Ryegrass Staggers in Japan Induced by Consumption of Ryegrass Straw Imported from America

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Abstract

From 1997 to 1999, we experienced 29 cases of disorders in cattle and horses that had been fed ryegrass straw imported from the USA. These animals showed symptoms resembling ryegrass staggers and the clinical signs disappeared after removal of the straw. To clarify the cause of these cases, we examined the suspected straw for the presence of endophytic fungi and toxins. Endophytic hyphae were detected in the seeds of all straw samples that were responsible for the clinical cases. Lolitrem B concentrations in the straw ranged between 972 and 3740 ppb. Ergovaline concentrations were between 355 and 1300 ppb. It is known that *N. lolii*-infected perennial ryegrass contains both lolitrem B and ergovaline. Our result was comparable with the observations of previous reports. Turner et al. (1995) proposed the threshold levels of lolitrem B for toxicity as 1800–2000 ppb in the total diet. Even though the concentrations of lolitrem B were lower than this proposed threshold in almost all clinical cases, we diagnosed the episodes as ryegrass staggers from the clinical signs and other epidemiological findings. Namely, neurologic disorders were diminished by deprivation of the suspected straw. Vitamin B1, calcium or magnesium deficiency, white muscle disease and nitrate poisoning were ruled out by the clinical examinations. Furthermore, experimental feeding of the straw produced typical neurologic signs.

Key words: ryegrass stagger, cattle, horse, lolitrem B, ergovaline.

Toxicoses in livestock that have grazed on *Neotyphodium* (formerly *Acremonium*) - infected grasses are well known as ryegrass staggers and fescue toxicosis¹⁾. Indole isoprenoid alkaloid lolitrems, primarily lolitrem B²⁾, found in *N. lolii*-infected perennial ryegrass (*Lolium perenne*) have been implicated as the causative toxin of ryegrass staggers³⁾. Lolitrems are neurotoxic and induce a nervous disorder of cattle⁴⁾, sheep⁵⁾ and horses⁶⁾. As for fescue toxicosis, ergot alkaloids, primarily ergopeptine alkaloid ergovaline⁷⁾, detected in *N. coenophialum*-infected tall fescue (*Festuca arundinacea*) have been shown to have pharmacological effects^{8,9)}. The toxic effects include poor weight gain, low milk production, poor reproduction, necrosis of the peritoneal fat tissue, depressed immune response and direct endocrine effects¹⁰⁾.

From 1997 to 1999, we experienced 29 cases of disorders in cattle and horses fed imported ryegrass straw. The place of origin of the straw was Oregon, USA, when the origin was

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indicated. The affected animals showed symptoms resembling ryegrass staggers. To clarify the cause of these cases, we examined the suspected straw for the presence of endophytic fungi by a microscopic method. Lolitrem B and ergovaline concentrations in the straw were determined by using high-performance liquid chromatography. Several samples of imported ryegrass straw that were associated with no clinical disorder were also examined to confirm the degree of contamination with endophyte toxins.

Among the 29 cases that we examined, 26 cases were disorders of cattle (Table 1). The cattle's main diet was imported ryegrass straw. Low nutritional feed such as ryegrass straw is not used for fattening cattle or milking cows. Ryegrass straw is used mainly as a fibrous feed for calves, growing cattle and breeding cows of Japanese black cattle, a typical breed for beef. Therefore, all the cattle cases we experienced were disorders of the cattle in those stages. In most of the clinical cases, the affected animals were under full feeding of ryegrass

Table 1 Summary of outbreaks of ryegrass staggers in Japan.

Case No.	Location	Time of outbreak	Number of cattle			LB ¹⁾ concentration	EV ²⁾ concentration	Remarks
			at risk	affected	dead			
1	Kagoshima	Feb-97	150	41	0	1250	791	cattle
2	Gifu	Aug-97	84	22	0	1460	781	cattle
3	Tokushima	Sep-97	30	15	0	1440	1100	cattle
4	Hyogo	Oct-97	200	3	1	1400	1300	cattle
5	Kagoshima	Oct-97	50	7	0	1080	534	cattle
6	Kagoshima	Oct-97	35	3	0	2320	920	cattle
7	Kagoshima	Oct-97	12	4	0	2580	680	cattle
8	Miyagi	Nov-97	240	6	0	1230	513	cattle
9	Okinawa	Nov-97	2950	11	0	3200	875	cattle
10	Yamagata	Dec-97	30	1	0	3740	1070	cattle
11	Yamagata	Dec-97	75	3	0	3640	368	cattle
12	Okinawa	Mar-98	38	4	1	1829	557	cattle
13	Okinawa	Mar-98	300	4	1	1068	355	cattle
14	Hyogo	Apr-98	4	3	0	2645	1236	cattle
15	Kagoshima	Apr-98	38	4	0	1961	693	cattle
16	Kagoshima	Apr-98	3	1	0	2684	1198	cattle
17	Kagoshima	Apr-98	5	5	0	2265	1101	cattle
18	Okinawa	Apr-98	330	1	0	1174	565	cattle
19	Miyazaki	Apr-98	4	4	0	2220	1172	horse
20	Okinawa	May-98	32	4	0	2620	910	cattle
21	Kagoshima	May-98	50	1	0	1160	500	cattle
22	Saga	May-98	10	2	1	2370	1040	cattle
23	Miyazaki	Jun-98	3	1	0	972	524	horse
24	Okinawa	Sep-98	150	3	0	1406	498	cattle
25	Okinawa	Oct-98	250	2	0	2133	941	cattle
26	Okinawa	Nov-98	5	2	0	2531	680	horse
27	Oita	Mar-99	35	2	0	1699	702	cattle
28	Hiroshima	Mar-99	10	3	0	1861	798	cattle
29	Kyoto	Apr-99	101	9	0	2535	ND ³⁾	cattle

1): Lolitrem B concentration expressed as ppb.

2): Ergovaline concentration expressed as ppb.

3): Not determined.

straw with a small amount of concentrated feeds. The remaining three cases were of horses. They also had been eating straw freely.

In the mild cases, the affected animals showed head tremors, hyperesthesia and a staggering gait. Severely affected animals showed tremors of the general musculature, falling down after staggering, and tetanic spasms (Fig. 1). Serum AST, CK and LDH activities of severely affected animals were higher than those of normal animals^{11,12)} (Table

Fig. 1 Affected cow falling down with ankylosis of the legs (case 9, photo by Dr. H. Koshimoto).

Table 2 Changes in serum enzyme activities in affected cattle.

A) Case 9¹⁾

Cattle	AST(IU/I)	LDH(IU/L)	CK(IU/I)
Affected (n=6)	272**	3610*	3720
Not affected (n=6)	55	1570	128

B) Case 1,5,15,16 and 17²⁾

Cattle	AST(IU/I)	LDH(IU/L)	CK(IU/I)
Affected			
Mild (n=9)	73.3	2090	381.0
Severe (n=12)	165.9	2210	853.5
Control (n=7)	66.5	1710	152.1

1): From reference 11.

2): From reference 12.

2). These clinical signs were consistent with those of a previous report¹³⁾. The affected animals recovered within 10 days after deprivation of ryegrass straw. In cases 2 and 8, experimental feeding of the straw was performed for the aid of diagnosis. The straw that cases 2 and 9 had been feeding on was used for experimental feeding of other cattle. These animals presented the same clinical signs that were observed in the field.

Endophyte infection of the suspected straw samples was inspected with a microscopic method. Seeds in the straw were collected and stained with rose bengal to detect the endophytic hyphae¹⁴⁾. Sparingly branched hyphae seen between the aleurone cells were identified as endophytic fungi (Fig. 2). The endophytic hyphae were detected in the seeds of all straw samples that were responsible for the clinical disorders.

Lolitrein B concentrations in the straw were determined by the method of Gallagher et al.¹⁵⁾, slightly modified. We used dichloromethane : acetonitrile : water (85 : 15 : 0.2) as the mobile phase to obtain a good separation of lolitrein B. Ergovaline analyses were performed according to the method of Shelby and Flieger¹⁶⁾ with a 4 mm × 150 mm STR-ODS column (Shimadzu, Japan) and methanol : water : ammonium hydroxide (62 : 38 : 0.04) for the mobile phase. Both lolitrein B and ergovaline were detected in ryegrass samples associated with clinical disorders. Lolitrein B concentrations ranged between 972 ppb and 3740 ppb and ergovaline ranged between 355 ppb and 1300 ppb (Table 1). It is known that *N. lolii*-infected perennial ryegrass contains both lolitrein B and ergovaline. Our results were comparable with a observation of previous report¹⁷⁾.

The threshold levels of lolitrein B for toxicity were reported as 1800-2000 ppb in the total diet¹⁸⁾. In all cases that we examined, other feeds such as formula feeds were fed with the straw. Therefore, the final concentrations of lolitrein B in the total diets should have been lower than the values shown in Table 1. Even though the concentrations of lolitrein B were

Fig. 2 Endophytic hyphae detected in a ryegrass seed. (case 17).

lower than the proposed threshold in almost all clinical cases, endophyte-infected perennial ryegrass straw was suspected as the cause of the disorders from the clinical signs and epidemiological findings. Namely, ryegrass staggers-like disorders were diminished by the deprivation of the suspected straw. Vitamin B₁, calcium or magnesium deficiency and nitrate poisoning were ruled out by the clinical examinations. Furthermore, the experimental feeding of the suspected straw reproduced the disorders found in the field. The sensitivity of the cattle to the endophyte toxins might be due to differences between strains, ages, sexes or other factors. Sensitive individuals might be affected with lolitrem B less than the threshold level proposed by the Oregon State University¹⁸.

Ryegrass or fescue straw is a waste product of turf-type grass seed production in the US and has been exported to Japan as a cheap feed. A fungal endophyte has been introduced into turf-type perennial ryegrass and tall fescue to enhance plant hardiness, pest resistance, and drought tolerance. We have been importing 300,000 tons of straw every year from America. Therefore, it was expected that most of the imported straw contains endophyte toxins to some degree. We also examined imported straw samples collected from farms with cattle that showed no disorders. All of 9 samples examined were endophyte-infected. Although endophyte toxins were detected in these samples, the concentrations of the toxins were lower than those found in the clinical cases (270–1220 ppb of lolitrem B).

As an interim measure for the prevention of the disorders, import companies have been requesting information on the concentration of the toxins. These analyses are being done by Dr. A. M. Craig of Oregon State University. These companies are importing ryegrass straw only when the concentrations of lolitrem B and ergovaline are lower than the thresholds proposed by Oregon State University.

Another endophyte problem of interest is the public health significance of the endophyte toxins. There have been many studies on the pharmacokinetics of the ergot alkaloids in which non-ruminant experimental animals were used. Ergotamine, one of the ergopeptine alkaloids, has been used as an anti-migraine. Since it has been reported that ergotamine is excreted in human milk, breast-feeding should be discontinued during treatment with the drug¹⁹. In ruminants, ergopeptine alkaloids, such as ergovaline, can be metabolized to non-peptide ergot alkaloids by the ruminal microflora²⁰. Further research is needed to examine the fate of metabolized ergot alkaloids. As for lolitrem alkaloids, their pharmacokinetics is unknown. The other biologically active substances found in endophyte-infected perennial ryegrass or tall fescue, such as ergosine, loline and paxilline, should also be considered. Until the pharmacokinetics of the endophyte toxins in cattle is investigated, we will not be able to secure the safety of the meat or milk produced from cattle consuming endophyte-infected perennial ryegrass or tall fescue.

In this report, we showed that perennial ryegrass straw imported from the United States, especially from Oregon, contained endophyte toxins and the toxins are suspected as being the main cause of some clinical disorders.

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