

## Cutaneous *Alternaria infectoria* infection in a dog in association with therapeutic immunosuppression for the management of immune-mediated haemolytic anaemia

Carla Dedola\*, Alasdair P. G. Stuart<sup>†</sup>,  
Alison E. Ridyard<sup>†</sup>, Roderick W. Else<sup>‡</sup>,  
Adri H. M. van den Broek\*, Jong Soo Choi<sup>§</sup>,  
G. Sybren de Hoog<sup>¶</sup> and Keith L. Thoday\*

\*Dermatology Group, The Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Division of Veterinary Clinical Sciences, Hospital for Small Animals, Easter Bush Veterinary Centre, Roslin, EH 25 9 RG, Midlothian, UK

<sup>†</sup>Internal Medicine Group, The Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Division of Veterinary Clinical Sciences, Hospital for Small Animals, Easter Bush Veterinary Centre, Roslin, EH 25 9 RG, Midlothian, UK

<sup>‡</sup>Diagnostic Pathology Group, The Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Division of Veterinary Clinical Sciences, Easter Bush Veterinary Centre, Roslin, EH 25 9 RG, Midlothian, UK

<sup>§</sup>Department of Dermatology, Yeungnam University College of Medicine, Daegu city, 705-035 South Korea

<sup>¶</sup>CBS-KNAW Fungal Biodiversity Centre, PO Box 85167, 3508 AD Utrecht, the Netherlands

Correspondence: Dr Carla Dedola, The Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Division of Veterinary Clinical Sciences, Hospital for Small Animals, Easter Bush Veterinary Centre, Roslin, Midlothian, UK. E-mail: carladedola@hotmail.com

### Sources of Funding

This study was self funded.

### Conflict of Interest

No conflicts of interest have been declared.

### Abstract

**A 4-year-old, ovariohysterectomized, English springer spaniel on immunosuppressive therapy was re-examined for the review of its immune-mediated haemolytic anaemia and the recent development of skin lesions. For the 3 months since hospital discharge, the dog had been receiving 1.3 mg/kg prednisolone and 2.6 mg/kg ciclosporin, both administered orally twice daily. Physical examination revealed hepatomegaly and multiple, purulent, crusting, erosive to ulcerative lesions over different body areas. Onychorhysis had occurred on one digit and the underlying corium had blackened. There were two proliferative and one plaque-like lesions in the mouth. Thick walled fungal hyphae were detected in impression smears from all skin lesions and staining with periodic acid-Schiff's stain confirmed the presence of multiple fungal hyphae and spores in all biopsies examined. Fungal culture isolated a heavy, pure growth of an *Alternaria* sp. which was identified as *A. infectoria* by sequencing the internal transcribed**

**spacer 1 region of the rRNA gene. The animal's condition prevented detailed investigation of the oral lesions. Withdrawal of the ciclosporin and reduction of the prednisolone dosage resulted in spontaneous resolution of the skin lesions within 40 days. Further gradual decrements in the prednisolone dosage to zero were carried out without recurrence of the immune-mediated haemolytic anaemia. After 12 months, there has been no recurrence of either the skin lesions or the anaemia. To the authors' knowledge, this is the first reported case of *A. infectoria* infection in a dog.**

Accepted 28 October 2009

### Introduction

*Alternaria* spp. are dematiaceous (pigmented) hyphomycetes that are distributed worldwide. Hundreds of species have been described, many of them being common soil saprophytes or recognized pathogens of plants.<sup>1-3</sup> Some species may infect humans,<sup>1</sup> cats<sup>4-7</sup> and, less commonly, dogs in which they may cause cutaneous, subcutaneous, cerebral, corneal or disseminated phaeohyphomycosis.<sup>8</sup> These fungi also form one of the most common airborne allergen groupings and are frequently associated with allergic respiratory disease in humans.<sup>2,9</sup>

*Alternaria alternata*, *A. infectoria* and *A. tenuissima* are the *Alternaria* spp. most frequently isolated from mammals and, with expertise, they can be differentiated one from the other by the morphological characteristics of their colonies and conidia.<sup>1,3</sup> Molecular techniques have also been used for speciation, especially when poorly sporulating species such as *A. infectoria* are isolated.<sup>1,10</sup>

Cell mediated immunity is critical in protection against fungal infections, although recent studies have also shown a potential role for the humoral response.<sup>11</sup> Debilitating and immunosuppressive factors are believed to favour the development of infections with soil saprophytes, such as *Alternaria* spp., not otherwise recognized as pathogens.<sup>11</sup> However, other factors must play a role, especially in cats, as in the majority of reported feline cases an underlying predisposing condition could not be identified.<sup>4-6,12</sup>

This report describes a case of subcutaneous *A. infectoria* infection in a dog which was receiving prolonged immunosuppressive therapy for the management of

immune-mediated haemolytic anaemia (IMHA) and the spontaneous resolution of the infection once the dosage of the immunosuppressive agents was reduced. To the authors' knowledge, there are no previous published case reports of *A. infectoria* infection in dogs.

## Case report

A 4-year-old, ovariohysterectomized, English springer spaniel was re-referred to the Internal Medicine Service, The Hospital for Small Animals, The Royal (Dick) School of Veterinary Studies [R(D)SVS], The University of Edinburgh for the further evaluation of anaemia and crusting skin lesions affecting different areas of the skin and nasal planum. Three months previously, the dog had been diagnosed with severe, non-regenerative IMHA. Because of the non-regenerative nature of the anaemia, this had been initially managed with prednisolone without azathioprine. However, there was no response after 5 days and ciclosporin was therefore added to the therapeutic regimen.

On re-presentation (Day 1), the dog was receiving 2.6 mg/kg ciclosporin (Atopica™; Novartis Animal Health, Little Royston, UK), 1.3 mg/kg prednisolone (Prednicare™; Animalcare, Dunnington, UK), 50 mg/kg sucralfate suspension (Antepsin™; Chugal Pharmaceutical, London, UK) and 0.5 mg/kg famotidine (Pepcid™; Merck Sharp & Dohme, Hertfordshire, UK), each administered orally twice daily. In addition, the dog was also

receiving 12.5 mg/kg of clavulanic acid-potentiated amoxicillin (Synulox™; Pfizer Animal Health, Sandwich, UK), administered orally twice daily, which the referring practitioner had introduced in an attempt to manage a number of non-pruritic, non-painful, crusting, erosive to ulcerative, purulent skin lesions that had developed 2 weeks previously.

On general physical examination, the dog weighed 18.5 kg, had a rectal temperature of 38.6 °C and was bright, alert and responsive. The mucous membranes were pink and the capillary refill time was less than 2 s. The submandibular lymph nodes were prominent. No abnormalities were detected on thoracic examination. The abdomen was palpably enlarged due to generalized hepatomegaly but no pain was elicited on palpation. Despite the chronic administration of glucocorticoids, the dog showed no evidence of weakness or obvious muscle atrophy. A complete blood cell count and routine serum biochemical examinations were carried out; the results are presented in Table 1. These revealed a mild, regenerative anaemia and a stress leucogram and some biochemical changes consistent with prolonged prednisolone usage. On abdominal ultrasonography, there was marked hepatomegaly and the adrenal glands were considered to be atrophic (3–4 mm width). No other abnormalities were detected.

Provisional diagnoses of iatrogenic hyperadrenocorticism and steroid hepatopathy secondary to management of the immune-mediated haemolytic anaemia were

**Table 1.** Haematological and biochemical blood analyses. Abnormal values are shown in bold

Parameters (reference range)	Day 1	Day 2	Day 3	Day 4	Day 6	Day 8	Day 10	Day 12	Day 19	Day 33
Packed cell volume (0.39–0.55 L/L)	<b>0.31</b>	<b>0.31</b>	<b>0.30</b>	<b>0.31</b>	<b>0.28</b>	<b>0.22</b>	<b>0.25</b>	<b>0.29</b>	<b>0.34</b>	<b>0.32</b>
Red blood cells (5.5–8.5 × 10 <sup>12</sup> /L)	<b>4.21</b>	–	–	–	<b>3.80</b>	<b>2.95</b>	<b>3.33</b>	<b>3.72</b>	<b>4.44</b>	<b>4.26</b>
Haemoglobin (12–18 g/dL)	<b>11.3</b>	–	–	–	<b>10.0</b>	<b>7.9</b>	<b>8.8</b>	<b>10</b>	<b>11.7</b>	<b>10.9</b>
Reticulocytes (0 × 10 <sup>12</sup> /L)	<b>0.24</b>	–	–	–	<b>0.07</b>	–	<b>0.05</b>	<b>0.26</b>	–	–
White blood cells (6–15 × 10 <sup>9</sup> /L)	<b>20.1</b>	–	–	–	<b>17.2</b>	13.3	<b>27.1</b>	<b>20.7</b>	<b>19.6</b>	<b>23.5</b>
Neutrophils – segmented (3.6–12 × 10 <sup>9</sup> /L)	<b>18.69</b>	–	–	–	<b>16.16</b>	<b>12.23</b>	<b>24.39</b>	<b>16.97</b>	<b>18.03</b>	<b>20.68</b>
Neutrophils-band (0 × 10 <sup>9</sup> /L)	<b>0.4</b>	–	–	–	0	0	0	0	0	0
Lymphocytes (0.7–4.8 × 10 <sup>9</sup> /L)	<b>0</b>	–	–	–	1.03	<b>0.13</b>	<b>0</b>	<b>0.41</b>	<b>0.19</b>	0.70
Monocytes (0–1.5 × 10 <sup>9</sup> /L)	1.00	–	–	–	0	0.93	<b>2.71</b>	<b>3.10</b>	1.37	<b>1.64</b>
Eosinophils (0–1.00 × 10 <sup>9</sup> /L)	0	–	–	–	0	0	0	0.20	0	0.47
Platelets (200–500)	<b>569</b>	–	–	–	409	362	<b>551</b>	<b>531</b>	<b>735</b>	<b>578</b>
Total protein (18–37 g/L)	<b>53</b>	–	–	–	<b>47.3</b>	<b>44.8</b>	<b>54.5</b>	<b>52.6</b>	<b>52.9</b>	<b>56.0</b>
Bile acids (0–7.0 µmol/L)	<b>132.1</b>	–	–	–	<b>148.0</b>	<b>39.6</b>	<b>97.2</b>	<b>127.2</b>	<b>10.8</b>	5.2
Bilirubin (0–6.8 µmol/L)	<b>17.7</b>	–	–	–	<b>13.1</b>	<b>10.7</b>	<b>8.5</b>	<b>9.1</b>	3.7	2.3
Creatinine (40–132 µmol/L)	64	–	–	–	71	57	66	72	73	81
Glucose (3.0–5.0 mmol/L)	4.6	–	–	–	<b>5.7</b>	<b>6.2</b>	4.7	<b>5.2</b>	5.0	<b>5.3</b>
Urea (1.7–7.4 mmol/L)	<b>8.6</b>	–	–	–	4.3	3.9	4.8	6.0	4.5	6.3
Alkaline phosphatase (20–60 IU/L)	<b>1319</b>	–	–	–	<b>1091</b>	<b>1066</b>	<b>1412</b>	<b>1465</b>	<b>687</b>	<b>153</b>
Alanine aminotransferase (21–102 IU/L)	<b>526</b>	–	–	–	<b>504</b>	<b>355</b>	<b>673</b>	<b>796</b>	<b>424</b>	82
Calcium (2.3–3.0 mmol/L)	2.40	–	–	–	2.40	2.13	2.41	2.42	2.63	2.63
Sodium (139–154 mmol/L)	147	152	152	147	143	145	150	146	147	144
Potassium (3.6–5.6 mmol/L)	5.2	4.5	5.2	5.4	4.6	4.4	4.9	5.2	5.2	5.0
Chloride (99–115 mmol)	101	106	106	105	101	104	107	105	105	107

–, not done

made. The dog was subsequently evaluated by the Dermatology Service of the R(D)SVS because of the presence of the skin lesions.

The largest lesion (4 × 2 cm) was localized over the dorsal muzzle and extended ventrolaterally down the left side of the nasal planum (Figure 1). Three other circular lesions of approximately 1.5 cm diameter were present on the ventral neck, left flank and left thigh. Purulent exudate drained from under thick crusts which covered the lesions on the ventral neck and the left thigh, and removal of the crusts revealed ulcerations. The lesions were not pruritic or painful on palpation. Onychorrhexis had occurred on the fourth digit of the left forefoot and the exposed corium was blackened (Figure 2). Small num-



**Figure 1.** The largest skin lesion, covered with crust and draining a small amount of purulent exudate.



**Figure 2.** The left forefoot showing a number of erosive and crusty lesions and, on the fourth digit, onychorrhexis and blackening of the corium (arrow).

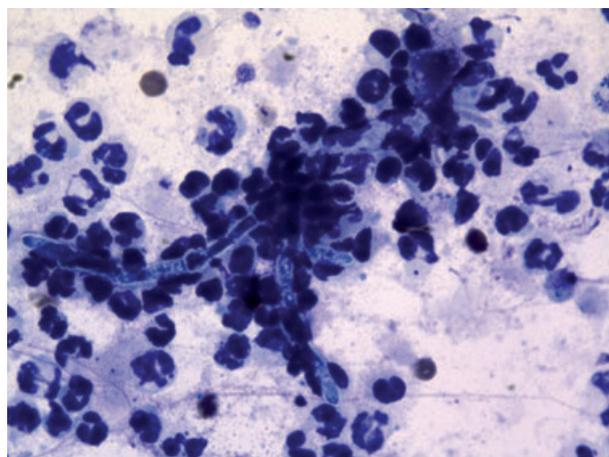
bers of crusts and erosions were present on the digits of the same foot and extended proximally up the limb. The remaining skin and haircoat were macroscopically normal and, despite the prolonged administration of high dosages of glucocorticoids, there was no alopecia, skin atrophy or calcinosis cutis.

There were two bilaterally symmetrical proliferative lesions in the mouth, on the upper labial mucosa adjacent to the canine teeth, each with a central area of ulceration. A white, plaque-like lesion was present on the left side of the lateral border of the apex of the tongue extending towards both the dorsally and ventrally (Figure 3).

Skin scrapings and hair plucks were negative for *Demodex* mites and no fungal spores were observed. Impression smears were made from the ulcerated skin lesions and stained with a modified Wright/Giemsa stain (Diff Quik™; Dade Behring, Milton Keynes, UK). Microscopic examination of all samples revealed pyogranulomatous inflammation with numerous intra- and extracellular rods and cocci, which were particularly abundant in samples from the nasal lesions. Thick walled fungal hyphae were also detected in all the impression smears (Figure 4). Cytological examination of material from the lesions on the lips and tongue sampled by swab smears and stained



**Figure 3.** The plaque-like lesion on the tongue.



**Figure 4.** Cytology from the ulcer on the thigh showing thick walled fungal hyphae surrounded by degenerate neutrophils with intracellular cocci visible in some of them. Modified Wright/Giemsa stain, magnification × 1000.

with Diff Quik™ showed a mixed bacterial population of rods and cocci with no inflammatory cells. Swabs collected from the lesions on the nose and in the mouth were sent for bacterial and fungal culture.

Cutaneous lesions from the nose, the left flank and the left thigh were biopsied under local anaesthesia with lidocaine (2% w/v Lidocaine sterile solution; B. Braun Medical, Sheffield, UK) using disposable 8 mm biopsy punches (Stiefel Laboratories, High Wycombe, UK). Areas to be sampled for bacterial and fungal culture were surgically prepared prior to biopsy and the samples placed into sterile tubes and submitted immediately. Areas to be biopsied for histopathological examination were gently clipped but not cleaned and biopsies preserved in 10% buffered formol saline. The animal's poor general condition prevented general anaesthesia for fine needle aspiration and biopsy of the oral lesions.

A diagnosis of multifocal cutaneous, possibly subcutaneous and mucosal bacterial and fungal infection secondary to immunosuppressive treatment with ciclosporin and prednisolone was made. The physical appearance of the lesion on the tongue was suggestive of *Candida* spp. infection.

While awaiting the results of other tests, the dog was hospitalized, the ciclosporin withdrawn and the prednisolone dosage decreased from 1.3 mg/kg twice daily to 1.3 mg/kg in the morning and 0.7 mg/kg in the evening; the clavulanic acid-potentiated amoxicillin, famotidine and sucralfate were maintained as before. The packed cell volume (PCV) and serum electrolyte concentrations were checked daily (Table 1) and no further significant changes were detected, with the values remaining stable throughout the following 3 days.

On day 4, the prednisolone dosage was further reduced to 0.7 mg/kg twice daily and the dog discharged while awaiting further laboratory results.

On day 6, the owner reported that the dog had been slightly depressed for 24 h. On general physical examination the heart rate was 104 beats/min and the dog was pyrexia (39.6 °C) and tachypnoea (40 breaths/min). The skin and oral lesions were unchanged, there were no new lesions and the biopsy sites were healing by first intention. There were no further significant changes in the complete blood count and routine biochemical determinations compared with the previous results (Table 1). Arterial blood gas analysis indicated hypoxia (PaO<sub>2</sub> 72 mmHg) which was poorly oxygen responsive. Echocardiography revealed no obvious cardiac or pulmonary arterial abnormalities and thoracic radiography revealed a mild bronchial pattern of unknown significance.

Due to the clinical suspicion of pulmonary thromboembolism, heparin sodium (Heparin Mucous Injection™; LEO Laboratories Limited, Princes Risborough, UK) was given at a dosage of 100 IU/kg three times daily, initially intravenously and then by subcutaneous injection. Supportive therapy also included nasal oxygen therapy and intravenous fluid therapy with compound sodium lactate solution (Aquapharm™; Animalcare Limited, York, UK) at a dosage of 2 mL/kg/h.

Bacterial culture of the cutaneous nasal lesions isolated a pure growth of *Serratia odorifera*, sensitive to gentamicin and ciprofloxacin. A mixed bacterial growth with no

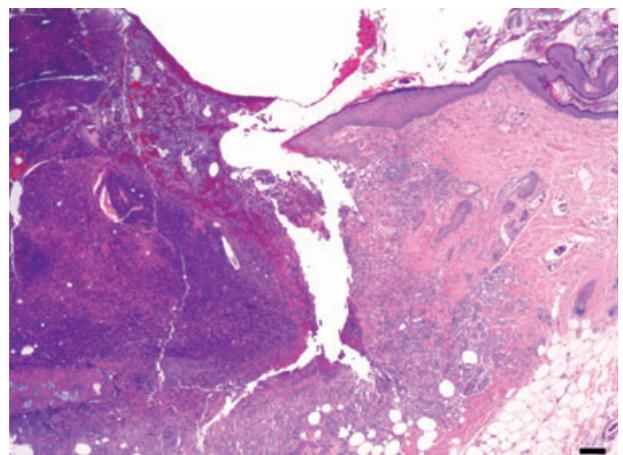
predominant organism was isolated from the lesions on the flank, lips and tongue but was not considered significant. Because of the possibility of systemic, pulmonary or urinary tract bacterial infections, the clavulanic acid-potentiated amoxicillin was stopped, and blood and urine samples were collected for culture. Intravenous marbofloxacin (Marbocyl™; Vetoquinol, Buckingham, UK) at a dosage of 2 mg/kg once daily was begun while awaiting the culture results. The possibility of a pulmonary fungal infection was also considered but the radiographs did not support this.

The punch biopsies of ulcerated and crusted lesions were routinely processed in paraffin wax, sectioned at 4 µm, and stained with haematoxylin and eosin (H&E), periodic acid–Schiff (PAS) and Ziehl–Neelsen (ZN) stains.

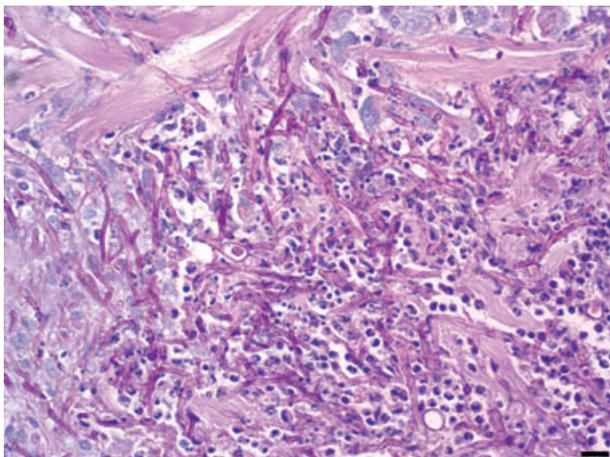
Examination of H&E stained biopsies from the lateral thigh and shoulder regions were similar, and demonstrated infiltrations of bloated macrophages with irregular foci of mature neutrophils in the dermis immediately below and contiguous with the degenerate epidermis, extending down to involve the upper sub-dermal fat layer. This cellular infiltration disrupted the dermal collagen and obliterated the adnexae. In sections from the shoulder, the dermal cellular reaction was frequently semi-nodular with central necrosis surrounded by neutrophils and macrophages.

The nasal biopsy sections showed a uniform, slightly melanotic epidermis which exhibited mild hyperkeratosis and an intact basement membrane. In the underlying dermis, there were irregularly nodular and coalescent, non-encapsulated foci of bloated macrophages with more sparse, irregular infiltrations of mature neutrophils, again with obliteration of dermal collagen and adnexae. Occasional solitary giant cells with central eosinophilic cytoplasmic inclusions were seen. The cellular reaction extended a short distance into the sub-dermal fat layer, in a manner similar to that observed in the other skin lesions (Figure 5).

There were no acid-fast organisms present in the ZN-stained sections. Staining with PAS, however,



**Figure 5.** Histopathology from the lesion on the lateral left thigh. Note the extensive ulceration and the diffuse inflammatory infiltrate extending from the dermis down into the sub-dermal fat layer. Haematoxylin and eosin stain, magnification × 40. Bar = 100 µm.



**Figure 6.** Histopathology from the ulcerated lesion on the lateral left thigh. Note the presence of many fungal hyphae and sporulating body-like forms in the section. Periodic acid–Schiff stain, magnification  $\times 400$ . Bar = 20  $\mu\text{m}$ .

revealed many fungal hyphae and sporulating bodies in the centres of the nodular and more diffuse cellular reactions (Figure 6). Fungal hyphae, together with inflammatory cells, extended up to the basement membrane but did not breach the epidermis nor were they detected in the crusts over the degenerate epidermis.

Over the following 5 days, the dog's physical condition gradually improved. No microorganisms were cultured from either blood or urine. A complete blood count and serum biochemical examinations were performed every 48 h (Table 1). Indices of hepatic status remained markedly elevated, but did not rise further.

On day 13, the prednisolone dosage was reduced from 0.7 mg/kg twice daily to the same dose once daily in the morning. The skin and oral lesions were drying and contracting. Daily baths with a shampoo containing 2% chlorhexidine and 2% miconazole (Malaseb™ shampoo; Dechra, Shrewsbury, UK) were introduced to speed up resolution.

Samples from both the exudate and the cutaneous biopsies were plated directly onto Mycosept™ Agar (E&O Laboratories Ltd, Stirlingshire, UK) and Potato Dextrose Agar (Oxoid, Basingstoke, Hampshire, UK poured in house) and incubated at 25 °C. Pure growths of numerous colonies of a species of the genus *Alternaria* were obtained from the exudate and biopsies of cutaneous lesions. No fungi were cultured from the samples obtained from the oral lesions. In order to confirm the causal fungal species, paraffin embedded tissue was submitted for polymerase chain reaction (PCR) analysis. Briefly, after DNA extraction, ITS regions of ribosomal RNA gene were amplified using a nested PCR with primer pairs V9G/LS266 and ITS5/ITS4. The reaction products showed multiple bands in electrophoresis with 1% agarose gel. Bands between 450 and 900 bp were excised from the gel and purified. After cloning, two different sequences were obtained. With BLASTn search in GenBank, one showed 100% similarity with *Candida sake* (AJ549822), while another had more than 99.4% homology with *Alternaria infectoria* (523/524 with AY154688,



**Figure 7.** Nasal lesion 40 days after initial presentation.

514/517 with Y17067)<sup>a</sup> and only 92% (422/454) homology with *Alternaria alternata* (U05195).

The dog was discharged on day 13 on the medications and dosages as above. Further re-inspections were performed after a further 3 (day 16) and 5 (day 18) days and subsequently every 2 weeks for the following 4 months. The dog's physical condition and blood parameters continued to progressively improve (data not shown). The marbofloxacin was stopped after 1 month. The prednisolone was gradually reduced by small decrements and, together with the famotidine and sucralfate, was stopped 5 months after initial re-presentation.

The skin lesions showed gradual improvement at each re-inspection with absence of exudate and gradual reduction in size. Cytological examinations were performed at each re-inspection and were consistently negative for fungal elements from day 30, initially showing merely pyogranulomatous inflammation from the healing lesions. On day 40, all cutaneous and lingual lesions had healed with the exception of a 10 by 5 mm erosion on the dorsal muzzle (Figure 7); no fungi or bacteria were isolated on culture and this lesion resolved in a further 14 days. The claw of the fourth digit of the left forefoot remained black but no signs of active infection were visible and normal claw tissue was starting to grow.

Twelve months after clearance of all the skin lesions, the dog remains well but has a continuing hepatopathy with persistent elevations in serum alanine aminotransferase and alkaline phosphatase for which the owner has declined further investigations. The dog is receiving no immunosuppressive therapy and there has been no recurrence of either the cutaneous or oral lesions or of the immune-mediated haemolytic anaemia.

## Discussion

Dematiaceous fungi are responsible for a variety of cutaneous, subcutaneous and systemic conditions. In humans, infection may result from either marked (e.g. penetrating wounds) or minor (e.g. abrasions) skin trauma. Predisposed sites are, therefore, the extremities

<sup>a</sup>The sequences of the isolated *Alternaria infectoria* will shortly be deposited in GenBank.

of the arms and legs.<sup>13–15</sup> However, although traumatic damage is hypothesised in the published reports it is rarely proven. A similar distribution has been seen in the occasional reports of canine phaeohyphomycosis.<sup>16,17</sup> In the present case, the lesions detected on the muzzle and forelegs could be easily attributed to minor traumatic injury. However, those on the lateral thigh, the ventral neck and the flank are more difficult to explain as these areas are covered by a thicker hair coat and would generally be expected to be subject to less accidental trauma. Despite the fact that there was no macroscopic or microscopic skin thinning, it is possible that an increased susceptibility to infection resulted from general cutaneous debility or impaired local immunity due to the prolonged use of glucocorticoids.<sup>18</sup>

In general, disseminated cutaneous and systemic forms of phaeohyphomycosis are thought to involve either haematogenous or lymphatic spread of an infection initially localized to the skin, as well as possible respiratory exposure with subsequent haematogenous spread.<sup>13,16,17,19–23</sup> In the present case, no compatible systemic signs were detected and undetected systemic involvement is unlikely to have occurred considering the rapidity of recovery after discontinuation of the immunosuppressive medication. Possible explanations for the presence of the cutaneous lesions in numerous areas of the body would be a local topical seeding of the infection caused by the dog during grooming or repeated infection from the environment associated with depressed immune status and poor cutaneous barrier function as described above.

In the present case, a number of different macroscopic skin and mucosal lesions were seen. Skin erosions, ulcers and crusts on the trunk and limbs, onychorrhexis and claw pigmentation, a plaque on the tongue and ulcerated nodules on the lips were all present. However, a casual agent was identified only from the ulcers and crusts on the skin. Despite this, it is likely that the lesions all had a common aetiology. Ulcers and crusts have also been reported in previous canine cases of cutaneous and subcutaneous phaeohyphomycosis.<sup>16,17</sup> Isolated nodular lesions are more often described in cats.<sup>4,5</sup>

In affected humans, infection with *Alternaria* spp. may result in melanonychia, onychomycosis, onycholysis and onychomadesis.<sup>24,25</sup> Concurrent paronychia was also described in a cat affected by cutaneous alternariosis, but this was not confirmed as being caused by the fungal infection.<sup>4</sup> In the present case, samples from the affected pigmented claw were not submitted for mycological examination and, although possible, it was considered unlikely that the claw was affected by onychomycosis. Fungal infections of claws usually require a minimum of 4–6 months to heal completely, while in this case normal claw tissue started to be obvious after approximately 40 days.

An unspciated organism of the genus *Alternaria* was reported to cause a single, oral, nodular lesion in an affected human.<sup>26</sup> In the present case, the oral lesions were, perhaps, macroscopically more comparable with the lesions described in candidiasis of humans<sup>27</sup> and, more rarely, dogs.<sup>28</sup> However, material from the lip and tongue lesions failed to yield fungal elements on culture

and, as biopsy would have required general anaesthesia, this was not carried out on this compromised patient. Thus, the cause of the oral lesions was not definitively diagnosed and they may have been due to bacteria, *Alternaria infectoria*, *Candida* spp. or to a different organism to those isolated from this case. Although DNA sequencing revealed the presence of *Candida sake* in the cutaneous lesions, this result was regarded as clinically not significant as there was no histological evidence of dermal candidiasis (such as budding spores or pseudohyphae) and it was not cultured from the cutaneous or oral lesions. To the authors' knowledge, infection with *Candida sake* has never been reported in dogs. This organism is known to be a common part of the normal flora of human skin.<sup>29</sup> Its presence in this case is likely to have resulted from contamination.

*Alternaria* spp. are commonly found on the coat of both dogs and cats. In studies of fungal carriage in healthy dogs and cats with no skin lesions, between 20% and 80% of isolates were *Alternaria* spp.<sup>30–34</sup> This genus has also been reported as a common laboratory contaminant.<sup>35</sup> *Alternaria infectoria*, in particular, is a complex of closely interrelated entities that are commonly found colonizing hard plant debris, particularly wheat straw.<sup>36</sup> Thus, the definitive diagnosis of infection requires demonstration of fungal hyphae in histopathological preparations, isolation on fungal culture and PCR analysis to confirm the identity of *Alternaria infectoria*.

Agents of phaeohyphomycosis usually form pigment in culture, but, as in the present case, pigmentation is not always evident in H&E stained tissues.<sup>16</sup> Fontana-Masson staining may be helpful for diagnosis of phaeohyphomycosis as it enhances the staining of the melanin in lightly pigmented organisms.<sup>37</sup> However, in the present case, this was not necessary as the species of fungal organism was identified by sequencing the internal transcribed spacer 1 region of the rRNA gene.

In humans, treatment for cutaneous alternariosis has not been standardized as different outcomes have been seen in different individuals in response to various medications.<sup>22,35,38</sup> As a general rule, reduction of immunosuppression, when possible, is considered fundamental for the resolution of infection. Surgical excision, on the other hand, is the best way to deal with a localized lesion that can easily be removed. In the present case, the fungal species responsible for the infection was not known until day 13 after presentation and, although the majority of the fungal infections can be treated successfully with the azole group of drugs, there are a number that may not respond to this group of compounds.<sup>39</sup> Because systemic antifungal medications have the potential to be hepatotoxic and this patient's hepatic status was compromised, it was decided to withhold, at least initially, systemic antifungal medication until the responsible species was known and the effects of reducing the immunosuppressive drug dosage had been evaluated. While a topical combination of 2% chlorhexidine and 2% miconazole (Malaseb™ shampoo; Dechra, Shrewsbury, UK) is licensed as an aid in the control of *Microsporum canis* infection in cats, all investigative studies were carried out in conjunction with systemic therapy.<sup>40</sup> It is unlikely that the use of this preparation

alone would have resulted in clearing of the infection in this case. Reduction of the immunosuppressive therapy resulted in gradual spontaneous resolution of the problem and within 40 days fungal elements were no longer isolated.

Case reports of phaeohyphomycosis in the human literature have increased significantly in the last few decades.<sup>29</sup> Immunosuppressive treatment following organ transplantation, human immunodeficiency virus infection and administration of chemotherapeutic agents for the management of malignancy are the most common predisposing factors for opportunistic infections.<sup>41,42</sup> Four reported cases of phaeohyphomycosis in dogs caused by *Curvularia lunata* on two occasions and *Ochroconis gallopavum* and *Drechslera spicifera* in the others were associated with immunosuppression: in two due to prolonged treatment with glucocorticoids;<sup>16,21</sup> in one a combination of glucocorticoids and ciclosporin;<sup>17</sup> and in one severe hepatic disease.<sup>43</sup> Prolonged administration of prednisolone together with ciclosporin is thought to have been responsible for the development and maintenance of the *A. infectoria* infection in the present case.

*Serratia odorifera* is well recognized as a common, opportunistic, nosocomial infection in humans<sup>44</sup> (where it is most often isolated from debilitated subjects) and animals.<sup>45</sup> It is a Gram-negative rod, considered to have low pathogenicity in otherwise healthy subjects but having the potential to cause severe infections in individuals with debilitating underlying conditions. In the current case, *Serratia odorifera* could not be considered as a nosocomial infection as it was isolated from the dog's skin from the sample collected immediately on admission. Compared with the situation in humans, the organism seemed of low pathogenicity and cleared rapidly with antibacterial treatment, in spite of the existing level of immunosuppression in this case. Despite only testing the sensitivity of this organism against one fluoroquinolone (ciprofloxacin), the dog was treated with marbofloxacin which cleared the infection rapidly. It has been common for veterinary laboratories, including our own, to use sensitivity standards for ciprofloxacin as representative of those of other fluoroquinolones used in animals. Recent work, however, has suggested that this procedure is actually inaccurate, at least for some bacterial organisms.<sup>46,47</sup>

The major protective mechanism against both the development and resolution of fungal infections is cell mediated immunity although the humoral response is also thought to play a role.<sup>11</sup> Both glucocorticoids and ciclosporin particularly impair cell mediated immunity. Glucocorticoids decrease neutrophil, monocyte and lymphocyte chemotaxis, macrophage phagocytosis, and activation and cytotoxicity of T-cells.<sup>48</sup> Ciclosporin reduces cytokine gene activation preventing the synthesis of various cytokines, in particular interleukin (IL)-2. It also inhibits early T-cell activation and stimulates the production of transforming growth factor (TGF)- $\beta$ , which is a potent inhibitor of IL-2-stimulated T-cell proliferation.<sup>48</sup>

This case highlights once more the potential for the development of opportunistic infections following immunosuppressive therapy, as well as the importance of iden-

tifying and removing, where possible, potential causes of immunosuppression in animals with infectious diseases. Stopping ciclosporin administration and reducing the dosage of prednisolone resulted in spontaneous resolution of the fungal infection, illustrating the importance of inherent immunity against opportunistic infections. Cases in which the immunosuppressive stimulus cannot be removed may carry a poor prognosis even when managed with the correct therapeutic agents.

## Acknowledgements

The authors are grateful to WD Gram for his help with some dermatological aspects of the case and to JW Simpson and AG Gow who assisted with the management of the immune-mediated haemolytic anaemia.

## References

- Pastor FJ, Guarro J. *Alternaria* infections: laboratory diagnosis and relevant clinical features. *Clinical Microbiology and Infection* 2008; 14: 734–46.
- Bush RK, Prochnau JJ. *Alternaria*-induced asthma. *Journal of Allergy and Clinical Immunology* 2004; 113: 227–34.
- Simmons EG. *Alternaria: an identification manual* 1st edn. Utrecht: CBS Fungal Biodiversity Centre, 2007: p. 1–775.
- Dye C, Johnson EM, Gruffydd-Jones TJ. *Alternaria* species infection in nine domestic cats. *Journal of Feline Medicine and Surgery* 2009; 11: 332–6.
- McKay JS, Cox CL, Foster AP. Cutaneous alternariosis in a cat. *Journal of Small Animal Practice* 2001; 42: 75–8.
- Outerbridge CA, Myers SL, Summerbell RC. Phaeohyphomycosis in a cat. *Canadian Veterinary Journal* 1995; 36: 629–30.
- Roosje PJ, de Hoog GS, Koeman JP *et al.* Phaeohyphomycosis in a cat caused by *Alternaria infectoria* E.G. Simmons. *Mycoses* 1993; 36: 451–4.
- Grooters AM, Foil CS. Miscellaneous fungal infections. In: Green CE, ed. *Infectious diseases of the dog and cat*, 3rd edn. St. Louis, Saunders Elsevier, 2006: 647–9.
- Andersson M, Downs S, Mitakakis T *et al.* Natural exposure to *Alternaria* spores induces allergic rhinitis symptoms in sensitized children. *Pediatric Allergy and Immunology* 2003; 14: 100–5.
- de Hoog GS, Horr r R. Molecular taxonomy of the *Alternaria* and *Ulocladium* species from humans and their identification in the routine laboratory. *Mycoses* 2002; 45: 259–76.
- Blanco JL, Garcia M. Immune response to fungal infections. *Veterinary Immunology and Immunopathology* 2008; 125: 47–70.
- Tennant K, Patterson-Kane J, Boag AK *et al.* Nasal mycosis in two cats caused by *Alternaria* species. *Veterinary Record* 2004; 155: 368–70.
- Romano C, Vanzi L, Massi D *et al.* Subcutaneous alternariosis. *Mycoses* 2005; 48: 408–12.
- Vieira R, Veloso J, Afonso A *et al.* Cuaneous alternariosis in a liver transplant recipient. *Revista Iberoamericana de Micolog a* 2006; 23: 107–9.
- Halaby T, Boots H, Vermeulen A *et al.* Phaeohyphomycosis caused by *Alternaria infectoria* in a renal transplant recipient. *Journal of Clinical Microbiology* 2001; 39: 1952–5.
- Herr rez P, Rees C, Dunstan R. Invasive phaeohyphomycosis caused by *Curvularia* species in a dog. *Veterinary Pathology* 2001; 38: 456–9.
- Swift IM, Griffin A, Shipstone MA. Successful treatment of disseminated cutaneous phaeohyphomycosis in a dog. *Australian Veterinary Journal* 2006; 84: 431–5.
- Machet L, Jan V, Machet MC *et al.* Cutaneous alternariosis: role of corticosteroid-induced cutaneous fragility. *Dermatology* 1996; 193: 342–4.

19. Mariani CL, Platt SR, Scase TJ *et al.* Cerebral phaeoophomycosis caused by *Cladosporium* spp. in two domestic shorthair cats. *Journal of the American Animal Hospital Association* 2002; 38: 225–30.
20. Bouljihad M, Lindeman CJ, Hayden DW. Pyogranulomatous meningoencephalitis associated with dematiaceous fungal (*Cladophialophora bantiana*) infection in a domestic cat. *Journal of Veterinary Diagnostic Investigation* 2002; 14: 70–2.
21. Singh K, Flood J, Welsh RD *et al.* Fatal systemic phaeoophomycosis caused by *Ochroconis gallopavum* in a dog. *Veterinary Pathology* 2006; 43: 988–92.
22. Montserrat G, Bartralot R, Torres JM *et al.* Cutaneous alternariosis in transplant recipients: clinicopathologic review of 9 cases. *Journal of Allergy and Clinical Immunology* 2005; 52: 653–9.
23. Revankar SG, Sutton DA, Rinaldi MG. Primary central nervous system phaeoophomycosis: a review of 101 cases. *Clinical Infectious Diseases* 2004; 38: 206–16.
24. Romano C, Paccagnini E, Difonzo EM. Onychomycosis caused by *Alternaria* spp. in Tuscany, Italy from 1985 to 1999. *Mycoses* 2001; 44: 73–6.
25. Veer P, Patwardhan NS, Damle AS. Study of onychomycosis: prevailing fungi and pattern of infection. *Indian Journal of Medical Microbiology* 2007; 25: 53–6.
26. Cardoso SV, Campolina SS, Guimaraes AL *et al.* Oral phaeoophomycosis. *Journal of Clinical Pathology* 2007; 60: 204–5.
27. Janik MP, Heffernan MP. Yeast infections: Candidiasis and Tinea (Pityriasis) Versicolor. In: Wolff K, Goldsmith LA, Katz SI *et al.*, eds. *Fitzpatrick's Dermatology in General Medicine*, 7th edn. New York, McGraw-Hill, 2008: 1822–3.
28. Jadhav VJ, Pal M. Canine mycotic stomatitis due to *Candida albicans*. *Revista Iberoamericana de Micología* 2006; 23: 233–4.
29. de Hoog GS, Guarro J, Gené J *et al.* *Atlas of Clinical Fungi*, 2nd edn. Utrecht/Reus: ASM press, 2001.
30. Philpot CM, Berry AP. The normal fungal flora of dogs. A preliminary report. *Mycopathologia* 1984; 87: 155–7.
31. Moriello KA, DeBoer DJ. Fungal flora of the coat of pet cats. *American Journal of Veterinary Research* 1991; 52: 602–6.
32. Cabanes FJ, Abarca ML, Bragulat MR *et al.* Seasonal study of the fungal biota of the fur of dogs. *Mycopathologia* 1996; 133: 1–7.
33. Simpanya MF, Baxter M. Isolation of fungi from the pelage of cats and dogs using the hairbrush technique. *Mycopathologia* 1996; 134: 129–33.
34. Bernardo F, Lanca A, Guerra MM *et al.* Dermatophytes isolated from pet, dogs and cats, in Lisbon, Portugal (2000–2004). *Revista Portuguesa de Ciências Veterinárias* 2005; 100: 85–8.
35. Lyke KE, Miller NS, Towne L *et al.* A case of cutaneous ulcerative alternariosis: rare association with diabetes mellitus and unusual failure of itraconazole treatment. *Clinical Infectious Diseases* 2001; 32: 1178–87.
36. Andersen BS, Sørensen JL, Nielsen KF *et al.* A polyphasic approach to the taxonomy of the *Alternaria infectoria* species-group. *Fungal Genetics and Biology* 2009; 46: 642–56.
37. Kwon-Chung KJ, Hill WB, Bennett JE. New, special stain for histopathological diagnosis of cryptococcosis. *Journal of Clinical Microbiology* 1981; 13: 383–7.
38. Sharkey PK, Graybill JR, Rinaldi MG *et al.* Itraconazole treatment of phaeoophomycosis. *Journal of the American Academy of Dermatology* 1990; 23: 577–86.
39. Chen SCA, Sorrell TC. Antifungal agents. *Medical Journal of Australia* 2007; 187: 404–9.
40. Paterson S. Miconazole/chlorhexidine shampoo as an adjunct to systemic therapy in controlling dermatophytosis in cats. *Journal of Small Animal Practice* 1999; 40: 163–6.
41. Revankar SG, Patterson JE, Sutton DA *et al.* Disseminated phaeoophomycosis: review of an emerging mycosis. *Clinical Infectious Diseases* 2002; 34: 467–76.
42. Gerdson R, Uerlich M, de Hoog GS *et al.* Sporotrichoid phaeoophomycosis due to *Alternaria infectoria*. *British Journal of Dermatology* 2001; 145: 484–6.
43. Kwochka KW, Maron BCM, Ajello L *et al.* Canine phaeoophomycosis caused by *Drechslera spicifera*: a case report and literature review. *Journal of the American Animal Hospital Association* 1983; 20: 625–33.
44. Mermel LA, Spiegel CA. Nosocomial sepsis due to *Serratia odorifera* biovar 1. *Clinical Infectious Diseases* 1992; 14: 208–10.
45. Lobetti RG, Joubert KE, Picard J *et al.* Bacterial colonization of intravenous catheters in young dogs suspected to have parvoviral enteritis. *Journal of the American Veterinary Medical Association* 2002; 220: 1321–24.
46. Riddle C, Lemons CL, Papich MG *et al.* Evaluation of ciprofloxacin as a representative of veterinary fluoroquinolones in susceptibility testing. *Journal of Clinical Microbiology* 2000; 38: 1636–7.
47. Wildermuth BE, Griffin GE, Rosenkrantz WS *et al.* Susceptibility of *Pseudomonas* isolates from the ears and skin of dogs to enrofloxacin, marbofloxacin and ciprofloxacin. *Journal of the American Animal Hospital Association* 2007; 43: 337–41.
48. Gregory CR. Immunosuppressive agents. In: Bonagura JD, Twedt DC, ed. *Kirk's Current Veterinary Therapy XIV*. MO, Saunders Elsevier, 2009: 509–13.

**Résumé** Une Springer spaniel anglais de 4 ans, ovariohystérectomisée, sous thérapie immunosuppressive, a été ré-examinée pour le suivi de ses anémies hémolytiques à médiation immune et le développement récent de lésions cutanées. Au cours des 3 mois depuis sa sortie de l'hôpital, l'animal a reçu 1.3 mg/kg de prednisolone et 2.6 mg/kg de ciclosporine par voie orale toutes les 12 heures. L'examen général a révélé une hépatomégalie et de multiples lésions purulentes, crouteuses, érosives à ulcéraires sur différentes régions du corps. Un doigt était atteint d'onychorrhéxis et la peau sous-jacente avait noirci. Il y avait deux lésions prolifératives et une lésion en plaque dans la gueule. Des hyphes fongiques à paroi épaisse ont été mis en évidence par calque direct de toutes les lésions cutanées et une coloration à l'acide périodique de Schiff a confirmé la présence de multiples hyphes et spores fongiques dans toutes les biopsies examinées. La culture mycologique a permis l'isolement pur d'*Alternaria* sp. identifié par séquençage comme *A. infectoria*. L'état de l'animal n'a pas permis d'explorer les lésions orales. L'arrêt de la ciclosporine et la diminution de la prednisolone a permis une guérison complète des lésions cutanées en 40 jours. Une diminution jusqu'à l'arrêt de la prednisolone a été réalisée sans réapparition de l'anémie hémolytique à médiation immune. Après 12 mois, aucune récurrence des lésions cutanées ou de l'anémie n'a été observée. A la connaissance de l'auteur, il s'agit du premier cas d'infection à *A. infectoria* chez un chien.

**Resumen** Una perra de 4 años de edad, ovariohisterectomizada de raza Springer Spaniel Inglés en terapia inmunosupresora fue reexaminada para evaluar la anemia hemolítica inmunomediada y el desarrollo reciente de lesiones de la piel. Durante tres meses tras ser dispensada del hospital la perra había estado recibiendo 1,3 mg/kg de prednisolona y 2,6 mg/kg de ciclosporina administradas por vía oral cada 12 horas. El examen físico reveló hepatomegalia y lesiones múltiples, purulentas, costrosas, erosivas a ulcera-

tivas en diferentes zonas del cuerpo. Se había producido onicorrexis en un dedo y el corion interno estaba de color negruzco. Había dos lesiones proliferativas y una lesión tipo placa en la boca. Se detectaron hifas fúngicas de pared gruesa en extensiones por impresión de todas las lesiones de la piel y la tinción con ácido periódico de Schiff confirmó la presencia de numerosas hifas fúngicas y esporas en las biopsias examinadas. El cultivo fúngico aisló una población elevada y pura de *Alternaria* sp. que se identificó como *A. infectoria* mediante secuenciación de la región 1 del espaciador de transcripción interna del gen de rRNA. La condición del animal evitó una investigación mas detallada de las lesiones orales. La interrupción del tratamiento con ciclosporina y la reducción de la dosis de prednisolona resultaron en la resolución espontánea de las lesiones de la piel en 40 días. Subsecuentes reducciones graduales de la prednisolona hasta cero se llevaron a cabo sin recurrencia de la anemia hemolítica inmunomediada. Tras 12 meses no ha habido recurrencia ni de las lesiones de la piel ni de la anemia. A nuestro entender, este es el primer caso descrito de infección *A. infectoria* en un perro.

**Zusammenfassung** Eine vier Jahre alte, kastrierte English Springer Spaniel Hündin, die immunsuppressive Therapie erhielt, wurde nochmals untersucht, um ihre immun-medierte hämolytische Anämie und die jüngste Entwicklung der Hautveränderungen zu überprüfen. In den drei Monaten seit sie aus dem Tierspital entlassen worden war, erhielt die Hündin 1,3mg/kg Prednisolon und 2,6 mg/kg Ciclosporin alle 12 Stunden per os. Bei der Untersuchung bestanden Hepatomegalie und multiple, purulente, krustige, erosive bis ulzerierte Veränderungen an verschiedenen Körperstellen. Eine Zehe zeigte Onychorrhhexis und die darunterliegende Lederhaut war schwarz verfärbt. Im Maul bestanden zwei proliferative und eine Plaque-ähnliche Veränderung. Dickwandige Pilzhyphen wurden auf Abklatschpräparaten aller Hautveränderungen gefunden und mittels „Periodic Acid Schiff“ Färbung das Vorhandensein von multiplen Pilzhyphen und Sporen auf allen untersuchten Biopsien bestätigt. Durch eine Pilzkultur wurde ein starkes, reines Wachstum einer *Alternaria* sp. isoliert, die durch Sequenzierung der internen transkribierten Spacer 1 Region des rRNA Gens als *A. infectoria* identifiziert wurde. Der Zustand des Tieres machte eine genaue Untersuchung der oralen Läsionen unmöglich. Das Absetzen von Ciclosporin und eine Reduktion der Prednisolon-Dosis resultierten in einer Spontanheilung der Hautveränderungen innerhalb von 40 Tagen. Eine weitere graduelle Absenkung der Prednisolon-Dosis auf null konnte ohne ein Wiederauftreten der immun-medierten hämolytischen Anämie durchgeführt werden. Nach 12 Monaten kam es weder bei den Hautveränderungen zum Rezidiv noch kam es zum Wiederauftreten der Anämie. Nach dem Wissen der Autoren handelt es sich hierbei um den ersten beschriebenen Bericht einer *A. infectoria* Infektion bei einem Hund.