

Patch test responses to *Malassezia pachydermatis* in healthy basset hounds and in basset hounds with *Malassezia dermatitis*

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The effects of the patch test application of *Malassezia pachydermatis* extracts were evaluated in seven healthy basset hounds and in seven basset hounds with *Malassezia dermatitis*. Antigens (4 and 0.4 mg/ml) and saline controls were applied for 48 h using filter paper discs in Finn chambers. One healthy basset hound and five affected hounds showed positive patch test reactivity to the yeast antigens. Positive patch test reactions were characterized histologically by mild epidermal hyperplasia and mild to moderate perivascular, periadnexal and interstitial infiltrates of neutrophils and CD3⁺ lymphocytes. Immediate intradermal test reactivity to *M. pachydermatis* antigens was seen in one healthy and one affected hound, whereas delayed intradermal test reactivity was seen in six healthy hounds and five affected hounds. This study indicates that patch test reactivity to *M. pachydermatis* antigen may occur in healthy basset hounds, and in contrast to delayed intradermal test reactivity, is more frequent in basset hounds with *Malassezia dermatitis*.

Keywords dog, *Malassezia pachydermatis*, patch test, intradermal test, skin

Introduction

In recent years, the lipophilic yeast, *Malassezia pachydermatis*, has been recognized as a very common cause of dermatitis in dogs [1–4]. Diseases associated with *M. pachydermatis* are usually characterized by marked pruritus, often have a chronic, relapsing course and many affected dogs require life-long therapy. Population densities of the yeast have been shown to be markedly increased in lesional skin of affected dogs [1,5]. Breed predilections have been identified and concurrent skin diseases, especially canine atopy, are often recognized but the factors which favour proliferation of the yeast are poorly understood [2,4,6–8]. The immunology of skin diseases associated with

Malassezia spp. in humans [9] and dogs [10] has been recently reviewed.

Basset hounds are predisposed to *Malassezia dermatitis*, characterized clinically by an erythematous, greasy, malodorous, pruritic dermatitis that frequently affects the body folds, ventral neck, axillae, groin and limbs [1,7]. Skin and mucosal population sizes of *M. pachydermatis* in healthy basset hounds often exceed those of healthy dogs of other breeds but are usually lower than those found in basset hounds with *Malassezia dermatitis* [5,11]. Affected basset hounds show poor peripheral blood mononuclear cell proliferative responses to *Malassezia* antigens *in vitro* in comparison to those of healthy basset hounds, but serum IgG titres to the yeast are increased in affected hounds [12]. Whilst immediate intradermal test reactivity to *M. pachydermatis* antigens is often seen in dogs with atopic disease [13–15], it was observed in only two out of 17 basset hounds with *Malassezia dermatitis* [16]. By contrast, delayed (24 h) intradermal test reactivity is frequent in healthy and affected basset

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hounds, whereas healthy beagle dogs are less reactive [16,17]. However, delayed intradermal test reactivity was seen to become more pronounced in healthy beagle dogs exposed to *M. pachydermatis* by cutaneous challenge, either directly or under occlusion [17]. An elucidation of the spectrum of immune responses that occur in dogs of different breed and disease status might explain the factors that determine whether dogs are susceptible or resistant to disease caused by *M. pachydermatis*, and might allow the subsequent development of novel immunotherapeutic and prophylactic interventions that avoid the need for long-term antifungal therapy in affected dogs.

Patch testing, classically used to assess contact sensitivity to allergens of importance in allergic contact dermatitis, has recently been developed as an investigative tool for evaluating immunity in human atopic dermatitis and other skin diseases; both crude and recombinant *Malassezia* allergens have been used, along with other important environmental allergens such as dust mite preparations [18–20]. In humans, positive patch test reactivity to *Malassezia* antigens has been shown to be a feature of atopic dermatitis and pityriasis versicolor, but not of seborrhoeic dermatitis and normal skin [18,21,22]. We have recently demonstrated that two out of eight healthy beagle dogs reacted to *M. pachydermatis* antigens following a 48 h patch test [23]. Four out of six dogs that did not react to the first patch test showed reactivity when retested on day 8. Positive patch test reactions were characterized clinically by erythematous macules and plaques, and histologically by mild epidermal hyperplasia, superficial dermal oedema and mild to moderate perivascular, periadnexal and interstitial infiltrates of neutrophils and CD3⁺ lymphocytes. Four of the dogs showed delayed intradermal test reactivity to *M. pachydermatis* antigens but intradermal and patch test reactivity did not correlate.

We hypothesized that patch testing might detect the presence of contact hypersensitivity responses to *M. pachydermatis* in healthy basset hounds and basset hounds with *Malassezia* dermatitis, and that patch test reactivity would be stronger and/or more frequent in basset hounds when compared with healthy beagles, in parallel with the stronger delayed intradermal test responses and higher skin population densities of the yeast seen in the former breed. The purpose of the present study was to investigate and compare the frequency of and clinical and histopathological features of patch test responses to *M. pachydermatis* antigens in healthy basset hounds, and in basset hounds with *Malassezia* dermatitis, with those seen previously in healthy beagle dogs, and to determine whether there

was any correlation between patch test and intradermal test responses to *M. pachydermatis* antigens in basset hounds.

Materials and methods

Animals

The Royal Veterinary College's Ethics and Welfare Committee approved the use of animals in this study. The written informed consent was obtained from each owner prior to enrolment in the study. The subject dogs included seven healthy Basset Hounds, aged between 2 and 9 years (median 4 years), comprising one entire male, two neutered males, three entire females and one neutered female. These dogs had no history of skin disease and on examination had no significant skin lesions, other than occasional discoloration of the hair coat or minimal erythema in intertriginous areas.

The affected group consisted of seven Basset Hounds aged between 4 and 11 years (median 7 years), of which one was a neutered male, one was an entire female and five were neutered females. These dogs had a history of non-seasonal pruritus of more than one year's duration, primarily affecting the ears, ventral neck, axillae, groin, and feet, in varying combinations. On dermatological examination, all affected dogs had some degree of erythematous-ceruminous otitis externa, and erythema with variable greasy exudation of the axilla and groin. Erythema with greasy exudation also affected the ventral neck in five hounds, the feet in five hounds, and folded areas on the limbs in two hounds. The clinical signs were consistent with previous descriptions of seborrhoeic dermatitis associated with *M. pachydermatis* in Basset hounds [1]. Each owner reported that their hounds improved when treated with a 2% miconazole and 2% chlorhexidine shampoo (Malaseb, VetXX, Princes Risborough, Bucks, UK), a product previously shown to be highly effective in the treatment of *Malassezia* dermatitis in Basset hounds [1].

Cutaneous population densities of *M. pachydermatis*

Contact plates consisting of 28 mm diameter universal bottle lids filled with modified Dixon's agar were used to determine population densities of the yeast on the skin of the subject dogs, as previously described [24]. Contact plates were obtained from the left and right axilla of each dog, and from additional lesional areas in affected dogs, and incubated at 32°C for 4 days. *M. pachydermatis* was identified by gross colony and microscopical morphology, and by the ability to grow when sub-cultured on Sabouraud's dextrose agar. Colony counts were expressed as colony-forming units

(cfu)/cm²; when confluent growth was obtained a value of >82 cfu/cm² was recorded.

Intradermal testing

Intradermal tests were performed on the lateral thorax of the subject dogs using test solutions prepared from mechanically-disrupted *M. pachydermatis* CBS 1879 cells, and from a field isolate (41/1192) obtained from a dog with skin disease, as described previously [16]. Histamine phosphate (0.1 mg/ml) and phenol saline served as positive and negative controls respectively. In the present study, the testing concentration for the yeast antigens was 20 µg/ml; the 200 µg/ml concentration used in previous studies was omitted to avoid the potential for strong delayed reactivity noted previously in certain individuals of this breed [16]. Test sites were inspected at 15 min, 24 and 48 h after injection for evidence of immediate and delayed hypersensitivity reactions. Sites were inspected clinically and the horizontal and vertical diameters of any wheals, papules and plaques were measured and recorded. Lesions at 15 min were considered significant if their diameters were equal to or greater than the midpoints of the diameters of the positive and negative controls. Significant lesions were subjectively graded as ++, +++ or ++++ depending on their size relative to the positive control. Delayed reactions at 24 and 48 h were graded on a scale of 0=no lesion, + = erythematous lesion of less than 4 mm diameter; ++ = erythematous macule of 4–5 mm in diameter; +++ = erythema or indurated plaque between 5 mm and 9 mm diameter; and ++++ = erythema or indurated plaques more than 9 mm diameter, as described previously [23]. Reactions of grade ++ or greater were considered significant.

Patch testing

Test antigens were prepared from mechanically-disrupted *M. pachydermatis* CBS 1879 cells, a type culture originally obtained from a dog with otitis externa, as described previously [23]. The protein concentrations of the test solutions, as determined using the bicinchoninic acid assay (BCA Protein Assay Kit, Pierce, Rockford, IL, USA), were 4.0 and 0.4 mg/ml. Antigens were prepared in 0.05% phenol saline.

Aliquots (20 µl) of the antigens or phenol saline control were applied to 6 mm filter paper discs and placed in 8 mm aluminium Finn chambers mounted on Scanpor tape with ten chambers per tape (Epitest Ltd., Tuusula, Finland), as previously described [23]. Hair was removed from the test sites on the lateral thorax of the subject dogs using electric clippers. The filter discs

containing antigen and saline controls were applied directly to the skin with no prior treatment, and after tape-stripping the sites 15 times using clear adhesive tape (Scotch tape), and after gentle scarification using a 27 gauge needle in a criss-cross pattern of four lines. The Finn chambers on Scanpor tape were held in place using adhesive dressings for 48 h. A standard layout of antigens and controls was adopted to minimize the potential for erroneous arrangement of the discs. The tape was orientated on the skin with five rows of two discs (Fig. 1). Three skin sites in the top two rows were scarified, three sites in the second and third rows were tape-stripped, whereas skin sites in the bottom two rows were not treated prior to patch test application.

The patch test sites were examined 20 min and 24 h after removal of the patches for evidence of contact hypersensitivity reactions. Sites were inspected clinically and the nature and severity of skin lesions were recorded on a subjective scale of 0, +, ++, +++ and ++++ where 0=no reaction; + = minimal/mild erythema; ++ = moderate erythema, with or without small papules; +++ = marked erythema, with or without moderate papules or plaques; ++++ = marked erythema and large plaques with or without



Fig. 1 Layout of patch test sites in basset hounds tested using *Malassezia pachydermatis* antigens. 1, scarified, saline control; 2, scarified, 0.4 mg/ml; 3, scarified, 4.0 mg/ml; 4, tape-stripped, saline control; 5, tape-stripped, 0.4 mg/ml; 6, tape-stripped, 4.0 mg/ml; 7, no site preparation, saline control; 8, no site preparation, 0.4 mg/ml; 9, empty chamber; 10, no site preparation, 4.0 mg/ml.

vesicle formation, as described previously [23]. Reactions of grade ++ or greater were considered significant.

Skin biopsy specimens

Patch-tested sites in two affected dogs with positive reactions were prepared for skin biopsy sampling by the subcutaneous injection of lidocaine (20 mg/ml). Skin specimens were obtained from a patch test site with a significant inflammatory reaction (grade ++ or stronger), and from its matched saline control, using a skin biopsy punch of 6 mm diameter (Steifel, High Wycombe, Bucks, UK), approximately 24 h after the patch was removed. Each skin incision was closed by nylon sutures that were removed after 10 days.

The biopsy specimens were placed in 10% neutral-buffered formalin, processed using routine methods and stained with haematoxylin and eosin. Sections from each specimen were labelled with antibodies to the T-cell marker CD3 (dilution 1 in 500, Dako Cytomation, Ely, UK), the B-cell marker CD79a (dilution 1 in 100, Dako Cytomation), or lysozyme (non-specific granulocytic and macrophage marker, dilution 1 in 1500, Dako Cytomation) using a polymeric two-step labelling method (ChemMate™ EnVision™ Detection Kit, Dako Cytomation). The detection reagent was a peroxidase-labelled dextran coupled with goat antibodies to mouse immunoglobulin. Endogenous peroxidase was blocked by incubation with 3% hydrogen peroxide solution. The immunoreaction was developed with 3,3'-diaminobenzidine substrate, and sections were counterstained with Gill's haematoxylin.

Sections were examined by a pathologist who was unaware of the study design (JPK). Epidermal hyperplasia was graded on a scale of 0–3 (normal, minimal, mild, moderate to severe). Dermal inflammation was graded on a scale of 0–4 (normal, minimal, mild, moderate, severe). Mast cell numbers in the superficial dermis were graded as 0 (<6 high powered field [hpf]⁻¹), 1 (6–10 hpf⁻¹) or 2 (>10 hpf⁻¹). A total grade was obtained by adding the scores for each criterion together. In immunolabelled sections, numbers of CD3-positive T lymphocytes in the dermis were graded as 0 (<10 hpf⁻¹), 1 (10–20 hpf⁻¹) or 2 (>20 hpf⁻¹).

Statistical analyses

Mann-Whitney U-tests were used to compare the population densities obtained using contact plate counts in the two groups of dogs. The diameters of lesions at intradermal test sites were compared between groups by Mann-Whitney U-tests, and within groups at

different time points by Wilcoxon signed rank tests. Frequency data were compared using Fisher's exact tests. Analyses were done using the Unistat v3.0 (Unistat Ltd., London, UK) statistical software package with $P < 0.05$ for significance.

Results

Cutaneous population densities of M. pachydermatis

M. pachydermatis was isolated from the left and right axilla of each dog. Population densities in the axillae of the healthy dogs ranged from 0.33 cfu/cm² to >82 cfu/cm² (confluent growth) (median, 1.80 cfu/cm²). The population densities in the axillae of the diseased hounds were significantly higher ($P = 0.02$) than those of healthy hounds, and ranged from 0.82 to >82 cfu/cm² (median, >82 cfu/cm²). Each affected hound had *M. pachydermatis* counts in skin lesions, either in the axillae or elsewhere, in excess of 14.1 cfu/cm² (Table 1).

Intradermal testing

All dogs showed a wheal at the histamine injection site at 15 min post-injection; wheal diameters ranged from 9.5–14 mm (mean $\pm$ SE; 11.3 ± 0.3 mm). Reactivity at the histamine injection sites was not observed at 24 and 48 h. None of the dogs reacted to the saline control at any time.

One affected dog (BH8) showed immediate (15 min) reactivity to both *M. pachydermatis* extracts used (Table 1). The CBS1879 allergen provoked an 8.5 mm (grade ++++) wheal whereas the 42/1192 extract provoked a 10 mm (grade ++++) wheal. One healthy dog (BH11) showed weak (grade ++, 7.5 mm wheal) immediate (15 min) reactivity to the 42/1192 extract but no reaction to the CBS1879 extract at this time (Table 1).

At the 24 h assessment, five healthy dogs and four diseased dogs showed delayed skin test reactivity to both *M. pachydermatis* antigens; a further one dog in each group was reactive to only the 42/1192 extract (Table 1). Reactivity was more evident at 24 h when compared with 48 h. Significant intradermal test reactivity to both antigens remained at the 48 h assessment in two healthy dogs and two affected dogs; in addition, one affected dog (number 2) that reacted to only the 42/1192 extract at 24 h continued to show reactivity at 48 h. Lesions at intradermal test sites at 24 and 48 h were characterized by indurated or erythematous plaques, or erythematous macules, of up to 11 mm diameter. All dogs with lesions at the 48 h assessment had reactivity at the 24 h assessment. The

Table 1 Intradermal and patch test responses to *Malassezia pachydermatis* antigens in seven healthy basset hounds and in seven basset hounds with *Malassezia* dermatitis.

Dog no.	Status	Yeast count [#]	Intradermal test			Intradermal test			Patch test		Patch test		Patch test	
			CBS 1879			42/1192			SC 4 mg/ml		TS 4 mg/ml		NP 4 mg/ml	
			15 min	24 h	48 h	15 min	24 h	48 h	20 min	24 h	20 min	24 h	20 min	24 h
4	H	2.5	0	+++	0	0	+++	0	0	0	0	0	0	0
5	H	1.8	0	+++	0	0	+++	0	0*	0*	++*	0	++	++
6	H	0.5	0	0	0	0	0	0	0	0	0	0	0	0
9	H	0.8	0	++++	+++	+	++++	+++	0	0	0	0	0	+
11	H	1.7	0	0	0	++	++	0	+	0	+	0	+	0
13	H	34.6	0	++	0	0	++	0	+	0	+	0	0	0
14	H	>82	0	+++	+++	0	+++	+++	0	+	0	0	0	0
1	MD	>82	0	0	0	0	0	0	++	+	+	++	++	++
2	MD	>82	0	+	0	0	+++	++	0	0	0	+	0	+
3	MD	>82	0	+++	0	0	++++	0	++	++	++	+	++	+
7	MD	4.6 [§]	0	++	+++	0	++++	+++	++	0	++*	0	+	0
8	MD	1.4 [¶]	+++	0	0	++++	0	0	0	0	0	0	0	0
10	MD	15.5	0	++	0	0	++	0	++*	++	0	0	0	0
12	MD	>82	0	+++	+++	0	+++	+++	NA	++	NA	0	NA	0

H, healthy; MD, *Malassezia* dermatitis; SC, scarified; TS, tape-stripped; NP, no site preparation.

Patch test reactions were graded on a scale of 0 (nil), + weak reaction, or positive on a scale of ++, +++ or ++++.

NA, not assessed at 20 min due to reactivity being observed at eight out of 10 sites.

*Grade ++ reaction observed to the *M. pachydermatis* antigen at the lower concentration of 0.4 mg/ml.

[#]Mean of *M. pachydermatis* count (cfu/cm²) obtained from the left and right axilla using contact plates.

[§]Populations in lesional skin of groin were 14.1 cfu/cm².

[¶]Populations in lesional skin of groin were > 82 cfu/cm².

affected dog which reacted to both antigens at 15 min (BH8) showed no reactivity at 24 and 48 h, whereas the healthy dog that reacted to the 42/1192 extract at 15 min (BH11) had delayed reactivity to this antigen at 24 h but not 48 h.

The lesion diameters at the sites injected did not vary significantly between healthy and affected basset hounds with either antigen at both 24 and 48 h (Table 2). However, lesion diameters at sites injected with 42/1192 extract at 24 h exceeded those at 48 h in both healthy ($P=0.043$) and affected ($P=0.046$) dogs; lesion diameters at sites injected with the CBS 1879 at 24 h exceeded those of 48 h in the healthy hounds

($P=0.043$) but were comparable in the affected hounds (Table 2).

Patch testing

The subject dogs showed good tolerance of the patches and dressings, and all patches remained in place for the 48 h period of application. When inspected 20 min after removal of the patches, one affected dog (BH12) showed erythema or mild plaque formation at the site of application of eight out of the 10 Finn chambers; this dog was omitted from the analysis at this time point. One healthy hound (BH13) showed some transient diffuse mild erythema over the upper half of the patch test site where the Scanpore tape had been applied, although normal skin was observed corresponding to the sites of application of the filter paper discs within this area; the erythema was absent when inspected 1 h after patch removal. None of the other dogs showed any reaction to the empty Finn chambers or to the Scanpor tape.

In the 13 dogs assessed at 20 min after patch removal (all dogs excluding dog 12), no lesions were seen at any sites patch-tested with phenol saline. Of the six affected dogs assessed at this time, four showed positive (grade ++) reactivity at two or three sites exposed to the *Malassezia* antigens (Table 1). Four dogs reacted to the

Table 2 Median (minimum, maximum) lesion diameters (mm) at 24 and 48 h after the intradermal injection of two *Malassezia pachydermatis* antigens (CBS 1879, 42/1192) at 20 µg/ml in seven healthy basset hounds and in seven basset hounds with *Malassezia* dermatitis.

	CBS 1879 20 µg/ml		42/1192 20 µg/ml	
	24 h	48 h	24 h	48 h
Healthy basset hounds	7 (0, 10)*	0 (0, 8)	7.5 (0, 10.5)*	0 (0, 8)
Affected basset hounds	5 (0, 8)	0 (0, 8)	6 (0, 11)*	0 (0, 8)

Comparison with 48 h response to the same antigen within group of dog. * $P<0.05$ (Wilcoxon signed rank test).

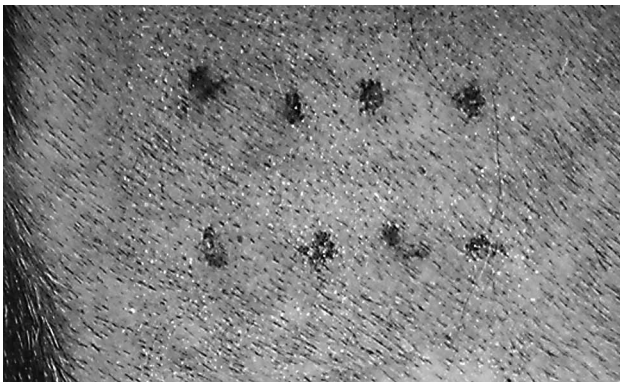


Fig. 2 Patch test responses to *Malassezia pachydermatis* antigens in a Basset Hound with *Malassezia* dermatitis (dog number 7) 20 min after removal of patches applied for a 48 h period. There is no visible inflammation at the site of application of a saline control to a tape-stripped site (top right). Moderate erythema (+ +) is present at the scarified site exposed to the *M. pachydermatis* antigen at 4.0 mg/ml (top left), and at tape-stripped sites exposed to 0.4 mg/ml (bottom left) and 4.0 mg/ml (bottom right).

4 mg/ml concentration at the scarified site, two at the tape-stripped site, and two at the site of no prior treatment. Reactivity to the 0.4 mg/ml concentration was seen at the scarified site in one affected dogs (dog 10) and at a tape-stripped site in another (dog 7, Fig. 2); in both cases, reactivity was also observed at the paired site tested with 4 mg/ml. Three out of the four affected dogs that showed reactivity at 20 min showed positive (grade + +) reactivity at 24 h at one site. One affected dog (number 1) had a grade + + reaction at 24 h at a site previously graded as + at 20 min. The two dogs with no reactivity at 20 min also showed no reactivity at 24 h. The dog with multiple reactivity at 20 min (dog 12) had a grade + + reaction at the scarified site exposed to 4 mg/ml of the antigen at 24 hours but no other lesions at this assessment. Of the five positive patch test reactions observed at 24 h in the affected dogs, three occurred at scarified sites, one at a tape-stripped site, and one at a site without prior treatment; all of these reactions occurred at sites exposed to 4 mg/ml.

Of the seven healthy hounds patch-tested, only one dog (number 5) showed positive reactivity (Table 1). This dog had four positive (grade + +) reactions at the 20 min assessment; positive reactivity occurred at the tape-stripped and untreated sites exposed to the *M. pachydermatis* antigen at 4 mg/ml, and at the tape-stripped and scarified sites exposed to 0.4 mg/ml. At the 24 h assessment, positive (grade + +) reactions were evident at the scarified site exposed to 0.4 mg/ml, and at the untreated site exposed to 4 mg/ml. The clinical features of the patch test reactions observed in

the healthy and affected basset hounds in the present study were comparable, and were also consistent with those observed previously in healthy beagle dogs [23].

The frequency of affected hounds with positive patch test reactivity at 20 min (four out of six dogs) did not vary significantly ($P=0.09$) from that of the healthy hounds (one out of seven). However, the frequency of positive reactions at all antigen-exposed patch test sites at 20 min in the affected dogs (10 out of 36 sites) exceeded ($P=0.04$) that of the healthy dogs (four out of 42 sites). The frequency of positive reactions at sites exposed to the higher (4 mg/ml) antigen concentration at 20 min in the affected dogs (eight out of 18 sites) exceeded ($P=0.02$) that of the healthy dogs (two out of 21 sites) whereas the frequency of positive reactivity to the lower (0.4 mg/ml) antigen concentration in the two groups of hounds was comparable ($P=0.6$). The frequencies of positive patch test reactivity in the two groups of dogs at 24 h did not vary significantly. Overall, positive patch test reactivity at one or more of the assessments was seen in one out of seven healthy dogs, and in five out of seven affected hounds ($P=0.05$).

Comparison of patch and intradermal test reactivity

Of the seven healthy hounds tested, five showed delayed intradermal test reactivity without patch test reactivity, one showed both intradermal and patch test reactivity, and one failed to react to either test (Table 1). Four out of seven affected hounds showed both positive patch test and delayed intradermal test reactivity, one showed positive patch test reactivity but no intradermal test reactivity, one showed delayed intradermal test reactivity but no patch test reactivity, and one hound with immediate intradermal test reactivity showed neither delayed intradermal nor patch test responses.

Histopathology

Skin biopsy samples were obtained from sites exposed to 4 mg/ml of yeast antigen and their matched saline control sites in two affected dogs; in one (dog 1), tape-stripped sites were sampled, and in the other (dog 3), scarified sites were sampled. In both control skin biopsy samples obtained from sites patch-tested with phenol saline, abnormalities consisted only of minimal (grade 1) dermal inflammation, with small numbers of mainly lymphocytes and plasma cells scattered around the superficial or mid-dermis and adnexal structures; both samples were given a total histological grade of 1. By contrast, both biopsy samples obtained from antigen-exposed patch test sites showed more marked

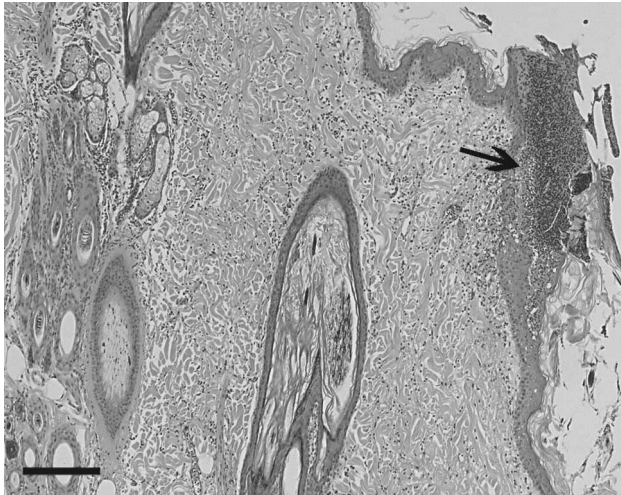


Fig. 3 Skin biopsy specimen from the reactive patch test site of dog 1, a basset hound with *Malassezia* dermatitis. There is epidermal hyperplasia with pustule formation (arrow), and moderate dermal inflammation (Haematoxylin and eosin, bar = 200 microns).

inflammation. There was mild (grade 2) epidermal hyperplasia with intracorneal pustules containing degenerate neutrophils (Fig. 3). There was moderate (grade 3) dermal inflammation comprising perivascular, periadnexal and interstitial infiltrates of neutrophils, with smaller numbers of lymphocytes and plasma cells (Fig. 4). Macrophages were noted in the specimen from one dog (dog 1) whereas small numbers of eosinophils were noted in the second dog (dog 3). Both specimens from antigen-exposed sites were given a total histological grade of 5. In immuno-stained

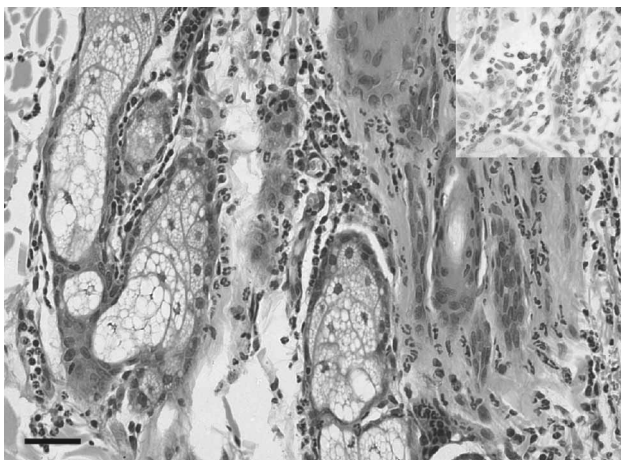


Fig. 4 Skin biopsy specimen from the reactive patch test site of dog 1, a basset hound with *Malassezia* dermatitis. There are moderate periadnexal and perivascular inflammatory cell infiltrates with predominance of neutrophils (Haematoxylin and eosin, bar = 30 microns). Inset: numbers of CD3+ lymphocytes are also significantly increased (immunoperoxidase).

sections, numbers of CD3+ lymphocytes were markedly increased (grade 2) in the dermis of both specimens.

Discussion

The frequent isolation of *M. pachydermatis* from the skin of basset hounds, both free of and affected by *Malassezia* dermatitis, is in accordance with previous studies of cutaneous population densities in this breed [5,11,25,26], but the specific features of the cutaneous environment of these dogs that render it so conducive to yeast growth remain to be determined. Similarly, the frequent occurrence of delayed intradermal test reactivity to *M. pachydermatis* antigens, especially at the 24 h rather than the 48 h assessment, and the infrequent immediate reactivity, is in accordance with previous studies of intradermal test reactivity in this breed [16].

Both the present study, and a previous study that used identical methods [23], have shown that some healthy dogs display positive patch test reactivity to *M. pachydermatis* antigens. Patch test responses to *M. pachydermatis* antigens in dogs might reflect prior contact sensitization in response to the presence of *M. pachydermatis* as a component of the commensal skin mycobiota, or an irritant effect of yeast-derived substances on keratinocytes; differentiation of these two mechanisms is often not straightforward based on clinical or histopathological features [23,27–29]. The clinical and histopathological features of positive patch test sites in the basset hounds with *Malassezia* dermatitis were very similar to those seen previously in patch test responses to *M. pachydermatis* in healthy beagle dogs [23].

There was a trend for patch test reactivity to *M. pachydermatis* antigens to be more closely associated with disease status than delayed intradermal test reactivity; delayed intradermal test reactivity is very frequently observed in both healthy and affected basset hounds, whereas patch test responses were more frequent in affected basset hounds than in healthy hounds. These observations might suggest that the patch test reactivity reflects contact hypersensitivity rather than irritancy; an irritant effect might have been expected in equal frequency in both healthy and affected dogs, although an increased susceptibility to an irritant as a consequence of other skin diseases could not be fully discounted. Contact sensitization might be promoted by the presence of high cutaneous population densities of the yeast often seen in affected hounds, and may contribute to the dermal inflammatory response in response to yeast proliferation in the stratum corneum. However, the absence of detectable

patch test reactivity in two affected hounds suggests that contact sensitization, at least under the conditions of the current test, is not a pre-requisite for a dermatitis response to *M. pachydermatis* in dogs.

In the present study, there was no clear difference in patch test reactivity at skin sites prepared by the three different methods, in accordance with the results of a previous study of healthy beagle dogs that utilized the same techniques [23]. In a recent study of patch test responses to house dust mite antigen and sodium lauryl sulphate in atopic dogs, physical disruption of the stratum corneum was not attempted but a white petrolatum vehicle that may have promoted antigen penetration was used [29]. The optimal method for skin preparation for patch testing dogs with *M. pachydermatis* antigens and other substances remains to be determined.

This study demonstrated that there was no clear correlation between patch test and intradermal test reactivity in basset hounds, as was the case in a recent patch testing study of healthy beagle dogs [23]. Delayed intradermal test reactivity was observed frequently in the healthy basset hounds, even with the relatively low antigen concentration used in the present study [15–17,23], whilst positive patch test reactivity was infrequent in this group. Whilst these responses may have varied had different antigen concentrations been used, or if clinically negative patch test sites had been evaluated histologically [29], these observations support the concept that distinct cellular mechanisms mediate responses to antigens applied epicutaneously and injected intradermally [30].

Atopic dogs differ from basset hounds with *Malassezia* dermatitis in that they frequently display immediate reactivity to the intradermal injection of *M. pachydermatis* antigens [13,15]. Patch test responses to *M. pachydermatis* should now be established in atopic dogs with and without *Malassezia* dermatitis.

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References

- Bond R, Rose JF, Ellis JW, Lloyd DH. Comparison of two shampoos for treatment of *Malassezia pachydermatis*-associated seborrhoeic dermatitis in Basset Hounds. *J Small Anim Pract* 1995; **36**: 99–104.
- Dufait R. Presence of *Malassezia pachydermatis* (syn. *Pityrosporum canis*) on the hair and feathers of domestic animals. *Bull Soc France Mycol Med* 1985; **14**: 19–22.
- Larsson CE, Gandra CRP, Larsson M, *et al.* Dermatitis in dogs caused by *Malassezia* (*Pityrosporum*) *pachydermatis*. *Ars Veterinaria* 1988; **4**: 63–68.
- Mason KV, Evans AG. Dermatitis associated with *Malassezia pachydermatis* in 11 dogs. *J Am Anim Hosp Assoc* 1991; **27**: 13–20.
- Bond R, Lloyd DH. Skin and mucosal populations of *Malassezia pachydermatis* in healthy and seborrhoeic basset hounds. *Vet Dermatol* 1997; **8**: 101–106.
- Aizawa T, Kano R, Nakamura Y, Watanabe S, Hasegawa A. Molecular heterogeneity in clinical isolates of *Malassezia pachydermatis* from dogs. *Vet Microbiol* 1999; **70**: 67–75.
- Bond R, Ferguson EA, Curtis CF, Craig JM, Lloyd DH. Factors associated with elevated cutaneous *Malassezia pachydermatis* populations in dogs with pruritic skin disease. *J Small Anim Pract* 1996; **37**: 103–107.
- Plant JD, Rosenkrantz WS, Griffin CE. Factors associated with a prevalence of high *Malassezia pachydermatis* numbers on dog skin. *J Am Vet Med Assoc* 1992; **201**: 879–885.
- Ashbee HR, Evans EG. Immunology of diseases associated with *Malassezia* species. *Clin Microbiol Rev* 2002; **15**: 21–57.
- Chen TA, Hill PB. The biology of *Malassezia* organisms and their ability to induce immune responses and skin disease. *Vet Dermatol* 2005; **16**: 4–26.
- Bond R, Lloyd D. The relationship between population sizes of *Malassezia pachydermatis* in healthy dogs and in basset hounds with *M. pachydermatis*-associated seborrhoeic dermatitis and adherence to canine corneocytes *in vitro*. In: Kwochka K, Willemse T, von Tscharner C (eds). *Advances in Veterinary Dermatology Volume 3*. Oxford: Butterworth Heinemann, 1998: 284–289.
- Bond R, Elwood CM, Littler RM, Pinter L, Lloyd DH. Humoral and cell-mediated immune responses to *Malassezia pachydermatis* in healthy dogs and dogs with *Malassezia* dermatitis. *Vet Record* 1998; **143**: 381–384.
- Morris DO, Olivier NB, Rosser EJ. Type-1 hypersensitivity reactions to *Malassezia pachydermatis* extracts in atopic dogs. *Am J Vet Res* 1998; **59**: 836–841.
- Morris DO, DeBoer DJ. Evaluation of serum obtained from atopic dogs with dermatitis attributable to *Malassezia pachydermatis* for passive transfer of immediate hypersensitivity to that organism. *Am J Vet Res* 2003; **64**: 262–266.
- Bond R, Curtis CF, Hendricks A, Ferguson EA, Lloyd DH. Intradermal test reactivity to *Malassezia pachydermatis* in atopic dogs. *Vet Record* 2002; **150**: 448–449.
- Bond R, Patterson-Kane JC, Lloyd DH. Intradermal test reactivity to *Malassezia pachydermatis* in healthy basset hounds and bassetts with *Malassezia* dermatitis. *Vet Record* 2002; **151**: 105–109.
- Bond R, Patterson-Kane JC, Lloyd DH. Clinical, histopathological and immunological effects of exposure of canine skin to *Malassezia pachydermatis*. *Med Mycol* 2004; **42**: 165–175.
- Tengvall Linder M, Johansson C, Scheynius A, Wahlgren C. Positive atopy patch test reactions to *Pityrosporum orbiculare* in atopic dermatitis patients. *Clin Exp Allergy* 2000; **30**: 122–131.
- Johansson C, Eshaghi H, Linder MT, Jakobson E, Scheynius A. Positive atopy patch test reaction to *Malassezia furfur* in atopic dermatitis correlates with a T helper 2-like peripheral blood mononuclear cells response. *J Invest Dermatol* 2002; **118**: 1044–1051.
- Johansson C, Sandstrom MH, Bartosik J, *et al.* Atopy patch test reactions to *Malassezia* allergens differentiate subgroups of atopic dermatitis patients. *Br J Dermatol* 2003; **148**: 479–488.

- 21 Rokugo M, Tagami H, Usuba Y, Tomita Y. Contact sensitivity to *Pityrosporum ovale* in patients with atopic dermatitis. *Arch Dermatol* 1990; **126**: 627–632.
- 22 Nicholls DSH, Midgley G, Hay R. Patch testing against *Pityrosporum* antigen. *Clin Exp Allergy* 1990; **15**: 75.
- 23 Bond R, Habibah A, Patterson-Kane JC, Lloyd DH. Patch test responses to *Malassezia pachydermatis* in healthy dogs. *Med Mycol* 2006; **44**: 175–184.
- 24 Bond R, Collin NS, Lloyd DH. Use of contact plates for the quantitative culture of *Malassezia pachydermatis* from canine skin. *J Small Anim Pract* 1994; **35**: 68–72.
- 25 Bensignor E, Jankowski F, Seewald W, *et al.* Comparison of two sampling techniques to assess quantity and distribution of *Malassezia* yeasts on the skin of Basset Hounds. *Vet Dermatol* 2002; **13**: 237–241.
- 26 Guillot J, Bensignor E, Jankowski F, *et al.* Comparative efficacies of oral ketoconazole and terbinafine for reducing *Malassezia* population sizes on the skin of Basset Hounds. *Vet Dermatol* 2003; **14**: 153–157.
- 27 Brasch J, Burgard J, Sterry W. Common pathogenetic pathways in allergic and irritant contact dermatitis. *J Invest Dermatol* 1992; **98**: 166–170.
- 28 Walder EJ, Conroy JD. Contact dermatitis in dogs and cats: pathogenesis, histopathology, experimental induction and case reports. *Vet Dermatol* 1994; **5**: 149–162.
- 29 Nogueria SAF, Torres SMF, Horne K, Jessen C. Patch test reactions to house dust mites in dogs with atopic dermatitis. In: Hillier A, Foster AP, Kwochka KW (eds). *Advances in Veterinary Dermatology Volume 5*. Oxford: Blackwell Publishing, 2005: 49–59.
- 30 Roitt IM. In: *Roitt's Essential Immunology*. Oxford: Blackwell Science; 1997: 346–348.