Disseminated *Penicillium radicum* infection in a dog, clinically resembling multicentric malignant lymphoma

Gedissemineerde Penicillium radicum *infectie bij een hond klinisch gelijkend op een multicentrisch maligne lymfoom*

[1]J.P. de Vos, [2]E. van Garderen, [3]H. Hensen, [4]I. Tange, [5]I. Curfs-Breuker, [1]B. Vandevelde, [5]J. F. Meis

[1]De Ottenhorst, Clinic for Companion Animal Medicine and Veterinary Oncology Referral Centre, van Diemenstraat 83, 4535 AR Terneuzen, The Netherlands
[2]Laboratory for Pathology and Histology, Animal Health Service, Postbus 9, 7400 AA Deventer, The Netherlands
[3]Veterinary Clinic IJzendijke, Minnepoortstraat 14, 4515 BC, IJzendijke, The Netherlands
[4]Zorgsaam Foundation Zeeuws-Vlaanderen, Laboratory of Medical Microbiology, Wielingenlaan 2, 4535 PA Terneuzen, The Netherlands
[5]Department of Medical Microbiology and Infectious Diseases C70, Mycologie, Canisius-Wilhelmina Hospital, Weg door Jonkerbos 100, 6532 SZ Nijmegen, The Netherlands

jpdevos@zeelandnet.nl

ABSTRACT

After detecting fungal organisms in smears of enlarged peripheral lymph nodes in a dog, determination was performed through culturing and genetic typing. The fungus was identified as *Penicillium radicum* by amplification of fungal DNA encoding of the ribosomal internal transcribed spacer region and comparison with DNA databases. In vitro susceptibility testing revealed multiresistance. In the literature, *P. radicum* is mentioned as a phosphate solubilizing agent used in agriculture for promoting plant growth. This is the first publication of a disseminated *P. radicum* infection in a dog. The generalized lymphadenopathy and hypercalcemia strongly resembled a multicentric lymphoma.

Hypercalcemia in granulomatous diseases, including disseminated fungal infections, is caused by activated macrophages possessing 1α-hydroxylase, which is able to convert 25-hydroxyvitamin-D into calcitriol. Although disseminated fungal infections are extremely rare in The Netherlands and Belgium, they should be included in the differential diagnosis of dogs with multicentric lymphadenopathy and hypercalcemia.

SAMENVATTING

In uitstrijkjes van vergrote perifere lymfeknopen van een hond werden schimmelstructuren aangetoond. De determinatie vond plaats via kweekmedia en een genetische typering. Het DNA dat codeert voor de "ribosomal internal transcribed spacer region" werd vermenigvuldigd, vergeleken met openbare DNA-databases en geïdentificeerd als overeenstemmend met dat van *Penicillium radicum*. *In vitro* gevoeligheidstesten toonden een multiresistente schimmel aan. Het gebruik van *P. radicum* in de landbouw wordt beschreven om plantengroei te stimuleren.

Door de gegeneraliseerde lymfeknoopzwelling en hypercalcemie vertoonde het ziektebeeld een sterke gelijkenis met een multicentrisch lymfoom. Hypercalcemie in granulomateuze ziektebeelden, inclusief gedissemineerde schimmelinfecties, wordt veroorzaakt door de omzetting van 25-hydroxyvitamine-D in calcitriol door geactiveerde macrofagen die 1α-hydroxylase bevatten. In Nederland en België komen systemische schimmelinfecties sporadisch voor maar ze dienen toch te worden opgenomen in de differentiaaldiagnose van honden met gegeneraliseerde lymfeknoopzwelling en hypercalcemie. Dit is de eerste publicatie van een gedissemineerde *P. radicum* infectie bij een hond.

INTRODUCTION

The diagnosis in animals of disseminated fungal infections, which are extremely rare in The Netherlands and Belgium, can be complicated. Most mycoses are easily confused with other disease entities, since the clinical picture of an animal with systemic mycosis is very unspecific. The causative agent needs to be promptly diagnosed and identified, usually by a combined microbiological and histopathological/cytological examination, if the infection is to be correctly treated and resolved. The principal route of infection for fungal diseases in dogs is inhalation, from which dissemination can occur. Occasionally, direct wound contamination and ingestion also play an important role in the pathogenesis of the disease, especially in histoplasmosis (Krohne, 2000).

The purpose of this report is to describe a case of

disseminated canine fungal infection. To the best of our knowledge, this is the first report of a disseminated *P. radicum* infection in a dog. Furthermore, infection in humans with this species is not mentioned in an extensive review of invasive disease due to Penicillium species other than *P. marneffii* (Lyratzopoulos *et al.*, 2002). In this series, *P. purpurogenum*, *P. brevicompactum* and *P. piceum* were among the most identified species. In humans, systemic infections with Penicillium species other than *P. marneffei* are rare, due to the inability of most non-marneffei species to grow at 37°C (Santos *et al.*, 2006; Horré *et al.*, 2001).

CASE REPORT

A 4-year-old male Dutch Braque with a body weight of 13.8 kg was presented in November 2007 with a mass, 3.5 cm in diameter, located in the left-ventral subcutaneous tissues of the neck. A complete physical examination showed no other abnormalities. A fine needle aspiration biopsy of the mass was performed and cytological examination revealed small lymphocytes, a moderate number of neutrophils, some plasma cells, and intracellular fungal hyphae in macrophages. A blood examination consisting of a complete blood count (CBC) and routine serum biochemistry profile showed no abnormalities, although no blood calcium concentration was determined at that time. The dog was treated with a total dose of 100 mg ketoconazole[a] once daily per os for 3 weeks.

One week after end of therapy, the dog was submitted again with fever, anorexia and a stiff gait. The mass in the neck had doubled in size. Treatment was started with 5 mg/kg enrofloxacin[b] once daily per os and 10 mg/kg amoxicillin[c] twice daily per os. The mass was surgically removed, and post-operative incision showed the aspect of an abscess. Histopathological examination was not performed at that time. Wound healing was uneventful and antibiotic therapy was stopped 10 days after surgery.

Two months after surgery the dog developed signs of general fatigue, polydipsia and polyuria. On physical examination there was generalized lymphadenopathy and splenomegaly. Abnormalities noted on the serum biochemistry profile were a moderate hypercalcemia (3.32 mmol/l; reference range 1.98–3.00 mmol/l), slightly elevated creatinin (98 µmol/l; reference 70 µmol/l), and an increased ALAT (257 U/l; reference range 0–113 U/l). No abnormalities were observed on a CBC. A cranial mediastinal mass was detected on thoracic X-rays. In addition, enlarged lymph nodes were visible in the hilus of the lungs.

The findings were considered not to be related to the initial fungal abscess, and as a result the dog was referred to De Ottenhorst Clinic in Terneuzen, the Netherlands, with strong suspicion of multicentric T-cell lymphoma. On physical examination, all peripheral lymph nodes were moderately enlarged. Body temperature was normal. Abdominal palpation revealed splenomegaly and severely enlarged medial iliac lymph nodes. On ultrasound, the spleen showed hypo-echogenic nodules with a diameter of 5 – 10 mm. To investigate the presence of lymphoma, ultrasound guided, fine needle aspiration biopsies were performed of peripheral lymph nodes, the spleen, and the mediastinal and medial iliac lymph nodes, and submitted for cytological examination.

At the pathology laboratory, the air-dried smears were stained according to May-Grünwald Giemsa. Some smears from each location were not stained, but were rather stored temporarily to allow immunocytological analysis of the presumed lymphoma, thus establishing its T-cell or B-cell origin. However, cytologically the smears of the lymph nodes and the spleen were not consistent with lymphoma, since the lymphoid cells demonstrated a heterogeneous morphology, which is indicative of lymphoid cells in various stages of differentiation. This finding is consistent with reactive hyperplasia of the lymphoid cells and not with neoplastic proliferation. In addition, moderate numbers of neutrophils and macrophages were present in the smears. The macrophages had frequently fused into multinucleated giant cells. Remarkably, the presence of ill-defined cytoplasmic structures was noted in a significant number of macrophages and giant cells. Although these structures were not very well stained in the May-Grünwald Giemsa stain, their branching morphology was highly suggestive of phagocytized fungi (Figure 1). As a consequence, a presumptive diagnosis of a systemic mycosis was made on the basis of the cytological findings. In order to study the microscopic aspects of these fungi in more detail, the smears of the lymph nodes and spleen that were temporarily stored were fixed overnight in 10% buffered formalin (pH 7.2) and stained with periodic acid Schiff reagent (PAS). The fungal hyphae that were ill-defined in the May-Grünwald Giemsa stain demonstrated affinity for the PAS stain, and their presence in macrophages and multinucleated giant cells was clearly outlined (Figure 2).

Several fine needle aspirates were performed again aseptically from the prescapular lymph nodes and submitted for fungal culture. The aspirated material remained in the syringes and was inoculated in a laminar flow safety cabinet in Sabouraud agar and Malt extract agar within 30 minutes and incubated at 24°C and 37 °C. After 5 days of incubation, fungal cultures were clearly visible (Figures 3 and 4).

Microscopic characteristics of *Penicillium spp.* were seen after 14 days of incubation, which prompted a molecular approach, because classical morphological identification is inherently difficult. DNA was isolated from a 15-day-old pure colony using a standard protocol (Boom *et al.*, 1990). The genomic DNA encoding the ribosomal internal transcribed spacer (ITS) region was amplified by using 5'-TCCGTAGGTGAACCTGCGG-3' and 5'-TCCTCCGCTTATTGATATGC-3' primers.[d] All amplicons were purified by using High Pure chemistry,[e] and sequenced by using a MegaBACE DYEnamic ET Dye terminator kit,[f] as suggested by the manufacturer. Reaction products were purified by ethanol pre-

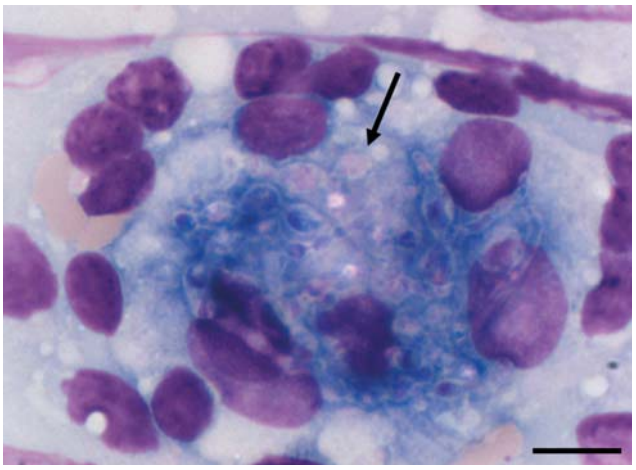

Figure 1. Smear of a fine needle aspirate of a prescapular lymph node, stained according to May Grünwald-Giemsa. In the center a giant cell is present that demonstrates cytoplasmic impressions of ill-defined fungal structures (arrow). The bar represents 10 µm.

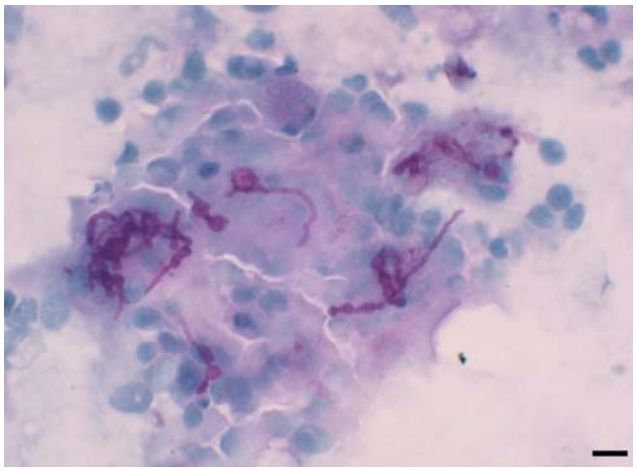

Figure 2. An air-dried smear of a prescapular lymph node was fixed overnight in 10% buffered formalin and stained with PAS showing fungi, macrophages and multinucleated giant cells. The bar represents 10 µm.

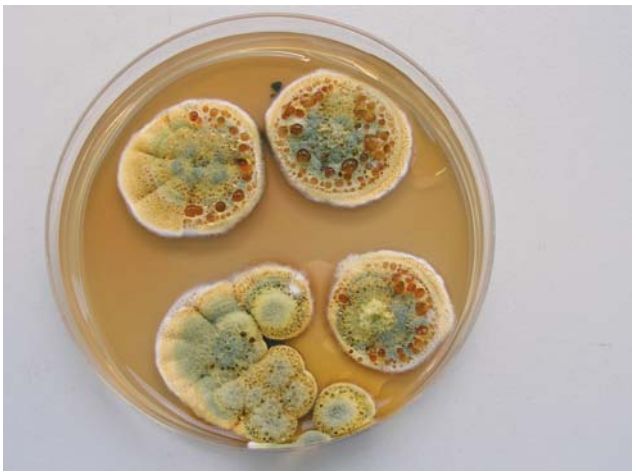

Figure 3. A colony of 14-day-old *P. radicum* on Sabouraud agar.

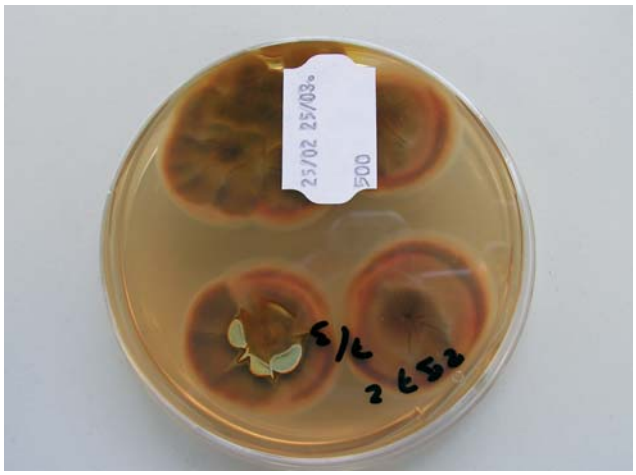

Figure 4. Reverse side of colony of *P. radicum*, with brownish-orange shades.

cipitation, dissolved in distilled water, and analyzed on a MegaBACE 500 capillary DNA analysis platform[g] under standard electrophoretic conditions. The ITS sequence obtained for the isolate was compared to the public DNA databases by using the BLAST interface at http://www.ncbi.nlm.nih.gov/BLAST/ (Altschul *et al.*, 1997), and proved to be 100% identical to previously reported *P. radicum* sequences. Molecular determination was confirmed by the CBS Fungal Biodiversity Center - KNAW, Utrecht, The Netherlands (http://www.cbs.knaw.nl), and the *P. radicum* isolate was deposited in the culture collection as CBS 122887.

The broth microdilution method was performed in duplo according to CLSI M38-A2 (Clinical and Laboratory Standards Insitute, 2008). Briefly, a spore suspension of the isolate was made in 0.9% sterile saline with 0.05% Tween 40. The optical density was adjusted to 80 to 82% T with a spectrophotometer at 530 nm, and then the isolate was diluted 1000 times in RPMI medium (final concentration 1-5 x 10^3 CFU/ml). One hundred microliters of the suspension were added to each well of a 96-well flat-bottom microtitration plate and incubated at 35°C for 48 hours. MIC was determined visually and spectrophotometrically as a ≥ 50% reduction of growth compared to that of the drug-free growth control for fluconazole. Also the lowest concentration of drug showing absence of growth was compared to that of the growth control for amphotericin B and the azoles. For caspofungin and anidulafungin, the MEC (minimum effective concentration) was determined microscopically as the lowest concentration in which abnormal, short and branched hyphal clusters were observed compared with the long, unbranched hyphal clusters that were seen in the growth control (Clinical and Laboratory Standards Insitute, 2008). The MIC or MEC for amphotericin B[i], itraconazole[j], fluconazole[k], voriconazole[k], anidulafungin[k], posaconazole[l], isavuconazole[m], and caspofungin[n] were 8, >16, >64, >16, 0.016, >16, 2, and >16 mg/L, respectively. The *P. radicum* isolate displayed resistance to the majority of the antifungals tested. Isavuconazole

had the lowest MIC (2 mg/L) compared with the other azoles and the MEC (0.016 mg/L) of anidulafungin suggested the isolate to be susceptible.

Meanwhile, the dog had difficulties in eating and was manually fed by the owner, but vomited almost after each meal. In expectation of the results of the antifungal susceptibility testing, therapy was started with 1 mg/kg maropitant[g] once daily as a subcutaneous injection and 5 mg/kg itraconazole[h] once daily per os. Vomiting stopped and three days after the beginning of therapy a moderate hypercalcemia (3.39 mmol/l; reference range 1.98–3.00 mmol/l) still remained.

Fourteen days after the beginning of itraconazole therapy, the condition of the dog still had not improved and the size of the lymph nodes had not decreased. The dog also developed uncontrolled muscular spasms in the head, an uncoordinated gait and an uncoordinated motoric activity when reaching out for food. Serum biochemistry profile abnormalities noted at that time included an increase in ureum concentration (14.8 mmol/l; reference range 2.3–9.1 mmol/l), a creatinin concentration of 133 µmol/l, a total serum calcium concentration of 3.71 mmol/l, alkaline phosphatase (151 E/l; reference range 0–130 U/L), alanine aminotransferase (214 U/l: reference range 0–133 U/L), and a normal albumin concentration (27g/l; reference range 22-35 g/l). CBC showed a normal leukocyte count (6.7 x 10^9/l; reference range 6.0-17.0 x 10^9/l), a normal lymphocyte count (2.6 x 10^9/l; reference range 0.7-5.0 x 10^9/l), neutropenia (2.5 x 10^9/l; reference range 3.6-13.0 x 10^9/l), and a normal number of monocytes (1.6 x 10^9/l; reference range 0.1-1.7 x 10^9/l). Considerable polydipsia and polyuria were still present, and forced diuresis through fluid therapy and furosemide treatment to attempt to normalize the hypercalcaemia was not yet started. Corticosteroid therapy was considered to be contra-indicated because it could possibly promote further deterioration through the suspected immunosuppression in this dog.

The day the results of the antifungal susceptibility testing became available, the creatinin level had increased to 171 µmol/l, with a total serum calcium concentration of 4.17 mmol/l. The peripheral and medial iliac lymph nodes, as well as the spleen, were severely enlarged and the total condition of the dog rapidly declined. Euthanasia was performed on the owner's request and no permission was obtained for necropsy.

DISCUSSION

Disseminated fungal infections in companion animals are extremely rare in The Netherlands and Belgium. In patients where such an infection clinically leads to generalized lymphadenopathy, splenomegaly and hypercalcemia, lymphoma should be included in the differential diagnostic work-up. This was especially true for the dog described in this case report, where there was no positive proof that the fungal organism detected on cytology specimens obtained from the mass in the neck was either the primary cause of or secondarily involved in the development of the abscess. Moreover, the time between the locally curative surgery and the development of the generalized lymphadenopathy justified putting lymphoma high on the list of differential diagnoses.

The genus Penicillium is among the most common fungi in the environment. Approximately 15 species are known to cause opportunistic human mycoses in immunocompromised patients (Lyratzopoulos *et al.*, 2002). In dogs, several cases of disseminated *Penicillium* infection have been described in recent years. Among them are *P. purpurogenum* (Zanatta *et al.*, 2006), *P. commune* (Kano *et al.*, 2006) and *P. brevicompactum* (Caro-Vadillo *et al.*, 2007). A review of opportunistic fungal infections in 10 dogs noted one case of non-specified penicilliosis (Watt *et al.*, 1995*)*.

P. radicum is mentioned in the literature as a phosphate solubilizing fungus used in agriculture for plant growth promotion (Wakelin *et al.*, 2007). The dog described in this article was living in an agrarian area, but no attempt was made to isolate the fungus from the dog's environment, or to investigate the use of *P. radicum* in locally applied fertilizers.

A common theme in the clinical outcome of a fungal infection is the response of the immune system. If the inoculum is small and the animal is immunocompetent, the infection is usually limited to the port of entrance and resolves with few or no clinical signs. As in human patients, we can hypothesize that canine patients with systemic mycotic infections lack a proper immune response to the fungus. Although not convincingly proved, there are indications that immunosuppression – for example, through the neutropenia – in the dog described in this case report was the main cause of the fungus becoming invasive. Clinical signs in canine patients with disseminated fungal infections are primarily determined by the loss of function of the infected and inflamed organ systems, which causes non-specific clinical symptoms. However, draining skin tracts and lymphadenopathy have regularly been reported in several systemic fungal infections (Krohe, 2000).

Hypercalcemia has been well described in a variety of neoplastic and granulomatous diseases. Serum calcium concentration is tightly regulated by a complex system of calcitropic hormones. Parathyroid hormone (PTH) and the biologically active form of vitamin D, calcitriol or 1,25-dihydroxyvitamin D ($1,25\text{-}(OH)_2D_3$), are the most physiologically relevant calcitropic substances, with actions at the levels of bone, kidney and gut. Neoplasms such as lymphoma can alter the calcium homeostasis indirectly through the production of endocrine factors that result in hypercalcemia of malignancy. This paraneoplastic condition is most often due to the synthesis and release of parathyroid hormone-related peptide by neoplastic cells and the ensuing increase of osteoclastic bone resorption. Another mechanism for this hypercalcemia operates through the excess production of 1,25-dihydroxyvitamin D from extra-renal sources (Clines *et al.*, 2005; Sharma, 2000).

Human patients with hypercalcemia during a dis-

seminated fungal infection are known to have their vitamin D metabolite levels increased (Spindel *et al.*, 1995). Calcitriol is a major biologically active metabolite of the vitamin D sterol family. Vitamin D precursors are either ingested in the diet or synthesized in the skin from 7-dehydrocholesterol through exposure to sunlight. Hydroxylation occurs in the liver to form 25-hydroxyvitamin D (25-$(OH)D_3$). 25-$(OH)D_3$ is hydroxylated in the kidney to form 1,25-$(OH)_2D_3$. The renal 1α-hydroxylation of 25-$(OH)D_3$ is the major recognized control point of vitamin D metabolism, responding to phosphate, PTH and calcitriol concentrations. Vitamin D receptors within renal proximal convoluted tubule cells are involved in an autocrine negative feedback loop whereby increased levels of 1,25-$(OH)_2D_3$ will down-regulate 1,25-$(OH)_2D_3$ production (Clines *et al.*, 2005). Other known important extra-renal sites of calcitriol production are the placenta and granulomatous tissue (Clines *et al.*, 2005).

However, hypercalcemia associated with granulomatous disease arises from an alteration of endogenous vitamin D metabolism. Macrophages activated in response to granulomatous inflammation are able to convert 25-hydroxyvitamin D, produced by the liver into calcitriol in an unregulated manner by possessing the 1α-hydroxylase enzyme (Sharma, 2000; Dusso *et al.*, 1991; Mellanby *et al.*, 2006; Monkawa *et al.* 2007).

In companion animals, hypercalcemia related to granulomatous disease has been reported in disseminated histoplasmosis, blastomycosis, coccidiomycosis, tuberculosis and schistosomiasis (Mellanby *et al.*, 2006; Dial, 2007; Dow *et al.*, 1986). Animals with hypercalcemia related to granulomatous disease are expected to have high serum concentrations of calcitriol and of total and ionized calcium. However, their PTH values will be low. Serum calcium concentrations return to normal with successful treatment.

This case report illustrates that in veterinary medicine for the specific identification of generalized or unusual fungal disease, cytology, histopathology and culture of the suspected organism from fluids or tissue samples is to be supplemented by newer identification techniques such as PCR, to obtain a proper determination of the causative organism. In vitro susceptibility testing is of value to make targeted treatment possible. The isolate of this patient had the lowest MIC for anidulafungin and isavuconazole, two human antifungals not yet registered for this indication, and not a treatment option in this case.

Commercial products mentioned in the paper

[a] Nizoral, Janssen-Cilag BV, Netherlands
[b] Baytril, Bayer BV, Netherlands
[c] Amoxicilline, Eurovet, Netherlands
[d] Eurogentec, Seraing, Belgium
[e] Roche Diagnostics, Almere, The Netherlands
[f] Amersham Biosciences, Roosendaal, The Netherlands
[g] Cerenia, Pfizer BV, Netherlands
[h] Trisporal, Janssen-Cilag BV, Belgium
[i] Bristol-Myers Squib, Woerden, The Netherlands
[j] Janssen Research Foundation, Beerse, Belgium
[k] Pfizer Central Research, Sandwich, United Kingdom
[l] Schering-Plough, Kenilworth, USA
[m] Basilea Pharmaceutica, Basel, Switserland
[n] Merck Sharp & Dohme BV, Haarlem, The Netherlands

REFERENCES

Altschul S.F., Madden T.L., Schäffer A.A., Zhang J., Zhang Z., Miller W., Lipman D.J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research 25*, 3389-3402.

Boom R., Sol C.J., Salimans M.M., Jansen C.L., Wertheim-van Dillen P.M., van der Noordaa J. (1990). Rapid and simple method for purification of nucleic acids. *Journal of Clinical Microbiology 28*, 495-503.

Caro-Vadillo A., Payá-Vicens M.J., Martínez-Merlo E., García-Real I., Martín-Espada C. (2007). Fungal pneumonia caused by Penicillium brevicompactum in a young Staffordshire bull terrier. *The Veterinary Record 160*, 595-596.

Clines G.A., Guise T.A. (2005). Hypercalcaemia of malignancy and basic research on mechanisms responsible for osteolytic and osteoblastic metastasis to bone. *Endocrine-Related Cancer 12,* 549-583.

Clinical and Laboratory Standards Institute. (2008). Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved standard M38-A2. In: *Clinical and Laboratory Standards Institute*. 2nd ed., Wayne, PA, USA.

Dial S. (2007). Fungal diagnostics: current techniques and future trends**.** *Veterinary Clinics of North America Small Animal Practice 37*, 373-392.

Dow S.W., Legendre A.M., Stiff M., Greene C. (1986). Hypercalcemia associated with blastomycosis in dogs. *Journal of the American Veterinary Medical Association 188*, 706-709.

Dusso A.S., Finch J., Brown A., Ritter C., Delmez J., Schreiner G., Slatopolsky E. (1991). Extrarenal production of calcitriol in normal and uremic humans. *Journal of Clinical Endocrinology & Metabolism 72*, 157-164.

Horré R., Gilges S., Breig P., Kupfer B., de Hoog G.S., Hoekstra E., Poonman N., Schaal K.P. (2001). Fungaemia due to Penicillium piceum, a member of the Penicillium marneffei complex. *Mycoses 44*, 502-504.

Kano R., Ito K., Imagi M., Watari T., Tokuriki M., Hasegawa A. (2006). Isolation of Penicillium commune from a pulmonary infection in a dog. *The Veterinary Record 159*, 779-80.

Krohne S.G. (2000). Canine systemic fungal infections. *Veterinary Clinics of North America Small Animal Practice 30*, 1063-1090.

Lyratzopoulos G., Ellis M., Nerringer R., Denning D.W. (2002). Invasive infection due to Penicillium species other than P. marneffei. *Journal of Infection 45*, 184-195.

Mellanby R.J., Mellor P., Villiers E.J., Herrtage M.E., Halsall D., O'Rahilly S., McNeil P.E., Mee A.P., Berry J.L. (2006). Hypercalcaemia associated with granulomatous lymphadenitis and elevated 1,25 dihydroxyvitamin D concentration in a dog. *Journal of Small Animal Practice 47*, 207-212.

Monkawa T., Yoshida T., Hayashi M., Saruta T. (2007). Identification of 25-hydroxyvitamin D3 1a-hydroxylase gene expression in macrophages. *Kidney International 58*, 559-568.

Santos P.E., Piontelli E., Shea Y.R., Galluzzo M.L., Holland

S.M., Zelazko M.E., Rosenzweig S.D. (2006). Penicillium piceum infection: diagnosis and successful treatment in chronic granulomatous disease. *Medical Mycology 44*, 749-753.
Sharma O.P. (2000). Hypercalcemia in granulomatous disorders: a clinical review. *Current Opinion in Pulmonary Medicine 6*, 442–447.
Spindel S.J., Hamill R.J., Georghiou P.R., Lacke C.E., Green L.K., Mallette L.E. (1995). Vitamin D-mediated hypercalcemia in fungal infections. *American Journal of the Medical Sciences 310*, 71-76.
Wakelin S.A., Gupta V.V., Harvey P.R., Ryder M.H. (2007). The effect of Penicillium fungi on plant growth and phosphorus mobilization in neutral to alkaline soils from southern Australia. *Canadian Journal of Microbiology 53*, 106-115.
Watt P.R., Robins G.M., Galloway A.M., O'Boyle D.A. (1995). Disseminated opportunistic fungal disease in dogs: 10 cases (1982-1990). *Journal of the American Veterinary Medical Association 207*, 67-70.
Zanatta R., Miniscalco B., Guarro J., Gené J., Capucchio M.T., Gallo M.G., Mikulicich B., Peano A. (2006). A case of disseminated mycosis in a German shepherd dog due to Penicillium purpurogenum. *Medical Mycology 44, 93-97*.

Uit het verleden

Op het eten van een kalfskop

Maakt je klaar, maak je klaar,
De kallefskop is gaar,
Ei, hoor hem lekker koken!
Mijn maagje danst op dat geluid,
De kop die wil de ketel uit,
Ik heb hem al geroken.

~~~

Wie zou niet, wie zou niet,
Als hij 't verhemelt ziet,
Een stukje daarvan wensen?
Wat zijn de harsens hagelwit,
Zie of er ook een kei in zit,
Als in de meeste mensen.

~~~

Gans en kip, gans en kip,
Kapoen en watersnip,
Wie zou er naar je talen?
Geen pluimgedierte, wild of tam,
Geen ossetong, geen hanekam,
Mag bij de koppen halen

Jan de Regt

Dit zijn drie van acht strofen die de lof van kalfskop bezongen lang voordat er sprake was van BSE. De kop verscheen op zijn geheel op tafel: met tong, kaakspiertjes en hersenen! De kei in de hersenen is een allusie op het 'keisnijden' dat toen nog toegepast werd bij sommige geesteszieken. Het werkje werd neergepend door Jan de Regt, een van die vrolijke poëten die Hollands' Gouden Eeuw afsloten. Hij stierf in 1715. Geen verwarring mogelijk dus met zijn naamgenoot, de eerste anatomieprof aan de Gentse veeartsenijschool, geboren te Rotterdam in 1899.

Uit: "*Geheime gedichten*" gekozen door Wim Zaal, De Arbeiderspers, Amsterdam, 1974, p. 36-37 die bij de Regt aantekende: *door zijn gebrek aan keurigheid raakte hij al in de achttiende eeuw vergeten*.