Field cases of aflatoxicosis in pigs

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SUMMARY: Five cases of aflatoxicosis in pigs in southern Queensland are described. One peracute case where aflatoxin concentrations of up to 5000μg aflatoxin B₁/kg were demonstrated in stomach contents was presumed to be caused by consumption of mouldy bread. High levels of toxins were also present in the livers. Two cases of acute toxicity were caused by feeding mouldy peanut screenings containing 22000μg aflatoxin B₁/kg. One case of subacute and one of chronic toxicity were caused by sorghum grain based rations with lower aflatoxin levels (4640 and 255 μg/kg). Peracute toxicity caused collapse and deaths within several hours, acute toxicity caused deaths within 12 h and with subacute toxicity deaths occurred after 3 weeks on a toxic ration. Anorexia and ill thrift affecting only growing animals were seen with chronic toxicity. Extensive centrilobular liver necrosis and haemorrhage occurred with peracute toxicity and in cases of acute poisoning there was hepatic centrilobular cellular infiltration, hepatocyte swelling and bile stasis. With subacute toxicity hepatocyte vacuolation together with bile stasis and bile ductule hyperplasia were seen.

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Introduction

The first report of toxicity in pigs, which in retrospect was probably due to aflatoxins, described acute and subacute disease in the United States of America in pigs grazing mouldy corn in the field (Sippel et al 1953). The acute disease was reproduced experimentally by dosing pigs for several days with corn on which cultures of Aspergillus flavus were grown (Burnside et al 1957). A more chronic syndrome occurred in weaner pigs fed rations containing imported peanut meal in England (Loosmore and Harding 1961). Subsequently aflatoxins were extracted from these imported peanut meals (Sargeant et al 1961).

Reports of aflatoxicosis in Australia have described acute disease in poultry fed imported peanut meal (Gardiner and Burnett 1981 and were from farms in the Brisbane (case 1), Burnett (cases 2, 3 and 4) and Darling Downs (case 5) areas in southern Queensland.

Pathology

Tissues for histological examination were fixed in 10% formalin, embedded in paraplast and cut at 5 μm. Sections were stained with haematoxylin and eosin and Perl’s Prussian Blue.

Analytical Procedures

Aflatoxin analyses were performed using the following methods. In cases 2, 3, 4 and 5, feed samples were analysed by the method of Blaney et al (1979) which involves aqueous acetone extraction, chloroform partition and visual estimation under ultra-violet illumination following two-dimensional, thin layer chromatographic separation.

The livers and stomach contents from case 1 were extracted by the method of Truckses and Stoloff (1979) which is similar to that of Blaney et al (1979), but gives slightly better recoveries of aflatoxin M₁. These extracts were then purified further by a Sep-pak* cleanup (Thean et al 1980) and analysed by reversed phase high performance liquid chromatography. The instrument used was a Spectra-Physics 8000B equipped with F.S. 970 fluorescence detector, and 10μl injection loop. The column was a Brownlee Labs. 25 cm R.P.-18 column operated at 35°C. The mobile phase was a mixture of methanol (20%), acetonitrile (20%) and water (60%) with a flow rate of 2 ml/min. The detector was fitted with a 418 nm emission filter, with excitation at 365 nm. Eluate from the Sep-pak was evaporated to dryness, then dissolved in 500 μl acetonitrile, and 10μl was injected into the chromatograph. This procedure allowed quantification of aflatoxins B₁, G₁ and G₂, after comparison with peak areas of standards. The remaining acetonitrile was evaporated, 20 μl of trifluoroacetic acid was added, and the mixture was then allowed to stand for 15 min. Next, 100 μl of hexane was added followed by a mixture of water (90%) and acetonitrile (10%), so that the volume of the aqueous layer was the same as that of the acetonitrile before evaporation. Injection of 10 μl of the aqueous layer allowed quantification of aflatoxins B₁ (as B₁), G₁ (as G₁) and M₁ (as M₁). This procedure is based on that of Beebe and Takahashi (1980).

Ochratoxin A was detected using a general screening method for a number of mycotoxins and was quantified by both thin layer chromatography and high performance liquid chromatography (C. J. Moore, unpublished data).

Materials and Methods

The cases occurred during the period May 1980 to June 1981 and were from farms in the Brisbane (case 1), Burnett (cases 2, 3 and 4) and Darling Downs (case 5) areas in southern Queensland.

Results

Case 1

History and Clinical Signs

The outbreak of toxicity occurred on a licensed swill feeding farm in a group of 380 pigs consisting of 150 adult sows, 200 baconers and about 30 porkers. Feed consisted of dry rendered hospital food scraps, stale bread returns from a bakery and vegetable scraps. The pigs were housed in a dirt yard and fed in an adjacent pen on a concrete floor which had space for about 100 pigs. A single early morning feed which was consumed in half an hour was the cause of

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toxicity. Fifty pigs died within 2h. 20 further pigs died within 24h of feeding and 10 pigs died over the following 3 days. Total mortality was 80 pigs, consisting of 70 baroners and adult sows and 10 porkers. The signs were frothing and bloody discharge from nose and mouth, coarse tremor and staggering followed by collapse and death as early as 10 min after onset of signs. Depression, vomiting and diarrhoea were seen in less acutely affected animals and there was evidence that some abortions occurred.

Pathology
Six pigs (2 mature sows, 2 baroners and 2 porkers) were autopsied 30 h after injection of the toxic feed. In the 4 older animals large volumes of dark blood stained fluid were present in thoracic and abdominal cavities. There was congestion of the submandibular and pharyngeal areas, extensive haemorrhage in diaphragm muscle and copious haemorrhage into the rumen of the lower half of the small intestine, the caecum and the upper part of the colon. Congestion and oedema of lungs and congestion of the liver with marked oedema of the gall bladder wall were constant findings. Stomachs were dilated with dirty grey semifluid material in which vegetable scraps were readily identified as well as large quantities of sand. Marked haemorrhage of a gastric lymph node was present in one pig and congestion and enlargement of the spleen was seen in another.

Both of the smaller sows had a firm congested liver and oedema of the gall bladder wall as well as blood in the caecum and anterior colon.

Histological examination of livers of all pigs revealed extensive acute centrilobular necrosis and haemorrhage with a few surviving hepatocytes in the periphery (Figure 1). Extensive subserosal oedema was seen in the gall bladders and there was pulmonary congestion and oedema. No significant changes were seen in kidney, small and large intestine, heart or brain sections.

Chemical Analyses
No bread or other feed material was available for analysis. The stomach contents and livers from 3 pigs, together with the liver from a sixth pig were stored at 4°C for 2 weeks prior to analysis for aflatoxins and metabolites of aflatoxins. The proportion of dry organic matter in the samples of stomach contents was determined as the loss during ashing of dried portions of the contents. This ranged from 9% to 11% in the 5 samples. The results of aflatoxin analyses expressed on an "as is" basis are shown in Table 1.

![Figure 1. Hepatic centrilobular necrosis and haemorrhage — case 1. Haematoxylin and eosin X50.](image)

### Table 1

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<th>G1</th>
<th>G2</th>
<th>M</th>
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* Not analysed
† Not detected (<5 G1, G2, ≤15M, for stomach contents; <0.1 G1, G2, B12 for liver; <1 G1, G2 for sorghum grain)

Case 2

History and Clinical Signs
A group of 8 large white sows in a pen and another group of 8 sows and one boar in a small paddock were fed a proprietary ration. One afternoon both groups were given a single feed of mouldy peanut screenings and this caused acute toxicity with signs of severe depression, vomiting, abortion and deaths. A total of 9 pigs died; 2 died within 12 h, another 6 died in the next 12 h and one died a week after knowing the toxic material.

Pathology
No material was available for pathological examination.

Chemical Analyses
Three samples of feed (each of one kg) were examined and found to contain a mixture of broken and whole peanut kernels, shells, peanut stubble and soil. Aflatoxin concentrations are shown in Table 1. Concentrations in vegetable matter would have been greater than shown since large amounts of soil were present in the samples.

Case 3

History and Clinical Signs
Acute poisoning occurred in a group of 60 large white pigs 3 months of age which had been purchased from various

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sources. The pigs were housed in dirt pens but feeding was done on concrete. A change of feed from a proprietary ration to mouldy peanut screenings was made and feed intake decreased markedly because the material was unpalatable. Three pigs were found dead 12 h after feeding and a further 5 pigs died over a period of 3 days. Many of the pigs appeared to be sick and depression, dyspnoea, apparent anaemia and dark faeces were seen. The peanut ration was replaced by the proprietary ration after 4 days and the sick pigs recovered.

Pathology

Five days after introduction of the peanut diet one sick pig was killed for autopsy. Dark yellow liver, very pale cream kidneys, pale mucous membranes and hyperemic gastric mucosa were seen. Histological examination of the liver showed swollen eosinophilic hepatocytes with swollen vesicular nuclei and scattered bile canaliculi were diluted with yellow pigment. In the centralzone area there was a deficit of hepatocytes and extensive infiltration with reticuloendothelial cells (Figure 2). Many of the latter cells contained greyish yellow pigment which gave a positive reaction to Perl's Prussian Blue stain for iron. Foamy vacuolation of the cytoplasm of some centrallobular hepatocytes was also present. In the kidney the epithelial cells of proximal tubules were abnormally eosinophilic and the lumen was dilated with proteinaceous material. Obvious necrotic cell debris and neutrophils were present in scattered tubules. Mild multifocal interstitial nephritis with infiltration of reticuloendothelial cells, plasma cells and fibroblasts was present in the cortex. In the medulla protein casts were common and a diffuse interstitial infiltration with reticuloendothelial cells and fibroblasts was present. There was colonic submucosal oedema but no lesions in the small intestine examined.

Chemical Analyses

Two samples of feed with similar appearance to those of case 2 were analysed and the results are shown in Table 1.

**Case 4**

**History and Clinical Signs**

Ten large white sows, one boar and 60 growers approximately 3 months of age were housed in pens. Signs of sickness followed introduction of a new batch of sorghum into the home mixed ration. The sorghum was unripe when harvested and had a 16% moisture content soon after. Overheating had occurred in the stored grain in spite of the use of an aeration spear. Inappetence, depression and growth depression were seen in all pigs and some pigs also had diarrhea and vomiting. Four growers died after ingesting the ration for a period of 3 weeks. Shortly after this the sorghum was mixed with an equal portion of corn but inappetence and depression continued until the sorghum was completely removed from the ration. When the pigs were slaughtered as baconers 2 carcasses were condemned due to jaundice and one of these was also cachectic.

**Pathology**

One of the pigs that died initially was autopsied. The liver was swollen and yellow and the kidneys were very pale. Subepicardial petechial haemorrhages and subperiosteal ecchymoses were seen. Subserosal oedema of the intestines, subepicardial oedema of the atria and perirenal oedema were present. Histological examination of the liver revealed cytoplasmic swelling and vacuolation, often with a foamy appearance, of a wide zone of centrallobular hepatocytes. Towards the periphery of the lobule hepatocytes were swollen but had less vacuolation and scattered megaloocytes with basophilic cytoplasm were present. Hyperplasia of bile ductule epithelium, focal hyperplasia of Kupffer cells and scattered deposits of bile pigment in distended canaliculi were present in the periphery of the lobule (Figure 3). The epithelial cells of the proximal tubules of the kidney were swollen with vacuolated cytoplasm, lumens of numerous other tubules were dilated and sometimes contained bile and protein casts.

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**Figure 2.** Hepatic centrilobular infiltration of reticuloendothelial cells — case 3. Haematoxylin and eosin X140.

**Figure 3.** Bile duct hyperplasia (arrows) in the periphery of the lobule — case 4. Haematoxylin and eosin X200.
Chemical Analyses
A sample of sorghum grain used in the ration was analysed for aflatoxins and results are included in Table 1. Ochratoxin A was also detected in the sample at a concentration of 100 µg/kg.

Case 5
History and Clinical Signs
Inappetence, depression and decreased growth rate occurred in growers in a 200-sow breeding and fattening unit. This was associated with introduction of a new batch of sorghum as the grain portion of the ration. The grain was harvested before it had completely ripened and stored in a silo for less than a month before feeding. Patches of black mould growth were present in the grain. Later the grain was donated using another batch of sorghum and fed without further problems.

Pathology
No material was available for pathological examination.

Chemical Analyses
A single sample of the sorghum grain and a pooled sample (2 kg) derived from samples collected at 13 different sites in the sorghum contained aflatoxin B₁ and B₂ (Table 1).

Discussion
Aflatoxins are products of secondary metabolism of toxigenic strains of A. flavus x A. parasiticus and can be produced in growing crops in the field and in harvested stored crops. Fusarial invasion of cereals grains and peanut kernels is dependent on moisture content and temperature but seed damage due to drought, insects or mechanical trauma during harvesting also predisposes to mould growth.

Analysis of aflatoxins in feed was not done in case 1 but mouldy bread was presumed to be the source of the toxins. Bread is apparently a very suitable substrate for production of aflatoxins, possibly because of its aerated structure, and it has been the source of poisoning in several cases of aflatoxicosis in dogs (Ketterer et al 1973).

Pea nut kernels are an excellent substrate for aflatoxin production and the peanut material fed in cases 2 and 3 were screenings from nut in shell purification procedures with a high proportion of kernel material. In addition the screenings came from a crop which was damaged by drought.

Storage problems with sorghum grain high with moisture content are a common occurrence in Queensland. The crop often is harvested late in the summer when temperatures are lower and less favourable for drying. In addition the crop tends to ripen unevenly so that frequently there is a proportion of immature grain with higher moisture content at harvest. A level of 12% moisture is considered the upper limit for safe storage (N. Heath personal communication). Aflatoxin concentration can vary greatly within a batch of mouldy sorghum as was shown by the greatly differing analysis results for sample 1, a single sample and sample 2, a pooled sample in case 1.

Relative acute toxicities in dactyloids of aflatoxin B₁, B₂, G₁, and G₂ are 1.0, 0.2, 0.5 and 0.1 respectively (Cornghan et al 1963) on this basis aflatoxin B₁ equivalent in the 5 stomach contents in case 1 varied from 679 to 5094 µg/kg with a mean of 2078 µg/kg. However, the peritoxic nature of the disease and the considerable dilution by gastric fluids suggests that much higher concentrations of aflatoxins were present in the ration fed. When the concentrations are expressed on the basis of dry organic matter in the contents, levels 10 times greater are obtained. The concentration of aflatoxins B₁, B₂, and M₁ in liver (especially in pig no. 4) were much higher than levels previously reported for pigs poisoned with aflatoxins. When a corn based diet containing 1500 µg/kg aflatoxin B₁ was fed to 4 pigs for 35 to 42 days aflatoxin M₁ was not detected in the livers and aflatoxin B₁ was detected in only one liver at a concentration of 8 µg/kg (Stoloff and Truckness 1979). The detection of aflatoxin B₂ in stored samples of stomach content is of interest. This compound is produced from aflatoxin B₁ by acetyl catalysed hydration and is much less toxic than the latter (Dutton and Heathcote 1966).

The average concentration of aflatoxin B₁ equivalent in the 3 samples of mouldy peanut screenings in case 2 was 22667 µg/kg and in case 3 the average in 2 samples of peanut screenings was 22675 µg/kg. Based on results of sorghum analysis and 80% sorghum level, the ration fed in case 4 contained aflatoxin B₁ equivalent of 4640 µg/kg and in case 5 the ration had 255 µg/kg (sample 2).

Pigs are among the animal species which are highly susceptible to aflatoxin poisoning. The single dose LD₅₀ of aflatoxin B₁ for weaners is 620 µg/kg bodyweight and a dose of 1000 to 2000 µg/kg results in death in 18 to 24 h (Newberne and Builer 1969). It is apparent that at least the latter dose rates occurred in cases 1 and 2 where porcine disease and high mortality rates followed a single feed of toxic material. In case 3, however, where peanut material with a similar toxin level to case 2 was fed for 5 days there was a much lower mortality rate. Decreased toxin intake because of poor palatability or resistance to the effects of the toxin due to genetic factors or nutritional status may have been responsible. Protein and vitamin K levels in the diet are known to affect the toxicity of aflatoxin B₁ in pigs (Edwards 1979).

Long-term feeding of low levels of aflatoxins as occurred in case 5 causes azotemia, decreased weight gains and decreased food conversion. Only young pigs in the herd are affected and suckers can show signs which persist after weaning due to injection of aflatoxin M₁ in the milk while sows are unaffected (Edwards 1979).

Aflatoxins, notably aflatoxin B₁, primarily affect liver cells but effects on blood coagulation are also important. Parenchymatous toxicity with extensive liver necrosis and haemorrhagic diathesis has been produced in pigs given high oral doses of crude aflatoxins or pure purified aflatoxin B₁ (Wilson et al 1967; Czywczik et al 1968). Subacute toxicity with fatty infiltration and bile ductule hyperplasia in the liver and jaundice, has been produced in pigs following short term oral dosing with lower levels of crude aflatoxins (Skik et al 1968). Chronic toxicity characterised by hepatic ancylosis, fibrosis and bile ductule hyperplasia has been described following long term feeding of low levels of aflatoxins (Gage et al 1988).

The differing liver pathology seen in cases 1, 3 and 4 results from the variations in toxin concentration in the feed, intake, time of exposure and time following exposure to toxins. In case 1, where many deaths occurred within 2 h of feeding, extensive pericase liver necrosis with haemorrhage was seen. The haemorrhage seen in the intestinal lumen and the diaphragm in this porcine case was possibly caused by a direct effect on the clotting mechanism by aflatoxins. In case 3 a small proportion of pigs in the group died within 18 h of the change to toxic feed and others took up to 5 days. The pig which was examined at 5 days had a deficiency of centrilobular hepatocytes with infiltration of reticuloendothelial cells containing haemorrhoids indicating that centrilobular necrosis and haemorrhage had occurred. Hepatocyte swelling was present throughout the rest of the lobule and this change probably caused the bile stasis in the canaliculi. In case 4, feed with lower aflatoxin concentration was ingested for 3 weeks prior to the post mortem examination. Focal vacuolization of a wide zone of centrilobular hepatocytes and cytoplasmic swelling of some hepatocytes in the lobule with resulting bile stasis was seen. In the periphery of the lobule hyperplastic bile duct epithelium and scattered megabacities were present at this stage.

Kidney tubule damage together with the typical liver lesions of aflatoxicosis have been reported with mouldy corn poisoning (Sippl et al 1953) and experimental aflatoxicosis (Skik et al 1968) in pigs. In case 2 and case 4 of the present paper, acute kidney tubule damage was observed. The involvement

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of another nephrotoxin such as the mycotoxin ochratoxin A was also considered possible and subsequently, the sorghum grain in case 4 was examined for ochratoxin A in the course of a survey of feeds for contamination by a number of mycotoxins (C. J. Moore, unpublished data). Ochratoxin A was detected at a concentration of 150 µg/kg. It has been reported by Krogh (1978) that 200 µg/kg of ochratoxin A in the ration for 4 months was required to produce kidney lesions in pigs. In case 4 it is possible that aflatoxins and ochratoxin A were additive or synergistic in their toxic effects on the kidney and there is evidence that this occurs in poultry (Huff and Dierk 1981).

The cases described have shown the potential of aflatoxin contaminated feeds to produce disease in pigs varying from a peracute fatal syndrome to a chronic, ill defined syndrome. Peanut-derived feedstuffs have long been recognised as a possible cause of aflatoxicosis but aflatoxins have also been demonstrated in a wide variety of cereals and other feedstuffs. In Queensland sorghum grain is widely used in pig rations and occurrence of 2 cases of aflatoxicosis caused by mouldy sorghum suggests that more widespread poisoning could occur if climatic and storage conditions were favourable for mould growth. Bread has very limited application as a feedstuff for pigs. However, extremely high levels of aflatoxins apparently can be produced in this material and measures should be taken to prevent this.

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References


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