1995) (ROC analysis not shown). In comparison, the Dachs TB-ELISA gave higher values for sensitivity (a 33 per cent improvement), positive predictive value, negative predictive value and accuracy at the same specificity of the Brock Test in this study. The cut-off value could be set in the Dachs TB-ELISA to emphasise its sensitivity or specificity, depending on the context of its use.

These preliminary results suggest that serodiagnosis of TB in badgers can be enhanced with the new test. Analysis of more sera using the Dachs TB-ELISA is required to reduce the confidence intervals reported here and confirm its superior performance to that of the Brock Test, which was originally evaluated on nearly 2000 sera (Clifton-Hadley and others 1995).

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Isolation of *Malassezia pachydermatis* from the skin of captive rhinoceroses

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Malassezia pachydermatis is a lipophilic, monopolar-budding yeast which was first isolated from the skin of an Indian rhinoceros (*Rhinoceros unicornis*) with exfoliative dermatitis in 1925 (Weidman 1925). *M pachydermatis* has been shown to be a component of the normal skin microbiota of a wide range of mammals and birds, particularly wild and domestic carnivores (Gustafson 1959, 1960, Baxter 1976, Dufait 1985, Hajsig and others 1985, 1990, Guillot and others 1994, 1997). It is a frequent opportunistic cutaneous pathogen of dogs (Dufait 1983, Larsson and others 1988, Mason and Evans 1991, Bond and others 1995). Six other *Malassezia* species are recognised as commensal organisms isolated from the skin of various hosts. These species differ from *M pachydermatis* in that their growth in vitro is dependent upon the presence of lipids.

Although the first reported isolation of the yeast was from the skin of a rhinoceros, to the authors' knowledge, skin colonisation and infection by *Malassezia* species has been studied in only small numbers of these animals. Kuttin and Müller (1994) isolated *M pachydermatis* from the external ear canal of wide-mouthed rhinoceroses. Guillot and others (1994) isolated *Malassezia* species from five of six Indian rhinoceroses, six isolates of *M pachydermatis* and two isolates of lipid-dependent *Malassezia* were recovered. Partial sequencing of the large subunit ribosomal RNA of five *M pachydermatis* isolates obtained from four wide-mouthed rhinoceroses showed that they had the same sequence type ('sequevar') designated Ic; isolates of this type were exclusively isolated from rhinoceroses (Guillot and others 1997). The aim of the present study was to evaluate the carriage of *Malassezia* species in the skin of three species of rhinoceroses kept in wildlife parks and zoological collections in the UK and Germany.

Forty-two rhinoceroses were sampled, consisting of 17 white rhinoceroses (*Cerathotherium simum simum*), 21 black rhinoceroses (*Diceros bicornis michaeli*) and four Indian rhinoceroses, of which 29 were female and 13 were male. Thirty animals were kept at two wildlife parks in the UK, eight at a wildlife park in Germany, and four in zoological collections (two in Germany and two in the UK). The rhinoceroses at the wildlife parks were kept in paddocks in groups, whereas the

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rhinoceroses in the zoological collections were kept in individual enclosures with daily access to outdoor paddocks. At all of the establishments the rhinoceroses were fed on diets of hay, pelleted horse and rhinoceros food, fruit, and various mineral and vitamin supplements. Water was available ad libitum. Two of the animals kept in zoos and two kept at wildlife parks showed skin lesions (erythema, scaling, erosions), the aetiologies of which were uncertain, whereas the skin of the other animals was normal.

The skin of each animal was sampled using contact agar plates containing a modified Dixon's agar (van Abbe 1964) supplemented with cycloheximide and chloramphenicol (Bond and others 1994). The plates, which had a surface area of 6·1 cm^2, were stored at 4°C and used within 10 days. Eight sites were sampled on each rhinoceros, comprising the dorsal and ventral midline, the left and right pinna, the left and right axilla, and the left and right precrural area. Sites were sampled by applying the plates to the skin for 10 seconds. Skin sites were not prepared before sampling in view of the potential hazards in handling captive rhinoceroses; sites with the least possible extraneous matter on the skin surface were selected within each anatomical area.

The plates were incubated aerobically at 32°C for seven days. Colonies with gross and microscopical morphologies suggestive of *Malassezia* species were counted and subcultured onto modified Dixon's agar and Sabouraud's dextrose agar (SDA) plates incubated at 32°C. Isolates with high convex or domed, yellow-buff colonies, composed of monopolar-budding, peanut-shaped yeast cells which formed similar colonies when subcultured on SDA, were identified as *M pachydermatis*. Counts were expressed as colony-forming units (cfu)/cm^2.

The growth of saprobic fungi was observed on many of the contact plates, associated with skin contamination from the environment and the inability to cleanse the sites before sampling. However, *M pachydermatis* was isolated from 30 of the 42 rhinoceroses sampled. All four of the black rhinoceroses kept in the zoological collections yielded *M pachydermatis*, and the yeast was isolated from 26 of the 38 animals kept at wildlife parks.

The frequencies of isolation from each anatomical site sampled ranged from 9 per cent from the ventrum to 30 per cent from the precrural area. The frequencies of isolation from the axillae, pinnae and dorsum were 15, 18 and 21 per cent, respectively. Population sizes of *M pachydermatis* on healthy rhinoceroses ranged from 0·2 to 6·1 cfu/cm^2. Population sizes of *M pachydermatis* on the four rhinoceroses with skin disease ranged from 18·4 cfu/cm^2 to confluent growth on the contact plate. Lipid-dependent *Malassezia* species were not isolated from any of the rhinoceroses in this study.

This study supports the results of previous, smaller surveys of *Malassezia* species carriage in rhinoceroses and confirms that *M pachydermatis* can be frequently isolated from these hosts. Before the present study, *M pachydermatis* had been isolated from wide-mouthed, Indian and white rhinoceroses but, to the authors' knowledge, there are no previous reports of the isolation of *M pachydermatis* from black rhinoceroses. The precrural site gave the highest frequency of isolation of the yeast, possibly reflecting a degree of folding of the skin at that site, thus creating local conditions more favourable for yeast proliferation.

In contrast to the report of Guillot and others (1994), lipid-dependent *Malassezia* species were not isolated from the rhinoceroses studied. However, the lipid-dependent *Malassezia* species are slower growing and more difficult to isolate than *M pachydermatis* and the frequent growth of saprobic fungi on the contact plates may have prevented isolation of these more fastidious species. In addition, the frequency of isolation of *M pachydermatis* may also have been reduced by the saprobic fungi overgrowing colonies of the yeast. It has been suggested that the skin of large, domestic animals is gently cleansed with soap and water or 70 per cent alcohol before the sampling of skin for fungi (Scott 1988), but this was deemed impractical for the captive rhinoceroses studied.

The presence of other microbes on the contact plates limits the value of the quantitative aspects of *M pachydermatis* growth in this study. However, the largest population sizes of the yeast were recovered from rhinoceroses with skin disease. Increased population densities of the yeast are associated with dermatitis in dogs (Bond and others 1994, Bond and Lloyd 1997). In the case described by Weidman (1925), exfoliative dermatitis in an Indian rhinoceros was associated with cytological evidence of large numbers of *M pachydermatis* cells, along with mycelial fungi. The potential for *M pachydermatis* proliferation to play a pathogenic role in the skin of rhinoceroses warrants further assessment.

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