Cutaneous Pythiosis

Missy Streicher, AAS, CVT, VTS (Dermatology) Auburn University Small Animal Teaching Hospital 334-844-4690 streiml@auburn.edu

Abstract: *Pythium insidiosum* is an important differential to consider when presented with a cutaneous nodule that rapidly progresses. This organism is present everywhere but should be a special consideration in the southeastern United States particularly in dogs that have access to water. Pythiosis can be treated but the prognosis is very poor for dogs that do not receive swift medical and surgical intervention. This presentation will discuss what an oomycete is and how it is able to infect the dog, differentials of cutaneous nodules, diagnostics and treatments for pythiosis.

Keywords: Pythium insidiosum, oomycete, opportunistic, dissemination

When a patient presents with a nodule in the skin, the differential list quickly becomes very long. The clinical history is necessary and important to differentiate between infectious and noninfectious causes, especially if an opportunistic infection may be involved. In these cases, especially when those such as *Pythium* are suspected, a quick diagnosis with medical and surgical intervention are needed for this aggressive organism that holds a potentially grave prognosis.

Pythium insidiosum is an aquatic organism that is most closely related to algae. It is also referred to as a "pseudofungus" for the way it invades plants and animal tissue with hyphal filaments, but unlike fungus, the *Pythium* cell wall lacks chitin and ergosterol which are the

1

target of antifungal medications. Pythium is an oomycete, a category which includes approximately 500 other organisms. In veterinary medicine, the oomycetes: Pythium, Lagenidium and Paralagenidium create opportunistic infections that can be fatal. Oomycetes are ubiquitous meaning they are found anywhere but thrive particularly in moist, humid climates such as the southeastern United States and reside in slow moving or standing water. Oomycetes play an important role in nature in the process of decaying matter. In water, via sexual reproduction, motile zoospores are produced that have flagella to propel them around. When a plant or animal with damage to the skin is near, the zoospore detects the increase of carbon dioxide and moves toward the source, in this case, the dog. This process is called chemotaxis. As the zoospore comes closer to its potential host, it becomes sluggish and loses the flagella. They cling to the animal or plant by using proteins called invasins and then use enzymes to degrade the proteins in the epidermis that hold the skin cells together and force themselves deeper into the tissue. Now the Pythium organism utilizes asexual reproduction behaving like a fungus and replicates spreading the infection with filamentous hyphae. Meanwhile, the body starts to recognize a foreigner is present and the immune system sends out leukocytes such as neutrophils and macrophages to combat the opportunist. This initial defense comes from innate immunity and then the body must employ an adaptive immune response to destroy it.

Innate immunity is composed of a variety of fast-acting mechanisms that react quickly and are the first responders when the immune system encounters foreign invaders. This kind of immunity is constantly waiting and ready to act. Inflammation is one of the mechanisms of innate immunity. During inflammation, defensive white blood cells and antimicrobial molecules migrate to where the tissue has been damaged and microbes have invaded. The white blood cells that circulate in the blood stream leave and go into the tissues to attach and destroy the invaders then they help repair the damaged tissues. While this is a very effective mechanism for the short term, there is not a guarantee there will be complete neutralization of the infection so the immune system is concurrently creating adaptive immunity.

Adaptive immunity starts during the innate response. Some of the cells bring information about the foreign material to the immune system where it learns how to recognize the pathogen quickly and also how to efficiently kill it for future exposures. The adaptive system is for long term immunity response. There are four types of lymphocytes involved in adaptive immunity. Three are categorized as T-lymphocytes (helper, regulatory, and effector) and the fourth is Blymphocytes which produce antibodies. When an opportunistic organism such as Pythium infiltrates the body, the role of the T-helper cells are very important. Antigen presenting cells (APC) trap and process foreign antigens and present them to T-helper cells to initiate an immune response. The T-helper 1 (Th-1) cells promote cell mediated immune responses such as activating macrophages and delayed hypersensitivity reactions. The immune system utilizes Th-1 cells for immunity to bacteria, viruses, protozoa and fungi. When a Th-1 response is activated, Th-2 cell responses are inhibited. Alternatively, T-helper 2 (Th-2) cells activate B-cells to produce antibodies but are less effective at cell mediated killing of invaders. Th-2 activation is associated with immunity to helminths, arthropods and allergic responses. When activated, Th-1 immunity is suppressed thus decreasing ability to provide immunity from infection(s).

When *Pythium* (or other pathogens) are detected, the desired immune response would be Th-1 mediated. However, proliferation of Th-2 cells occurs instead and this creates a more allergic response. In other words, the wrong T-helper cell for the situation has been activated and an allergic reaction and resulting inflammation can be quite damaging to the skin. Additionally, the Th-1 cell response is suppressed allowing the infection to flourish.

Initial presentation to the veterinarian may be for a "mass" or a non-healing draining wound and may be treated empirically for infection, inflammation or benign neglect and have the owner monitor for changes in the mass. This gives the hyphae more time to proliferate and potentially travel the lymph nodes allowing the infection to disseminate leading to a poorer prognosis.

When a patient presents with a skin nodule in dermatology, a broad differential list is created: cyst, tumor (neoplastic or non), granuloma (infectious or non), acral lick dermatitis, or a foreign body. These differentials have differentials of their own particularly for the infectious causes of bacterial, fungal, atypical bacteria and other opportunistic organisms.

History becomes extremely important as the time of inoculation with *Pythium* may have occurred several weeks to months prior to seeing the lesion. Young, male, large breed dogs, hunting dogs and dogs that live primarily outdoor with access to slow moving water have an increased risk of infection.

Presentation of cutaneous lesions are most often noted on limbs, rump and face. The nodules may be single or multiple which may coalesce and usually found on one area one the body. The solitary lesions are due to the requirement of damaged skin for zoospore entry. It is very unlikely that multiple zoospores enter multiple wounds at the same time. Nodules often rupture and drain and may wax and wane with treatment with antibiotics due to secondary bacterial infections.

A variety of initial diagnostics are done to yield a definitive diagnosis. Once confirmed, additional diagnostics are performed to check for disseminated disease prior to instituting therapy and surgery to remove the lesions which may include radical surgical resection or amputation. If lesion is draining, an impression cytology of the exudate is obtained that will probably yield pyogranulomatous inflammation (neutrophils, macrophages, lymphocytes with or without some secondary bacteria from contamination) but will not likely show hyphae. A fine needle aspirate (FNA) is generally the first step as results are obtained fairly quickly. However, a similar cell population as with the impression smear will be noted and hyphae still may not be detected. It is more likely to detect hyphae on FNA than a surface cytology. A FNA of the suspected draining lymph node to the lesion is often collected as well. Hyphal organisms detected here will add to a poorer prognosis for the dog as disease is more likely to be disseminated. In pythiosis, there is often an eosinophilic component to the inflammation and increased numbers of eosinophils may be present in the lymph node aspirate as well.

A blood sample submitted for a *Pythium* ELISA may be ordered. The values from the laboratory here at Auburn are that a positive result for *Pythium* is >70% positivity, and a negative result is <40% positivity however most healthy dogs are usually between 0-10%. However, even though negative, a small percentage of reactivity is noted as the test may detect other fungi that are present but not causing disease and may indicate the presence of another oomycete. The other two oomycotic infections that are similar have some interesting trends in their positivity results but this is not definitive. Dogs with *Paralagenidium sp.* tend to have 10-15% positivity on the ELISA and those with *Lagenidium sp.* are in the 40-50% positivity range.

The patient is then prepped for collection of multiple punch biopsies of affected tissue. Some of the tissue samples are placed in formalin and submitted for histopathology which requires special stains to find the organism. Other biopsy samples are collected for multiple types of cultures which are: aerobic, anaerobic, mycobacteria and systemic fungal culture. These are all diagnostics that can help in the case of a non-specific nodule. Handling the sample after collection is very important. At Auburn, the sample is not to be refrigerated and the lab should be alerted that *Pythium* or other oomycetes are suspected as the care for incubation is very involved. If another laboratory is used, some may require submission within 24 hours so the day of the week the sample is collected is a considering factor. Call the lab you are using before taking the samples for clear instructions on the temperature the specimen should be after collection and during transport, patient preparation, how many samples are required to get enough for all the cultures to be run and the preferred transport media to be used.

Suggestions of the species of oomycete may be made by the histopathologist based on the size of hyphae and type of stain used for optimal uptake of the organism. The culture results will give a definitive diagnosis of *Pythium insidiosum*. Once diagnosis confirmed, additional diagnostics are done for staging of the disease and baseline labwork including liver values before initiation of antifungal medications.

Radiographs of the chest and abdomen and abdominal ultrasound are recommended. These images will help document presence of dissemination of disease.

A complete blood count, full chemistry profile and urinalysis are completed for baseline values as well as serves as pre-anesthesia labwork. Two systemic antifungal medications which may adversely affect the liver may be prescribed as part of the medical therapies several months post operatively. Knowing the liver values prior to treatment is important in case there is evidence of hepatoxicity while receiving the medication.

A consultation with the surgery team for the removal of the affected area is next. If located on an extremity, the limb is recommended for amputation. In some areas, the lesion may be excised with wide margins and enough skin is present to close the incision. However, if on a region

where not able to excise completely due to size and ability to close, debulking the site may be necessary with a follow up surgery later. Because the immune system malfunctions and alerts the Th-2 cells to act that are governing an allergic reaction to the hyphae, anti-inflammatory doses of prednisone are often instituted. This may help to reduce edema and shrink the lesion before surgery is performed. Culture of tissue at the margins of the excised area is recommended to see if fungal disease extends to the margin. Additionally, regional lymph nodes will be removed and submitted for histopathology and/or fungal culture to determine spread of disease. If this is noted to be present, even more vigilance at monitoring for infections to other areas on the body is recommended.

As mentioned earlier, antifungal therapy will be instituted. A combination of itraconazole and terbinafine are used post operatively for several months, usually 2-4 months, until the *Pythium* ELISA is negative. This ELISA is monitored monthly until it drops into negative range. As previously mentioned, *Pythium* and other oomycetes lack chitin and ergosterol in the cell wall which is what antifungals target to kill the fungus. While this seems counterintuitive, in the face of such an aggressive infection, the antifungal drugs may be used to slow down any further growth while hoping that the body will mount an appropriate Th-1 immune response. After the ELISA is finally negative, samples are decreased in frequency and submitted every 3 months for one year and then annually thereafter to monitor for relapse.

There are some other therapy options to consider when treating *Pythium insidiosum* which are the *Pythium* vaccine and mefonoxam.

A commercially available vaccine for Pythium has been developed and may be implemented. It is a type of immunotherapy that helps modulate the body's Th 1/Th 2 response. More extensive studies have been done in horses and people with the vaccine but not for dogs. The efficacy of this therapy remains controversial and further studies are warranted.

Mefenoxam is a topical plant fungicide that has shown to be effective at killing Pythium in a laboratory setting. However, it is not approved for use in humans or animals and safety has not been established. This is experimental treatment requiring the owner to be counseled on potential side effects, advised of unknown long term effects, and potentially death. The owner must sign a document stating informed consent.

And finally, humane euthanasia is a consideration especially if disease is disseminated.

In summary, despite oomycetes existing ubiquitously and in particular more often in the southeastern United States, *Pythium* is often not on the differential list for a non-healing wound. Sadly, once diagnosed, the disease may have progressed to the point it has disseminated or the lesion has become too large to completely excise. When caught early, it can be treatable. Unfortunately, a lot of patients are diagnosed once the disease has progressed. That, along with a poor prognosis and cost of therapy and surgery, euthanasia is often elected.

References:

Miller WH, Griffin CE, Campbell KL. Small Animal Dermatology, 7th ed. St. Louis, MO. Elsevier. Muller & Kirk. 2013

Hnilica, KA, Patterson AP. Small Animal Dermatology, A Color Atlas and Therapeutic Guide, 4th ed. St. Louis, MO. Elsevier. 2017

Tizard, I. Veterinary Immunology, 10th ed. St. Louis, MO. Elsevier. 2018

Hicks, KD. Oomycoses. Auburn University, 2019