5: Bacterial toxins (ingested pre-formed) [Biological-origin toxins]

💣☑ *Corynetoxins (tunicaminyl-uracils)*

Core data

Syndromes:
- annual ryegrass toxicity (ARGT)
- flood-plain staggers
- Stewart's Range syndrome

Common sources:
- bacteriophage-infected *Rathayibacter toxicus*
- in nematode-induced seedhead galls
- on pasture grasses
  - *Lolium rigidum* (→ ARGT)
  - *Agrostis avenacea* (→ flood-plain staggers)
  - *Polypogon monspeliensis* (→ Stewart's Range syndrome)

Animals affected: sheep, cattle, horses

Mode of action:
- inhibit N-glycosylation of proteins
- impair cardiovascular function & vascular integrity

Poisoning circumstances: infected mature seedheads on standing pasture

Main effects:
- intermittent cerebral convulsions, commonly → death
- depressed wool production

Diagnosis:
- convulsive syndrome + high mortality + grass-dominant pasture carrying seed
- histopathology (brain, liver)
- *R. toxicus* antigen-capture ELISA for pasture, fodder, rumen contents and faeces
- microscopic pasture examination for seedhead galls
- pasture corynetoxin assay

Therapy/Management:
- gentle movement of flock/herd to safe paddock
- CSIRO cyclodextrin antidote under development

Prevention:
- monitor pasture for *R. toxicus*
- regular frequent inspection of at-risk flocks
- grazing management, mowing, burning, herbicides
- immunisation under development by CSIRO

The first record of toxicity from what were subsequently identified as corynetoxins was made in the mid-north of South Australia in 1955-57 where sheep and cattle were affected after grazing mature Lolium rigidum (Wimmera ryegrass) pastures, the seedheads of which were known to be parasitised by the nematode Anguina agrostis along with bacteria of three colony types, two of which were then identified as Pseudomonas fluorescens (McIntosh et al. 1967). A similar disease had been previously reported from North America as associated with nematode-infected seeds of Chewing's fescue (Festuca nigrescens) (Haag 1945, Shaw & Muth 1949, Galloway 1961), but has not been further reported or characterised. The corynetoxins were first isolated from seedhead gall material collected in Western Australia in 1979-80 (Vogel et al. 1981) and characterised as a previously-unknown mixture of tunicaminyl-uracil antibiotics in 1981 (Edgar et al. 1982).

Syndrome names:

- **Annual ryegrass toxicity** (ARGT) - WA, SA, V, NSW
- Flood-plain staggers - north-western NSW
- Stewart's Range syndrome - south-eastern SA

Chemical structure:

Corynetoxins are tunicaminyl-uracils, a subclass of nucleoside antibiotics which also includes tunicamycins and streptovirudins (Eckardt 1983). Corynetoxins were named after *Corynebacterium rathayi*, now classified as *Rathayibacter toxicus* [previously *Clavibacter toxicus* (Riley & Ophel 1992)]. Corynetoxins differ from the other tunicaminyl-uracils in the structure of the fatty acids linked to the amino group of the central tunicamine unit of the molecule (Frahn *et al.* 1984).

Sources:

- produced by **bacteriophage-infected *Rathayibacter toxicus*** bacteria infecting **seedhead galls** initiated by *Anguina agrostis* [= *Anguina funesta*] **nematodes** in pasture grasses (Riley & Gooden 1991, Riley & Orphen 1992) [bacteriophage = virus parasitic on bacteria]
- host grasses associated with toxicity:
  - ***Lolium rigidum*** (annual ryegrass) → Annual ryegrass toxicity (ARGT)
    - normal ryegrass seedheads have round-topped seeds which are almost as wide as the husks; infected seedheads have galls half as wide as the husk and with a pointed top in place of the seed. Bright yellow galls carry most toxin
    - proliferating bacteria may form a yellow slime or yellow-orange crust on seedheads, but this is easily washed off by rain
  - *Agrostis avenacea* (blown grass; blow-away grass) → Flood-plain staggers (Bourke *et al.* 1992, McKay *et al.* 1993, Davis *et al.* 1995)
  - *Polypogon monspeliensis* (annual beard grass) → Stewart's Range syndrome (Finnie 1991, McKay *et al.* 1993)
- water-damaged wheat has caused the syndrome in pigs on one occasion (Bourke 1987; Cockrum *et al.* 1989)

Lolium rigidum (annual ryegrass) inflorescence. [RAM Photo]

Toxicity:
- **sheep**, cattle
- horses (Creeper *et al.* 1996, 2000)
- high mortality (compared with perennial ryegrass staggers)
- toxic doses 3-5 mg/kg

Mode of action:
- tunicaminyl-uracils
  - **cumulative**
  - inhibit N-glycosylation of proteins (production of glycoproteins) and thus cell membrane synthesis
  - impair cardiovascular function & vascular integrity
  - **→ interfere with peripheral circulation & oxygen distribution**
  - brain most sensitive

Conditions of poisoning:
- infected mature seedheads on **standing pasture**
- most cases on pasture are in November-January, some as late as April
- signs first commonly occur 2-6 days, but may be up to12 weeks, after infected pasture access
- forced exercise, high temperatures → precipitate/exacerbate signs

- Co-deficient sheep are more susceptible to intoxication (Davies *et al.* 1993, 1995)
- oaten **hay** contaminated with galled *Lolium rigidum* retains toxicity (Nogawa *et al.* 1997), probably for the life of the hay (several years) (Edgar 1998)
- **feed grain** (wheat, barley, canola) contaminated with infected *Lolium rigidum* seed galls and the residue from cleaning such contaminated grain may be toxic (Edgar 1998). The increasing herbicide resistance of *Lolium rigidum* in cropping areas is exacerbating this problem.

Clinical signs:

Acute poisoning:

- **intermittent cerebral convulsions, commonly → death**
  - ataxia (high-stepping gait; "rocking-horse" gait)
  - muscle tremor
  - head nodding/swaying
  - collapse
  - nystagmus
  - neck ventroflexion
  - opisthotonus/tetanic spasms
  - limb paddling
- affected animals left undisturbed often regain their feet and return to apparent normality
- deaths may occur within a few hours or up to 8 days after onset of signs (including after removal from infected paddock)

Chronic effects: (Stewart 1998)

- sublethal doses can **depress wool growth and fibre diameter** (Davies *et al.* 1996)
- sublethal doses of tunicamycin cause testicular atrophy in rats, but not in rams (Stewart *et al.* 1998a)

Pathology:

- ± ↑ serum liver-associated enzymes
- perivascular oedema in brain
- hepatocyte vacuolation

Diagnosis:

- **convulsive syndrome + high mortality + grass-dominant pasture carrying seed**
- histopathology (brain, liver)
- antigen-capture ELISA for quantitative detection of *R. toxicus* in pasture and fodder samples and in rumen contents and faeces of fatally-affected animals (test kit to be marketed commercially throughout Australia under license from Agriculture Western Australia)
- seedhead galls difficult to identify →
  - 50 g seed threshed from pasture → lab microscopic examination
- corynetoxin detection → HPLC, Fast Atom Bombardment MS, immunoassay (Than *et al.* 1998)

Therapy/Management:

- careful removal from pasture, minimising stress
- anaesthetics/tranquillisers → suppress convulsions, but the need for repeat treatment and supportive measures for tranquillised animals make general use of these measures impractical.
  - magnesium sulphate IM @ 200 mg/kg → prevents convulsions for 12 hr. Repeat treatment as required. (Richards 1982)
  - chlordiazepoxide (Librium®; a benzodiazepine) PO @ 25 mg/kg → prevents convulsions, but sedates sheep for up to 14 days (Richards *et al.* 1979; Norris *et al.* 1981)
- cyclodextrin toxin-binding agent - CSIRO antidote (commercial product undergoing development) (May & Stewart 1998; Stewart *et al.* 1998b): cyclic glucose molecules, water-soluble and configured with a central cavity which is hydrophobic. Molecules which are relatively hydrophobic or with a hydrophobic structural feature (fatty acid side chain) partition into the cavity. The combination markedly increases the water solubility of the captured molecule. The current antidote formulation is hydroxypropyl β-cyclodextrin + magnesium gluconate (gelling agent) + magnesium sulphate given IP. Field trials have proved effective.

CSIRO ARGT antidote treatment regimen (under development)

Early in a poisoning incident, move affected flock (herd) to an area where they can be treated, provided with water and retained overnight after the first treatment. Re-treat the next day and move to a safe pasture.

A less-ideal regimen is to treat once and move the flock to a safe pasture immediately.

Prevention & control:

- monitoring for pasture *Rathayibacter toxicus* infection
- grazing management
    - graze annual ryegrass before it "hays off" (matures)
    - regular frequent inspection (twice-daily or daily) for clinical signs of flocks on potentially-toxic pasture
    - paddocks cut for hay less than 5 years previously are rarely toxic and may be used as "safe" pasture refuges
    - avoid suddenly increased stocking rates that may force consumption of infected seedheads previously avoided
- pasture management
    - mowing, burning, herbicides to remove seedheads or destroy toxic pasture
        - herbicide resistance has developed in *Lolium rigidum*, hindering control methods (Gill 1995)
    - encourage legume growth in the pasture
    - prevent spread to uninfected ryegrass
    - generally takes 10-15 years from introduction of infected nematodes to onset of stock poisoning
    - prevent spread through uncleaned seed, hay, uncleaned vehicles & machinery
- immunisation of sheep
    - CSIRO experimental immunogen is under evaluation by Agriculture WA; early results are promising (Tham *et al.* 1998b)
- cobalt supplementation (up to 4 mg per day) delays, but does not prevent, onset of clinical signs (Davies *et al.* 1993, 1995)
- biological control strategies considered
    - nematode-resistant ryegrass
    - *Dilophospora alopecuri* fungal pathogen of nematode
    - non-toxigenic strains of *R. toxicus* → compete with toxic strains
    - strains of *Anguina* not capable of carrying *R. toxicus* → compete with bacterial-carrying strains

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Se73

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*Tetrodotoxin (*q.v.*)*

Botulinum toxins