Perennial ryegrass staggers

Perennial ryegrass staggers has been recognised in New Zealand for more than 90 years. Gilruth, in 1906, described a neurological disorder in farm animals in which outbreaks were associated with perennial ryegrass dominant pasture[1]. Investigations by Cunningham and Hartley[2] confirmed that the disease was associated with the ingestion of perennial ryegrass pasture, usually dried up pasture with a small degree of slow growth. They suggested the disease was caused by a toxin in the grass. It was postulated as early as 1940[3] that an endophytic fungus may be involved. However, it was not until 1981, during a study of an outbreak of ryegrass staggers that occurred in a trial of ryegrass genotypes at Lincoln, that the disease was definitely associated with the presence of *Lolium* endophyte[4]. This finding was confirmed at Ruakura in 1982[5]. The fungus was identified as *Acremonium* (now *Neotyphodium*) *lolii*[6]. The main toxin involved appears to be the alkaloid lolitrem B[7][8].

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Taxonomy and biology

Within the tribe *Balensia* are numerous genera broadly classified according to the recognition of imperfect (asexual or anamorphic) or perfect (sexual) forms. The genus *Acremonium* is recognised as an anamorphic form of the endophyte *Epichloe*. Recently, on the basis of ribosomal DNA sequence analyses, it has been suggested[9] that anamorphic forms of *Epichloe* (some *Acremonium* spp including *A lolii*) should be reclassified as a new genus *Neotyphodium*.

The spread of *Neotyphodium* in ryegrass is vertical, with infection spread mainly by seed. After germination, the hyphae spread within the growing plant, with the highest concentration of hyphal elements found in the lower tillers and sheath parts. At seeding the hyphae grow up the stem and the embryonic seed becomes infected. The highest concentrations of lolitrem B are found in the base of the plant and in the seed. If seed is stored, the viability of the endophyte declines and the rate of decline is fastest in hot and humid conditions.

Aetiology

Endophytic fungi (*Neotyphodium* spp) are present in a range of grasses. They produce toxins that vary in action and type, and can be considered in two main groups: the vasoactive ergot alkaloids and the indole-diterpenoid tremorgens. The endophyte *N lolii*, which grows in perennial ryegrass (*Lolium perenne*), produces a range of indole-diterpenoid compounds; the most significant is lolitrem B - one of a number (>18)[10] of lolitrems. Structure-activity studies have shown that at least five of the indole-diterpenoids produced by *N lolii* are tremorgenic[10][11][12][13]. Lolitrem B, the most abundant lolitrem in endophyte-infected ryegrass, is thought to be the main tremorgen causing ryegrass staggers, but others may also contribute. Lolitrem B has been shown by parenteral administration to cause long lasting tremors in rats (Munday-Finch SC, pers comm), mice and sheep[13][14].

The ergot alkaloids, which are vasoactive, are also produced by the endophyte *N coenophialum* in tall fescue (*Festuca arundinacea*) and are responsible for the syndromes associated with fescue. In New Zealand, the commercial fescue cultivars are endophyte-free and only the wild type tall fescue creates a problem. Some ergot alkaloids are also produced by *N lolii* in ryegrass. The relative significance of the tremorgens and ergot alkaloids of endophyte-infected ryegrass in the aetiology and pathogenesis of ryegrass staggers and its symptomatology, is still uncertain. It is likely that a spectrum of symptomatology, between the uncomplicated neurological syndrome and those symptoms associated with the ergot alkaloids, will eventually be acknowledged.

The prevalence of staggers in sheep associates well with the presence or absence of endophyte in ryegrass[15] and correlates with the concentration of lolitrem B in the pasture being grazed[16].

Several tremorgens have also been shown to have effects that result from direct or indirect stimulation of smooth muscle. These include effects, both inhibitory and stimulatory, on the rumen, reticulum, pyloric antrum and duodenum[17][18], and increases in heart rate, respiratory rate and blood pressure. Blood pressure increases precede heart rate increases[19], suggesting a peripheral effect on the vascular smooth muscle. These effects on smooth muscle have been observed in vitro (McLeay LM, pers comm), and in the field[20] increased heat stress, reduced milk production and greater faecal smearing have been associated with endophytic infection of ryegrass. The relative importance of ergot alkaloids and tremorgens in these effects is still undetermined.

Clinical signs

Clinical signs usually appear in herbivores within 7 to 12 days of introducing stock to toxic pasture[21]. Fine head tremors and trembling of the muscles of the neck and limbs are the first signs to appear. As severity increases, mild postural incoordination, head nodding and swaying while standing develops. There is jerky, uncoordinated movement. Exercise exacerbates the signs and severely affected animals show hyperkinesia, a stiff-legged gait, may collapse and have marked muscular spasms. In the most severe cases nystagmus may occur. If left undisturbed the affected animals will recover and walk away.

Subclinical signs, which may result from the additional presence of ergot alkaloids in the *N lolii* infected pasture, include lowered daily weight gains and reduced hormonal levels: plasma testosterone in entire males[22] and serum prolactin levels in males and females[23]. In rams and bulls, ryegrass staggers causes profound reductions in sperm counts[22], even before clinical staggers are apparent.

Species affected and geographical distribution

Ryegrass staggers has been recorded in sheep[1], cattle[2], deer (red[24], fallow[25] and elk[24][26][27] (wapiti)), horses[28][29], swamp buffalo (*Bubalus bubalis*) (Julian AF, unpublished findings) and alpacas[30]. Experimentally tremors have been reproduced in mice, rats and sheep by parenteral administration.

The condition has been reported from New Zealand[1], Australia[25], North[31] and South America[32], South Africa[33] and Europe[28]. It should be considered as a possible cause of tremors or staggers in herbivores grazing perennial ryegrass (*L perenne*) anywhere in the world.

Pathology

There are no specific haematological or serum biochemical changes with ryegrass staggers. In the most severe cases there may be elevations in serum aspartate transaminase and creatine phosphokinase. No macroscopic changes are present in the nervous system.

Histology of brains has revealed lesions in Purkinje cell axons in cases of protracted ryegrass staggers. There are homogeneous, eosinophilic swellings of the axons ("torpedoes"). Purkinje cells may also exhibit chromatolysis. It has not been proven whether these changes are induced by the toxins directly or, as has been suggested, are the response to a number of factors including repeated anoxic insults[34].

Diagnosis

A diagnosis is made by detection of the clinical signs, in conjunction with the history of grazing perennial ryegrass pasture. Rapid recovery when animals are fed non-toxic feed helps support the diagnosis. Geographical, regional and local environmental conditions may also be important in the diagnosis.

The following need to be differentiated using history and clinical biochemistry: phalaris staggers, paspalum staggers, lead poisoning, hypomagnesaemia, annual ryegrass toxicity, mannosidosis and other storage diseases, fungal (penicillium and aspergillus) tremorgen staggers, drunken horse disease, dronk grass staggers, and other grass related diseases with tremors. Where appropriate, even transmissible spongiform encephalopathies might be considered.

Prognosis, treatment and control

The prognosis for animals affected with ryegrass staggers is generally good, once the affected animals have access to non-toxic feed. The only treatment is to remove the animals from the pasture known to cause the disease to safer pasture. This involves, in most circumstances, selecting endophyte-free or less ryegrass-dominant pastures, highly or totally leguminous (eg lucerne) pastures or hay. If no suitable feed is available, then selecting the pasture with the largest (but not seeding) growth may be the only option. Most ruminants will respond by improving within a few days. In the most severe cases in horses and elk, a permanent long-term persistence of staggers or peculiarity (unsteadiness) of gait has been observed.

To prevent the disease, avoid close grazing of endophyte-infected long-term or old ryegrass pastures. Select longer pastures for grazing, especially those with less dominance of perennial ryegrass. Stocking rates and rotation rates of grazing should also be adjusted, to avoid over-grazing and grazing of seeding pastures should also be avoided. Supplementation of tremorgenic ryegrass with other feeds might also help. The tremorgenicity of pastures is likely to persist in the hay or silage made from it.

Ryegrass staggers resistance in sheep is of high heritability. The formal identification of sires, by a challenge test or other means, is not yet available. However, the natural resistance and susceptibility of sires in field outbreaks should be noted and used in the selection of future sires. The agronomic advantages resulting from the insect and drought resistance conferred by the presence of the endophyte has complicated the management of this problem. Under New Zealand conditions, these advantages are perceived to outweigh the disadvantages of animal disease. In the meantime, attempts are being made to seek alternative solutions such as the incorporation of non-toxigenic endophytes that will still confer insect resistance (and hence persistence properties) to *L perenne*. Similar problems of grass persistence versus disease-causing potential accompany fescue toxicity, and international cooperative research, mainly between the USA and New Zealand, has been instituted.

Related conditions

The ingestion, by horses, of *Achnatherium inebrians* (drunken horse grass) in Asia causes the syndrome, drunken horse disease. High levels of ergonovine and lysergic acid amide and an endophyte similar to *N lolii* have been found in the grass[35].

A similar syndrome of intoxication of sheep occurs in Australia[36]. The grass (*Echinopogon ovatus*) associated with the disease has been shown to be infected with a *Neotyphodium* endophyte similar to that of ryegrass and fescue[37].

In South Africa, a staggers syndrome occurs in sheep grazing the grass *Melica decumbens* and indole-diterpenoid alkaloids other than lolitrem B have been found in the endophyte-infected grass[38].

In Argentina, *Poa huecu* is associated with a staggers syndrome and an endophyte and a non-lolitrem B tremorgen have been detected[39]. This syndrome has also been attributed to a toxic glycoprotein.

Assays for endophyte related toxins

The toxins associated with endophyte-infected plants may be detected by HPLC assay and lolitrem B assays are done by the Toxinology and Food Safety Research Group at AgResearch, Ruakura. Ergopeptide analyses, by HPLC, are done by AgResearch, Grasslands, Palmerston North. While relatively specific these methods require extraction and clean-up procedures, which do not readily lend themselves to routine analysis.

An important advance in endophyte research has been the development of sensitive immunoassays for the detection of the toxins. The direct and indirect competitive ELISA techniques used to assay these toxins can be either generic or specific in nature, require relatively little sample preparation and are very sensitive. Each test has a methodology developed specifically for the matrix involved (eg blood, serum, urine, grass) and they are ideally suited for screening, research or diagnostic purposes.

Detecting and characterising the endophyte

Endophytic hyphae may be detected in leaf material after staining with aniline blue or by immunochemical procedures. The cellular and molecular techniques involved in endophyte research have recently been reviewed[40]. This review includes immunoprobes for both the endophyte and the toxin and also gene probing procedures for characterising the genome.

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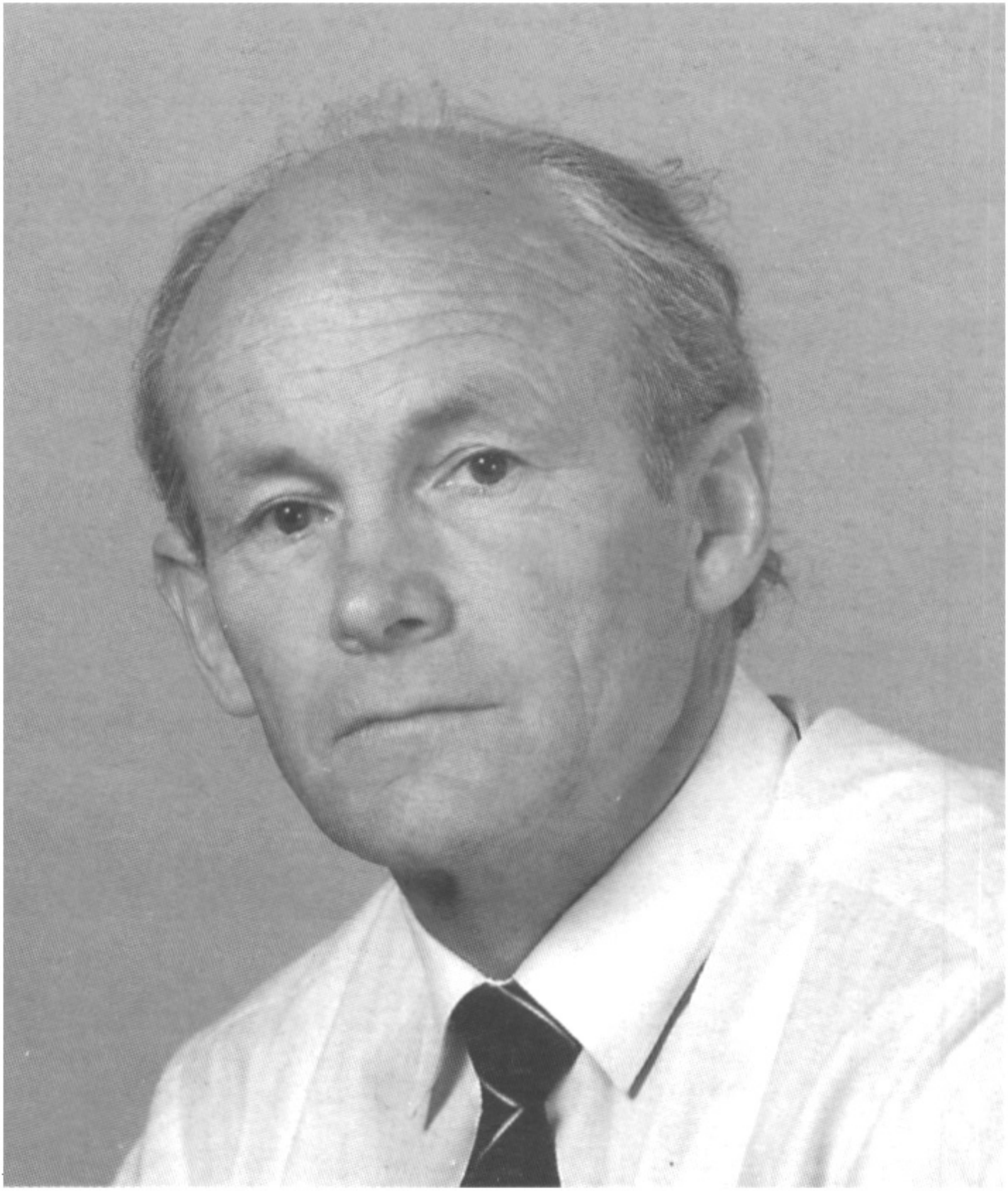

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