Clinical-Epidemiological Investigation of Moldy Corn Poisoning due to *Penicillium spp* in mules at Udayapur District, Nepal

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Abstract

A clinical epidemiological investigation of Acute sudden death syndrome due to which 31 mules from a herd of 900 died within the period of 12-07-2006 to 21-10-2006 in Udayapur District Nepal. These animals were being used for good transportation work in hilly region of Nepal. On rout these animals were being fed only whole maize and gram. Normally healthy looking animals started dying suddenly. Initially suspected for acute bacterial disease and treated with broad-spectrum antibiotics and vaccinated with bacterial vaccine.On close observation of herd their feed stuff revealed grains fed to these animal during rainy season was found 20% moldy in appearance. On Postmortem examination acute severe congestion and hemorrhages in liver, lung, spleen heart, intestinal mucosa were found. Histopathological examination of tissue from these organs revealed infiltration of mononuclear cell in tissue indicative of chronic nature of condition.Continous use of apparently 15-20% moldy grain (maize, gram) was used as feed. On laboratory analysis of sample of same grain was found to be containing 6^{10}-110^{10} CFU/gm of *Penicillium* spp of fungus. When remaining herd still using same feed treated with toxin binder (toxicurb, varishta), liver tonic like bioliv, digevet, mineral mixture promin and immunocare controlled the mortality may be indicative of the disease syndrome being caused by moldy corn poisoning similar to condition Equine Encephalomalacia.Laboratory findings and response of treatment indicates that during rainy season and immediately after rainy season feeding of stored grains are going to be detrimental to equine species. During this period care should be taken to feeding grains to this animal if treated with any toxin binder as well as herbal immunomodulater is going to reduce the chances of occurring this syndrome.

Keywords: Clinical-epidemiological investigation, Moldy Corn poisoning, *Penicillium Spp.*, Mule, Nepal.

History

The DLSO Udayapur reported the periodic death of total 31 adult mules during 12-07-2006 to 21-10-2006 with showing symptoms like twisting of head, star-grazing position, excessive sweating, aimless biting to inanimate objects. Death within 10-15 minutes after collapsing on the ground. These herd were being treated with antibiotics and vaccinated against anthrax during this period with unchecked mortality. On field investigation, obtaining a thorough history and completing a physical examination, it was discovered that the mules were being fed cracked and moldy corn.

Review of Literatures

Penicillium grain mold is probably the second most common grain mold pathogen. It may be caused by several species of *Penicillium*, including *P. oxalicum* and *P. chrysogenum*. *Penicillium* species are well adapted to survival in many types of storage facility. Kernel infection can occur in the field or in storage. Symptoms range from external mold development to internal discoloration ("Blue Eye") of the embryo. Symptoms caused by *Penicillium* are easy to confuse with those caused by *Aspergillus glaucus*. Mycotoxins most fungi produce a class of chemical compounds called secondary metabolites. These compounds have a wide range of biological activities including antibiotic (antibacterial and antifungal), acute and chronic toxicities (plant, animal, and humans), and hormone and growth regulation (plants and animals). It is not unusual for more than one species of grain mold pathogen to be present within a single storage bin and many grain mold pathogens can be active under the same environmental conditions providing for the opportunity to have more than one mycotoxin produced in a lot of contaminated grain. The nature of the toxic effects caused by mycotoxins varies

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greatly. Some mycotoxins cause acute toxicities (i.e., immediate effect) where a certain organ (e.g., liver, kidney) loses complete or partial function; other mycotoxins cause chronic toxicities (i.e., long-term) resulting in symptoms such as weight loss and reproductive dysfunction. Still other mycotoxins impair the immune system predisposing the affected animal to a variety of infections or other ailments. For some mycotoxins damage is not permanent and affected animals can recover from ingestion if the contaminated feed is removed from the diet.(*Jim Stack).*

Equine leukoencephalomalacia commonly called "Moldy Corn Poisoning", is a disease of the central nervous system that affects horses, mules, and donkeys. It is commonly associated with feeding of moldy corn over several days to weeks. The clinical signs associated with the neurologic form of Equine leukoencephalomalacia in horses include apathy, drowsiness, pharyngeal paralysis, blindness, circling, difficulty backing, staggering, hyper excitability, seizures and eventual recumbency. However, in some cases, sudden death may be the only clinical sign observed. Once animals show the neurological signs, death usually occurs within 48-72 hours. If an animal survives the acute syndrome, neurological deficits are observed. A recovered horse is sometimes referred to as a "dummy" because of its loss of intelligence. Histologically, there may be diffuse vacuolization of hepatocytes, fatty degeneration, centrilobular necrosis with inflammatory cell infiltrate, bile duct proliferation, bile stasis, increased mitotic figures within the hepatocytes, or periportal fibrosis. Equine leukoencephalomalacia is a generally fatal, rapidly progressing neurologic disease of horses (and other equids) caused by ingestion of fumonisin. Horses showing these signs will usually become recumbent and comatose in 1 to 10 days and may show clonic-tonic convulsions before dying. In some cases, frantic behavior such as head pressing, agitation, hyper excitability, profuse sweating and delirium may be observed. It is characterized by liquefactive necrosis of the cerebral white matter. Liver lesions can also occur. The extent of contamination of raw corn with fumonisins varies with geographic location, agronomic and storage practices, and the vulnerability of the plants to fungal invasion during all phases of growth, storage, and processing. The levels of fumonisins in raw corn are also influenced by environmental factors such as temperature, humidity, and rainfall during pre-harvest and harvest periods. High levels of fumonisins are associated with hot and dry weather, followed by periods of high humidity. High levels of fumonisins may also occur in raw corn that has been damaged by insects. Horses, along with rabbits, are the species most sensitive to the toxic effects of fumonisin. Ruminants, mink and poultry are more resistant than horses, rabbits, catfish and swine to fumonisin. Onset of clinical signs can occur from 1-21 weeks after beginning eating feeds containing fumonisin, but generally occur within 2-9 weeks. Time of onset depends on the concentration of fumonisins in the feed. Clinical signs of fumonisin poisoning in horses are usually related to liquefactive necrosis of the white matter of the brain and include progressive ataxia, depression, anorexia, delirium, aimless wandering, recumbency, coma and death. Death can occur from 12 hours-1 week after onset of clinical signs. At necropsy, lesions in the cerebral cortex can range from none to multifocal areas of hemorrhage and necrosis, to the presence of large cavitations of liquefactive necrosis. Histologically, there are multifocal areas of liquefactive necrosis within the cerebral cortex with infiltration of macrophages. Differential diagnoses should include rabies, equine encephalomyelitis, equine herpes virus, botulism, head trauma, hepatoencephalopathy, and bacterial meningoencephalitis (Dr. Steve Hooser, Dr. Duane Murphy 2003).

Material and Methods

Mule population and Husbandry in Udaypur: A total 900 mules are being used for domestic good transportation to hilly district under Mule Transporters Society. A total of 50 members are being involved in this bissunes.Mules are generally fed on dry whole maize,grame grains. These grains are generally purchased from market only small part of it is utilized from domestic production. These grains are having moldiness upto15-20% and are not processed before feeding.

Preliminary Field Investigation: Post-mortem examination of dead mules revealed severe congestion of liver, lung, speen, heart,serosanginous fluid in thoracic cavity. Haemorrhage in stomach mucosa. Preliminary cause of sudden death was suspected for moldy grain poisoning. For bacteriological culture of smear, swab,blood. Liver, lung, spleen, heart tissue for histopathology. Blood for bacteriological, parasitological examination.

Liver, lung, spleen,heart,intestine for toxicological analysis. Fecal samples for endo-

parasite examination. Serum for serological examination.

Feedgrains: Maize, Gram for mycological culture identification,quantitification were collected.

Mules in herds were provided with toxinbinder, adaptogen, immunomodulater, vitaminBcomplex as treatment and preventive measure.

Laboratory Investigation:

Bacteriological culture of tissue specimen, swab blood: revealed no growth of any bacteria.

Blood parasite: Negative.

Chemical toxin in tissue: negative.

Intestinal parasite: Mixed *Strongyles spp.*

Penicillium, Aspergillus, Candida spp on mycological media.

6^{10}-110^{10} CFU/gm *Penicillium* colonies recorded in feed samples.

Histopathological changes:

Liver: Fatty degeneration of hepatocytes. Mononuclear cells infiltration in the form of few nodules.

Lungs: Perivascular cuffing. Infiltration of mononuclear cells in the form of nodules.

Kidney: Deep medullary region reveals infiltration of mononuclear cells.

Spleen: The number of white cells seems to be increased in the area of white pulp. Suggestive of chronic/viral disease**.**

Serum sample : Revealed positive for Japanese's Encephalitis (ELISA).

Treatment and Control Measures

Advised for proper drying of grain before feeding. 2% Copper sulfate to be mixed in grain before feeding. Commercial toxinbinders,Varishta, Toxicurb @ 1kg/tone of grain for 15days along with Liver tonic, Immunomodulaters, Vitamin B complex.

Result and Discussion

Approximately 15-20% moldy corn infestated with *Penicillium spp* was being fed regularly to these herd.Intermittent but acute sudden death during post rainy season suggest the death due to fungal toxin.Histopathological changes in the tissue of lung, liver, kidney was similar to the findings of other workers. Histologically, a center of necrosis with no recognizable structure was observed. The transition between normal and necrotic tissue will often show hemorrhage, edema, congested blood vessels and neuronophagia. In animals with the hepatotoxic syndrome, livers will be swollen and a diffuse yellow-brown color. Irregular nodules and pale foci can be seen in hepatic parenchyma. (Dr. Steve Hooser, Dr. Duane Murphy 2003).

Pathogenicity of *Fusarium* revealed, that it causes hepatic congestion with mild triaditis, pulmonary congestion, and splenic lymphoid hyperplasia (Karki 2003). Furthermore, a positive response to treatment with toxin-binder, adaptogen, immunomdulater further confirm the sudden death was due to moldy corn poisoning. Further monitoring of finding of this investigation is suggested.

Conclusion

Findings of this investigation indicates that moldy feed grains and ingredients are infested with toxic fungus is emerging as a new health hazard for livestock and poultry. Simultonious use of toxin binder, adaptogen like livertonic,mineral mixture and Immunomodulaters drugs promises to help in minimizing health risk in livestock and poultry production should be looked into.

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