ITCCF-4821)] or in control group even after 30 days.

Figure 4. Experimental reproduction of Deg Nala disease in buffalo by feeding F. oxysporum infested rice straw showing gangrenous lesions on tail and legs.

4. Discussion

Deg Nala has been reported to occur in various rice growing parts of India and other parts of the world having similar climatological condition and is a cause of concern to the farmers having impact on rural economy[3, 6-7, 17]. Occurrence of Deg Nala disease appears to be associated with the feeding of mouldy rice straw, as it is suspected to play some role, directly or indirectly, in the development of the disease[11, 18]. During the present investigation, both in natural and experimental cases, the clinical symptoms and gross features of lesions recorded were in close conformity with the findings of earlier workers ([3, 7, 19-20]. Loss of body weight in animals fed with infested rice straw could be due to anorexia and increased tissue catabolism[21]. The progressive posterior weakness may be due to degenerative changes of adjoining musculature[22]. The occurrence of the disease in these regions might be due to liberation of certain toxic compounds from fungi under conducive environmental conditions like temperature, humidity and water content of rice straw. This may explain the confinement of the disease to a particular region which is its characteristic feature.

Since occurrence of the disease has been associated with the feeding of rice straw, selenium toxicity is suspected to play some role, directly or indirectly, in the development of the disease[8, 23]. To rule out this as a possible cause of Deg Nala disease, the selenium content of rice straw, soil and tissue samples collected from affected as well as unaffected areas were estimated and compared with the reported toxic level (0.9 to 6.7 ppm) in various fodder crops like berseem, paddy straw, lucern, maize green and oat fodder, causing selenium toxicity[24]. As the selenium content of the samples was much below the toxic level, this rules out the possibility of chronic selenium toxicity as a cause of Deg Nala disease. This notion is further confirmed by the fact that the symptom of chronic selenium toxicity takes a long period to develop i.e. several weeks to months[25-26] as compared to onset of Deg Nala disease. Further, the selenium contents in the tissues collected from experimental cases of Deg Nala disease were found to be very low and comparable with normal animals (Data not shown).

Since the cultural isolation and identification of the scrapings of the mouldy rice straw, collected from affected areas, revealed *Fusarium* spp. (*F. oxysporum* most predominant); an attempt was made for reproduction of the disease in buffaloes by feeding *Fusarium* infested rice straw. The group fed with *F. oxysporum* (F01) and *F. oxysporum* (F02) infested rice straw produced the disease within 20-23 days in three and one animals, respectively. However, the disease could not be reproduced in animals by feeding of rice straw infested with *F. equiseti* (ITCCF-2470) and *F. moniliforme* (ITCCF-4821). The variation in number of affected animals in two groups of *F. oxysporum* may be due to either variation in the susceptibility of the buffaloes or variability of pathogenecity of two strains. Although, the Deg Nala disease syndrome has been

reproduced clinically in buffalo calves within 10–15 days by feeding toxic rice straw collected from the affected herds[11], other workers reproduced the disease experimentally with onset of symptoms from 19 days onwards by feeding rice straw artificially infested with *F. equiseti*[27]. The marginal difference in time interval for reproduction of the disease may be due to involvement of different *Fusarium* species. However, in the present investigation, disease could not be reproduced in other two groups of *F. equiseti (*ITCCF–2470) and *F. moniliforme* (ITCCF–4821), probably due to absence of relevant toxic factor in the reference strains received from IARI, New Delhi.

This is so far the first report of reproduction of Deg Nala disease in buffalo with *F. oxysporum*. The field investigation and laboratory studies, including experimental production of Deg Nala disease suggest the possible involvement of mycotoxins. However, further investigations needs to be done to understand nature of the toxic factors involved in production of the Deg Nala disease.

Conflict of interest statement

We declare that we have no conflict of interest.

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