Annual ryegrass toxicity – an animal disease caused by toxins produced by a bacterial plant pathogen

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Annual ryegrass toxicity (ARGT) is an acute and often fatal neurological disease of livestock caused by consumption of annual ryegrass (*Lolium rigidum*) seed heads infected with the bacterium *Rathayibacter toxicus* (formerly *Clavibacter toxicus* and *Corynebacterium rathayi*). These toxic seed heads may be present in pasture, crop stubbles or provided feed (hay, grain, crop fines or pellets).

The disease occurs every year in the agricultural areas of Western and South Australia, and has been reported in South Africa. A disease considered clinically similar to annual ryegrass toxicity (ARGT) occurred in cattle grazing annual ryegrass and fescue in the Central Valley of California in 1996 and 1997[1].

The first deaths attributed to ARGT occurred in 1956 near Black Springs in the mid-north region of South Australia[2]. The first reported outbreaks in Western Australia occurred in 1970, although the first definite outbreak of the disease in this state was in 1968 in the Gnowangerup area[3]. Since those early outbreaks, the area in which the disease occurs in each state has grown considerably, particularly in Western Australia where it now occurs over an area of 10 million hectares[4], and the causative organisms have been detected over an even greater area[5].

When this disease occurs in livestock, it is the end result of a highly complex inter-relationship between three, and possibly four, organisms, and many outside influences including seasonal weather and paddock management.

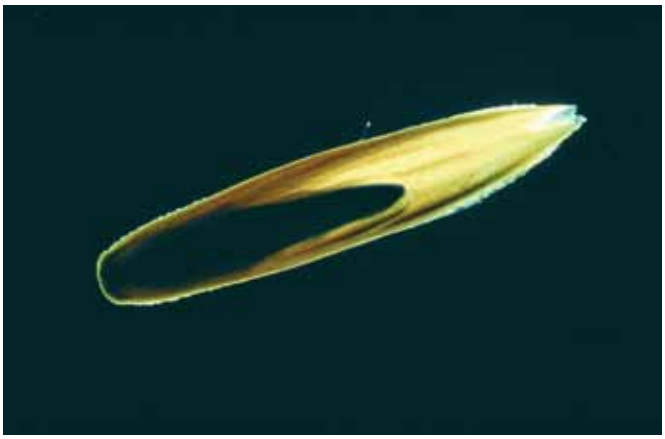
The toxigenic bacterium is normally soil-borne, and requires the seed-gall forming, parasitic nematode, *Anguina funesta*, to gain access to and infect the plant. This nematode reproduces in the seed head of ryegrass plants. During winter, the second stage larvae of *A. funesta* move over the soil in surface moisture and seek out newly germinated ryegrass plants. On locating host plants, the nematode larvae invade them and congregate at the vegetative apex. When the tillers elongate, the larvae are carried up with the growing point, and then move through the cavity formed by the emerging leaves to locate and penetrate a developing ovary within the forming inflorescence. Here they induce the formation of a gall, in place of a seed. Inside the gall the nematodes feed and moult three times to become adults, which mate, and the females produce hundreds of eggs. The larvae within the eggs moult once and hatch within the galls as second-stage larvae. The first larvae hatch as the first ryegrass heads reach flowering, and most eggs hatch over the next 10 days. The larvae feed and grow to produce a gall that is packed full of larvae that enter an anhydrobiotic state as the seed head matures and the ryegrass plant undergoes senescence. These galls are referred to as nematode galls, and the larvae survive the dry summer inside them. After the opening rains in the following winter, the wall of the nematode gall starts to break down, and eventually the second-stage larvae emerge to recommence the life cycle. The period taken for the wall of the gall to break down ensures that by the time the larvae emerge, new ryegrass plants will have germinated[6-8].

As the second stage larvae of *A. funesta* move over the soil surface, *Rathayibacter toxicus* organisms that they encounter become attached to the cuticle of the nematodes. In this way the bacterium is introduced to the galls and developing inflorescence. When introduced to a gall, the bacteria multiply rapidly soon after the gall is initiated, smother the larvae, and colonise the structure to form a bacterial gall. If the bacteria are deposited outside the galls but within the developing inflorescence, under suitable environmental conditions they can multiply to form a yellow slime that will become apparent on the surface of the plant at the time of head emergence[9]. The *R. toxicus* within bacterial galls produces the toxins that cause ARGT, called corynetoxins[10], during the very short period from the end of flowering, through

Normal ryegrass seed (left) and nematode (centre) and bacterial galls (right) as viewed on a light box.

Normal ryegrass seed.

Bacterial gall.



Nematode gall.

seed set, to maturity of the seeds. No further toxin production occurs after the seeds have matured[11]. It has been suggested that the bacterium only produces the corynetoxins when infected by a bacteriophage[11], but recent studies have not supported this[12], and further research is needed to establish the true situation. The bacterial galls remain toxic throughout the following summer and autumn, and if kept dry and protected from the weather can remain toxic for years[1]. Bacterial galls in paddocks at the start of winter break down as do the nematode galls, and release the bacteria onto the soil. These organisms are dispersed by rain droplets and moving surface water, and may encounter the free-living, second-stage larvae of *A. funesta*.

Corynetoxins, extracts of toxic seed heads and toxic ryegrass have proven to be lethal to all animal species exposed naturally, or tested, including sheep, cattle, horses, donkeys, pigs, guinea pigs, rats, mice and chickens[13]. They are cumulative toxins, so the total lethal dose is the same whether given as a single dose or as repeated, smaller doses up to two months apart[14].

Most cases of ARGT in grazing livestock occur between mid-October and mid-December (late spring to early summer), after the seed heads have developed maximum toxicity and while the pastures are undergoing senescence. Reasons for the reduced prevalence of the disease after this period are not known. It may be due to livestock changing their selection of diet after the pastures have senesced, or due to some loss of the bacterial galls onto the ground where they are less available for consumption. Later in summer, and in autumn, cases may occur in association with the introduction of livestock into crop stubbles containing toxic ryegrass, or following occurrences of summer rainfall. ARGT can occur at any time of the year in livestock being fed contaminated hay or grain[15].

Clinical signs in livestock appear abruptly, usually following some external stimulation. They include tremors, ataxia, adoption of a wide-based stance, stumbling and falling over, nystagmus, convulsions while recumbent and death. Animals may appear to recover between episodes of ataxia and convulsions. Sheep often exhibit a high stepping gait with their forelimbs, while the head is held high. When forced to run, some sheep have a stiff-legged or "rocking horse" gait. Some cattle appear disorientated and wander aimlessly between episodes of convulsions. Signs may appear as soon as four days after introduction to toxic pasture or feed. Animals may continue to exhibit neurological signs and die for up to 10 days after removal from toxic feed. Ewes may abort[13,16,17].

There is no satisfactory treatment for ARGT. Animals should be removed from the source of toxic feed immediately, and

provided good-quality feed and water in a shady location where they are unlikely to be disturbed. If possible, affected animals should be separated from unaffected animals.

Testing pasture for the presence of the causative organisms has been promoted for decades as a way to avoid ARGT from occurring. Feed can be physically examined for the presence of ryegrass seeds, and nematode and bacterial galls identified using a light box[18], but the test currently offered by diagnostic laboratories is an enzyme-linked immunosorbent assay (ELISA) that specifically detects and quantifies the presence of *R. toxicus* in feeds[19]. It can also be used to detect the presence of the organism in rumen contents and faeces[20]. An ELISA that detects and quantifies the corynetoxins in feed has also been developed[21], but it is not as quick and is more costly than the ELISA for *R. toxicus*, and studies have shown a good correlation between the results from both ELISAs (Masters, A.M., unpublished data). Therefore, the ELISA for the bacterium is still the preferred test. Relative risks of the feed causing ARGT are provided on the basis of the quantities of the bacterium detected, which is reported in terms of bacterial galls/kg. The validity of the result and advice offered is heavily dependent on the suitability of the sample submitted. For pasture or stubble, a recommended sampling strategy for paddocks up to 40 ha is to walk a "W" across the paddock, stopping to collect 3–5 seed heads at each of at least 100 locations along the "W". All the collected seed heads can be combined into a single sample for testing. If the ryegrass is unevenly distributed in the paddock, then more collections should be made in the areas of greater ryegrass density. If the paddock is greater than 40 ha it should be subdivided, and samples submitted for each subdivision[15].

There is a definite protocol for the testing of hay. This has been developed for the export industry, and since it was introduced there have been no occurrences of ARGT in livestock overseas fed hay from Australia. In the first instance, 15% of bales in a paddock should be sampled for testing[22]. People purchasing hay for local consumption should require a vendor declaration stating that the hay has been tested and is safe.

The main methods of preventing ARGT aim to reduce ryegrass by controlling it in cropping years, and to stop development of ryegrass in pasture before flowering commences. Both can be achieved by the strategic use of herbicides, or other management strategies. With the emergence of herbicide resistance in ryegrass populations, use of other management strategies will become increasingly important. These include very heavy stocking during winter and spring, cutting pasture for silage, hay or green manure at the booting stage, and collecting and burning crop residues. When pasture is cut, it should be heavily grazed to stop the regrowth of ryegrass that may become toxic. During the high-risk period, livestock on ryegrass pasture should be inspected daily and removed from the paddock immediately clinical signs are seen[1].

Two cultivars of *Lolium rigidum* that are resistant to *A. funesta* have been bred and released. These are Guard and Safeguard, with the latter being an earlier flowering cultivar. When sown according to recommendations they have the potential to significantly reduce the risk of ARGT[23]. In one study conducted in Western Australia the bacterial gall count in pasture was reduced from 10,361 galls/kg to 4 galls/kg in one year by planting Safeguard[24].

The twist fungus, *Dilophospora alopecuri,* is also used to control the causative organisms of ARGT. Spores of this fungus in the soil adhere to the second-stage larvae of *A. funesta* and are carried onto the ryegrass plant where they germinate, and the fungus colonises the inflorescence. In doing this the fungus substantially reduces the number of nematode and bacterial galls that are produced in the seed head, thus greatly reducing the risk of ARGT[25]. *D. alopecuri* is being distributed throughout regions of Western Australia where ARGT is a problem[26].

ARGT is not the only corynetoxicosis to occur in Australia and overseas. Similar diseases are Stewarts Range syndrome (annual beard grass [*Polypogon monspeliensis*], the nematode *Anguina paludicola*, *R. toxicus* and the corynetoxins) in south eastern South Australia and flood plain staggers (blown grass [*Lachnagrostis filiformis*, formerly *Agrostis avenacea*], *Anguina paludicola*, *R. toxicus* and the corynetoxins) in northern New South Wales[27,28]. Furthermore, a similar disease involving the association of a nematode and a grass (*Festuca nigrescens*, Chewings fescue) occurred in Oregon up to about 1960, but it has not been seen since[17]. Recent examination of stored specimens suggests that corynetoxin-like toxins produced by a variant of *R. toxicus* caused this disease[29]. Finally, a similar disease, apparently caused by corynetoxin-like toxins, occurred in pigs fed rain-damaged, mouldy wheat in New South Wales. The source of the toxins was not determined[30].

References

1. Allen, J.G. (2004) Annual ryegrass toxicity. In *Clinical Veterinary Toxicology* (Plumlee, K.H., ed), pp. 422–424, Mosby.
2. McKay, A.C. *et al*. (1985) Are farmers controlling annual ryegrass toxicity in South Australia? In *Plant Toxicology* (Seawright, A.A., Hegarty, M.P., James, L.F. and Keeler, R.F., eds), pp. 559–568, Queensland Poisonous Plants Committee.
3. Gwynn, R. and Hadlow, A.J. (1971) Toxicity syndrome in sheep grazing Wimmera ryegrass in Western Australia. *Aust. Vet. J.* 47, 408.
4. Roberts, W.D. *et al.* (1994) The distribution of annual ryegrass toxicity in Western Australia. In *Plant-Associated Toxins: Agricultural, Phytochemical and Ecological Aspects* (Colegate, S.M. and Dorling, P.R., eds), pp. 51–56, CAB International.
5. Kessell, D. (2007) Annual ryegrass toxicity; current situation. *Farmnote 259*, Department of Agriculture and Food Western Australia.
6. McKay, A.C. *et al*. (1981) Ecological field studies on *Anguina funesta*, the vector in annual ryegrass toxicity. *Aust. J. Agric. Res*. 32, 917–926.
7. Price, P.C. *et al*. (1979) Annual ryegrass toxicity; parasitism of *Lolium rigidum* by a seed-gall forming nematode (*Anguina* sp.). *Ann. Appl. Biol*. 91, 359–369.
8. Stynes, B.A. and Bird, A.F. (1982) Development of galls induced in *Lolium rigidum* by *Anguina agrostis*. *Phytopath*. 72, 336–346.
9. Price, P.C. *et al*. (1979) On *Anguina funesta* n. sp. and its association with *Corynebacterium* sp. in infecting *Lolium rigidum*. *Nematologica* 25, 76–85.
10. Vogel, P. *et al*. (1981) Isolation of a group of glycolipid toxins from seedheads of annual ryegrass (*Lolium rigidum* Gaud.) infected by *Corynebacterium rathayi*. *Aust. J. Expt. Biol. Med. Sci*. 59, 455–467.

11. Stynes, B.A. and Bird A.F. (1983) Development of annual ryegrass toxicity. *Aust. J. Agric. Res*. 34, 653–660.
12. Kowalski, M.C. *et al*. (2007) Development and application of polymerase chain reaction-based assays for *Rathayibacter toxicus* and a bacteriophage associated with annual ryegrass (*Lolium rigidum*) toxicity. *Aust. J. Exp. Agric*. 47, 177–183.
13. Purcell, D.A. (1985) Annual ryegrass toxicity: a review. In *Plant Toxicology* (Seawright, A.A., Hegarty, M.P., James, L.F. and Keeler, R.F., eds), pp. 553-558, Queensland Poisonous Plants Committee.
14. Jago, M.V. and Culvenor, C.C.J. (1987) Tunicamycin and corynetoxin poisoning in sheep. *Aust. Vet. J.* 64, 232–235.
15. Allen, J.G. (2002) Annual ryegrass toxicity. *Proceedings of ARGT Workshop*, El Caballo, Western Australia, pp. 1–11, Department of Agriculture Western Australia.
16. Bourke, C.A. (1994) Tunicaminyluracil toxicity, an emerging problem in livestock fed grass or cereal products. In *Plant-Associated Toxins: Agricultural, Phytochemical and Ecological Aspects* (Colegate, S.M. and Dorling, P.R., eds), pp. 399–404, CAB International.
17. Cheeke, P.R. (1998) *Natural Toxicants in Feeds, Forages, and Poisonous Plants*, Second Edition, pp. 260–263, Interstate Publishers Inc.
18. Bird, A.F. *et al*. (1980) A comparison of nematode; bacteria-colonised galls induced by *Anguina agrostis* in *Lolium rigidum*. *Phytopath*. 70, 1104–1109.
19. Masters, A.M. *et al*. (2011) Improvements to the immunoassay for detection of *Rathayibacter toxicus* in hay. *Crop Past. Sci*. 62, 523–530.
20. Allen, J.G. and Gregory, A.R. (2011) The diagnostic significance of detecting *Rathayibacter toxicus* in the rumen contents and faeces of sheep that may be affected by annual ryegrass toxicity. In *Poisoning by Plants, Mycotoxins and Related Toxins* (Riet-Correa, F., Pfister, J., Schild, A.L. and Wierenga, T., eds), pp. 325–330, CAB International.
21. Than, K.A. et al. (1998) Development of an immunoassay for corynetoxins. In *Toxic Plants and Other Natural Toxins* (Garland, T. and Barr, A.C., eds), pp. 49-54, CAB International.
22. Anonymous (2002) Code of practice for minimisation of the risk of corynetoxins contamination of hay and straw for export. Department of Agriculture, Fisheries and Forestry Australia.
23. Coles, D.J. (2002) Safeguard ryegrass. *Proceedings of ARGT Workshop*, El Caballo, Western Australia, pp. 87-95, Department of Agriculture Western Australia.
24. Allen, J.G. (2008) Evaluation and further development of biocontrol for the ARGT organisms. Final Report for Grains Research and Development Corporation Project Number DAW699, pp. 153–154.
25. Barbetti, M.J. and Riley, I.T. (2006) Field application of *Dilophospora alopecuri* to manage annual ryegrass toxicity caused by *Rathayibacter toxicus*. *Plant Dis*. 90, 229–232.
26. Yan, G. *et al*. (2010) Twist fungus reduces the risk of annual ryegrass toxicity. *Farmnote 416*, Department of Agriculture and Food Western Australia.
27. Edgar, J.A. *et al*. (1994) Identification of corynetoxins as the cause of poisoning associated with annual beardgrass (*Polypogon monspeliensis* (L) Desf.) and blown grass (*Agrostis avenacea* C. Gemelin). In *Plant-Associated Toxins: Agricultural, Phytochemical and Ecological Aspects* (Colegate, S.M. and Dorling, P.R., eds), pp. 393–398, CAB International.
28. Bertozzi, T. and Davies, K.A. (2009) *Anguina paludicola* sp. n. (Tylenchida: Anguinidae): the nematode associated with *Rathayibacter toxicus* infection in *Polypogon monspeliensis* and *Lachnagrostis filiformis* in Australia. *Zootaxa* 2060, 33–46.
29. Riley, I.T. *et al*. (2003) Poisoning of livestock in Oregon in the 1940s to 1960s attributed to corynetoxins produced by *Rathayibacter* in nematode galls in Chewings fescue (*Festuca nigrescens*). *Vet. Hum. Toxicol*. 45, 160–162.
30. Cockrum, P.A. *et al*. (1992) Identification of tunicaminyluracil antibiotics in water-damaged wheat responsible for the death of pigs. In *Poisonous Plants, Proceedings of the Third International Symposium* (James, L.F., Keeler, R.F., Bailey, E.M. Jr., Cheeke, P.R. and Hegarty, M.P., eds), pp. 611–615, Iowa State University Press.

Biography

Dr Jeremy Allen is the Principal Veterinary Toxicologist in the Department of Agriculture and Food Western Australia, and Group Leader of the Pathology and Biochemistry Sections of the Animal Health Laboratories. He has spent most of his career researching the pathology, toxicology and epidemiology of plant poisonings in livestock, with a particular emphasis on lupinosis and annual ryegrass toxicity.