Cutaneous pythiosis in a Red Brangus beef calf cured by immunotherapy

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ABSTRACT

Pythiosis in Southern USA have been increasingly reported in the past ten years. The infection occurs more frequently in dogs and horses inhabiting the endemic areas. Cases of the disease are rarely diagnosed in other species including humans. Herein, we describe the first case of bovine pythiosis in a breed other than Brahman successfully treated by the used of immunotherapy.

1. Introduction

Pythium insidiosum is the etiologic agent of pythiosis in humans and other animals [1,2]. The disease is enzootic in the Southern USA, especially in horses and dogs inhabiting states bordering the Gulf of Mexico [4,5] and has also been reported in Africa, Asia, Australia, Latin America and New Zealand [5]. Although the disease occurs in several species, bovine pythiosis is rarely diagnosed in endemic areas. The first case of bovine pythiosis in the USA was published by Miller et al. in 1985 [7] and more recently by Martins et al. [8]. In addition, two reports from Brazil [9] and Venezuela [10] exist, also involving Brahman beef calves. This report describes a new case of bovine pythiosis in the USA and the first diagnosed case in a breed other than Brahman successfully cured by immunotherapy alone.

2. Case

A three month-old male Red Brangus calf near Garrison, Texas, USA, a small town located on eastern Texas near the border with Louisiana, developed on day 0 a five mm-diameter ulcer over its metatarsus that rapidly increased in size. By day 15 the original lesion had reached 50 mm in size. On day 23 it was admitted to an equine Hospital with a ~200 mm in diameter ulcerated lesion (Fig. 1A). The affected area was edematous, but the metatarsal bones themselves did not appear to be involved. A profuse serosanguinous exudate was noted and the lesion was non-pruritic. Stony masses (kunkers) characteristic of pythiosis lesions in horses were absent. A presumptive clinical diagnosis of pythiosis prompted the collection of a biopsy. A wet mount preparation in 10% KOH showed the presence of few hyaline sparsely septate hyphae similar to that developed by P. insidiosum. Suspecting P. insidiosum, part of the biopsied tissue was cut into 2.5 mm diameter blocks washed three times with sterile distilled water and cultured on 2% Sabouraud dextrose agar incubating plates at 25 °C and 37 °C. Despite numerous efforts the isolation of P. insidiosum in culture was not possible.

Histopathological examination using H & E stains showed several micro-abscesses with numerous neutrophils, eosinophils and some giant cells. The histopathological changes were similar to those recorded by Martins et al. [8]. The presence of poorly stained short hyaline hyphal fragments difficult to visualize on H & E at lower and high magnification was main characteristic (Fig. 2A). The hyphal elements were always surrounded by an eosinophilic material. In Gomori Methenamine Silver (GMS) staining several 3.5–10 µm in diameter hyphal elements consistent with P. insidiosum were evident at the center and at the edges of the microabscesses (Fig. 2B). Despite several efforts P. insidiosum could not be isolated in pure culture. A serum sample from the infected calf evaluated by ELISA and Western Blot strongly detected anti-P. insidiosum IgG antibodies.

To further investigate the identity of the hyphae in the infected tissues, total genomic DNA samples were extracted following standard methodologies [10]. The extracted genomic DNA was PCR amplified using NS1 and NS3 universal primers and the amplicons were cloned and sequenced as previously [11]. BLAST analysis (NCBI, http://www.ncbi.nlm.nih.gov/) of the 500 bp 18 S rDNA amplicon showed 100% identity with the DNA sequences of other P. insidiosum strains. Phylogenetic analysis of the 18 S rDNA sequence with 20 other P. insidiosum DNA sequences available in NCBI, placed this particular strain in Cluster I of Schurko et al. [12].

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Immunotherapy was initiated with USDA-licensed Allergenic Extract, Code 9540.00. Three injections containing 20 mg/ml of *P. insidiosum* protein each were subcutaneously administered as follows: the first injection was given on day 23 and then repeated on days 30 and 37. No deleterious side effects were observed at the injection sites nor any other harmful manifestations detected. Seven days after the first injection (day 30), the serosanguinous secretion ceased and the lesion showed clear signs of drying out. Three weeks after the first injection (day 44) the ulcerated tissue completely dried out and no recurrence has thus far been reported (Fig. 1B).

**3. Discussion**

Numerous cases of pythiosis in dogs and horses are reported yearly during the summer months in the states bordering the Gulf of Mexico [6]. However, pythiosis in other species [4,5], including humans, inhabiting the same areas are only sporadically recorded [2]. This case of bovine pythiosis in a Red Brangus calf occurred 28 years after the first report of the infection in six USA Braham beef calves [8], suggesting a low rate of infection in this species. Given the high occurrence of the disease in dogs and horses [4–6,13,15] however, we believe cases of bovine pythiosis could occur every year, but probably are not recognized as such and misdiagnosed with diseases sharing similar clinical signs. A good example of this could be pododermatitis caused by aerobic and anaerobic bacteria [8].

*Pythium*-immunotherapy was first tested in the early 80’s to treat horses with cutaneous pythiosis in Australia and Costa Rica [14,15]. Ever since it has been successfully used in humans [4,16] and in dogs with cutaneous [16] or intestinal pythiosis [6]. It is important to mention, however, that this approach was not successful in three camels with the disease [17,18]. The cure rate is about ~75% in horses in the early stages of the disease [6], ~55% in humans [3] and ~60% in dogs [6]. Interestingly, when *Pythium*-immunotherapy was used in Venezuela beef calves with cutaneous pythiosis, around 90% of the infected cattle responded to this approach [6]. Chindamporn et al. [19] reported the detection of unique *P. insidiosum* antigens by the IgG present in sera of cattle with pythiosis. Garcia et al. [20] using electron microscopy found also that anti-*P. insidiosum* cattle IgG bound to different antigenic compounds within *P. insidiosum* hyphae than those in the IgG sera of cats, dogs, horses and humans with the disease. These findings suggest that the high cure rate to immunotherapy in cattle may be linked to the way *P. insidiosum* vaccine antigens are presented to the immune system in this species. In addition, spontaneous healing of cutaneous lesions caused by *P. insidiosum* in cattle has been mentioned [9]. In the current case, the dramatic improvement of the animal after the first injection suggests immunotherapy played a central role in the speedy recovery of the animal.

**Conflict of Interest**

All authors have reviewed the manuscript and approved submission for publication. The authors have declared no potential conflict of interest of the material included in this manuscript.

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References

[1] A.W.A.M. DeCock, L. Mendoza, A.A. Padiye, L. Ajello, L. Kaufman, *Pythium insidiosum* sp. nov., the etiologic agent of pythiosis, *J. Clin. Microbiol* 25 (1987) 344–349.

[2] W. Gaastra, L.J. Lipman, A.W.A.M. deCock, T.K. Exel, R.B.G. Pegge, J. Scheuwater J, et al., *Pythium insidiosum*: an overview, Vet. Microbiol 20 (2010) 1–16.

[3] W. Wanachiwanawin, L. Mendoza, S. Visuthisakchai, P. Mutsikapan, B. Sathapatayavongs B, A. Chaiprasert A, et al., Efficacy of immunotherapy using *antigens of Pythium insidiosum* in the treatment of vascular pythiosis in humans, *Vaccine* 22 (2004) 3613–3621.

[4] M.K. Chaffin, J. Schumacher, W.J. McMullan, Cutaneous pythiosis in the horse, *Vet. Clin. North Am. Equine Pract.* 11 (1995) 91–103.

[5] R.C. Thomas, D.T. Lewis, Pythiosis in dogs and cats, *Compend. Contin. Educ. Pract. Vet.* 20 (1998) 63–74.

[6] L. Mendoza, J.C. Newton, Immunology and immunotherapy of the infections caused by *Pythium insidiosum*, *Med Mycol.* 43 (2005) 477–486.

[7] R.I. Miller, B.M. Olcott, M. Archer, Cutaneous pythiosis in beef calves, *J. Am. Vet. Med. Assoc.* 186 (1985) 984–986.

[8] T.B. Martins, G.D. Kommers, M.E. Trost, M.A. Inkemllmann, R.A. Figlera, A.L. Schild, A comparative study of the histopathology and immunohistochemistry of pythiosis in horses, dogs and cattle, *J. Comp. Pathol.* 146 (2012) 122–131.

[9] J.M. Santurio, A.B. Monteiro, A.T. Leal, G.D. Kommers, R.S. de Sousa, J.B. Catto, Cutaneous Pythiosis insidiosi in calves from the Pantanal region of Brazil, *Mycopathologia* 141 (1998) 123–125.

[10] R. Pérez, J.J. Luis-León, J.L. Vivas, L. Mendoza, Epizootic cutaneous pythiosis in beef calves, *Vet. Microbiol* 109 (2005) 121–128.

[11] L. Mendoza, M. Arias, V. Colmenares, Y. Perazzo, Intestinal canine pythiosis in Venezuela confirmed by serological and sequencing analysis, *Mycopathologia* 159 (2005) 219–222.

[12] A.M. Schurko, L. Mendoza, C.A. Lévesque, L. Désaulniers, A.W.A.M. deCock, G.R. Klassen, A molecular phylogeny of *Pythium insidiosum*, *Mycol. Res.* 107 (2003) 537–544.

[13] N.A. Berreyessa, S.L. Marks, P.A. Pesavento, T. Krasnasky, S.K. Yoshimoto, E.G. Johnson, et al., Gastrointestinal pythiosis in 10 dogs from California, *J. Vet. Intern. Med.* 22 (2008) 1065–1069.

[14] R.I. Miller, Treatment of equine phycomycosis by immunotherapy and surgery, *Aust. Vet. J.* 57 (1981) 577–382.

[15] A. Thitithayanont, L. Mendoza, A. Chuansumrit, R. Pracharktam, J. Laothamatas, B. Sathapatayavongs, et al., Use of an immunogenic therapeutic vaccine to treat a life-threatening human arteritic infection caused by *Pythium insidiosum*, *Clin. Infect. Dis.* 27 (1998) 1394–1400.

[16] P. Hensel, C.E. Greene, L. Medleau, K.S. Latimer, L. Mendoza, Immunotherapy for treatment of multicentric cutaneous pythiosis in a dog, *J. Am. Vet. Med. Assoc.* 223 (2003) 215–218.

[17] J.F.X. Wellehan, L.L. Farina, C.G. Keoughan, M. Lafor tane, A.M. Grooters, L. Mendoza L, et al., Pythiosis in a dromedary camel (*Camelus dromedaries*), *J. Zoo. Wildl. Dis.* 35 (2009) 564–568.

[18] R. Videla, S. van Amstel, S.H. O’neill, L.A. Frank, S.J. Newman SJ, R. Vilela, L. Mendoza, Vulvar pythiosis in two captive camels (*Camelus dromedaries*), *Med. Mycol.* 50 (2012) 219–224.

[19] A. Chindamporn, R. Vilela, K.A. Hoag, L. Mendoza, Antibodies in the sera of host species with pythiosis recognize a variety of unique immunogens in geographically divergent *Pythium insidiosum* strains, *Clin. Vaccin. Immunol.* 16 (2009) 330–336.

[20] R.B. Garcia, A. Pastor, L. Mendoza, Mapping of *Pythium insidiosum* hyphal antigens and ultrastructural features using TEM, *Mycol. Res.* 111 (2007) 1352–1360.