

## Serum Antibodies to the Catalase Antigen of *Aspergillus fumigatus* in Cattle

*Aspergillus fumigatus* is an important agent of mycotic infection in cattle and a potent source of antigens. However, the efficacy of serological diagnosis of aspergillosis in cattle remains controversial. *Corbel* (1972) and *Knudtson et al.* (1974) considered a precipitin assay useful as a supplementary test in the diagnosis of mycotic abortion, whereas *Wiseman et al.* (1984) found the specificity too low to justify its routine use. We have studied 1) the antibody response to the catalase antigen of *A. fumigatus* in experimentally infected cattle and 2) the prevalence of catalase antibodies and *A. fumigatus* precipitins in healthy and diseased cattle. The aim was to ascertain how far detection of antibodies to a defined fungal antigen can contribute to the often difficult diagnosis of mycosis.

Four Jersey bull calves (weight 50-60 kg) and one artificially inseminated cow were inoculated intravenously with freshly harvested *A. fumigatus* conidii,  $10^3$  to  $10^8$  colony forming units per injection as detailed below. Conidii were suspended in sterile 0.9% saline with 0.01% Tween 80. Venous blood samples were obtained twice a week for up to 21 weeks, and animals were sacrificed at the end of experiments. Mycological cultures and histopathological examination were performed. Venous blood samples from healthy cattle ( $n = 98$ ) were obtained from a herd and slaughterhouse cattle. Seventeen diseased cattle admitted to the Department of Internal Medicine at the Royal Veterinary and Agricultural University were also investigated serologically.

Sera were stored at  $-20^{\circ}\text{C}$  until analysis. Precipitating antibodies to the catalase antigen of *A. fumigatus* were detected by an immunoelectrophoretic technique as previously described (*Schönheyder et al.* 1985). Immunoprecipitated catalase in the dried gel was visualized by addition of hydrogen peroxide, whereby a characteristic »bubble« reaction was seen. A hyperimmune rabbit serum was included as a positive control in each run. Precipitating antibodies to *A. fumigatus* were detected by double immunodiffusion (ID) as previously described (*Schönheyder et al.* 1982). Positive sera were tested in two-fold serial dilutions in both assays in order to determine end-point titres.

In none of the 5 experimental animals serum antibodies to *A. fumigatus* were detected prior to inoculation. A dose of  $10^3$  conidii was well tolerated in 2 calves and no antibody response occurred within 2 weeks. A second dose of  $10^7$  conidii on day 14 was also well tolerated, and only one of the calves developed catalase antibodies and precipitins (titres = 1) within 4 weeks. A third inoculation of this calf with  $10^8$  conidii (day 51) boosted titres to 4 and 16, respectively, and when sacrificed on day 70 hyphae and a granulomatous reaction was seen in the kidneys. A third inoculation of the other calf (day 137) elicited a weak precipitin response within 2 weeks, but no catalase antibodies were detected. When sacrificed on day 151 no evidence of mycosis was found.

Two additional calves were inoculated primarily with  $10^8$  conidii. Precipitins developed within 2 and 3 weeks, respectively, in ti-

tres up to 8, but no catalase antibodies were found. At autopsy on days 23 and 28, respectively, aspergillosis was confirmed mycologically and histopathologically in both animals. The pregnant cow was inoculated with  $10^8$  conidii on day 158 of gestation and precipitins became detectable on day 164. A plateau titre of 4 was maintained for a month, when abortion occurred on day 202. *A. fumigatus* was cultured from the placenta and foetus, and histologically necrotizing placentitis and hyphae were revealed. Catalase antibodies were not revealed in any of the serum samples.

None of 98 healthy cattle had a positive catalase antibody test, whereas 15 had *A. fumigatus* precipitins in titres 1 or 2. Of the 17 cattle admitted to the university clinic 6 (35%) had precipitins, but only one cow with a precipitin titre of 4 reacted for catalase antibodies (titre = 1). This cow was acutely ill with extensive decubital ulcers, but no aetiological diagnosis was obtained. In 2 cattle a postmortem diagnosis of acute mycosis was inferred from histopathological study, but neither *A. fumigatus* precipitins nor catalase antibodies had been found.

This investigation has confirmed a high prevalence of *A. fumigatus* precipitins in both healthy and diseased cattle (Wiseman *et al.* 1984). Spontaneous occurrence of catalase antibodies was recorded in only one cow, that might have had an occasional aspergillus infection. However, catalase antibodies developed in only 1 of 5 experimentally infected cattle, and thus the catalase antibody test appears not to be applicable to the serological diagnosis of aspergillosis in cattle. It

is noteworthy that cattle with *A. fumigatus* infection (inoculum  $\approx 10^8$  conidii) mounts a conspicuous precipitin reaction within 2 to 3 weeks, and longitudinal serological survey by this technique and e.g. immunoblotting might contribute to the diagnosis of mycotic abortion and other forms of aspergillosis in cattle.

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