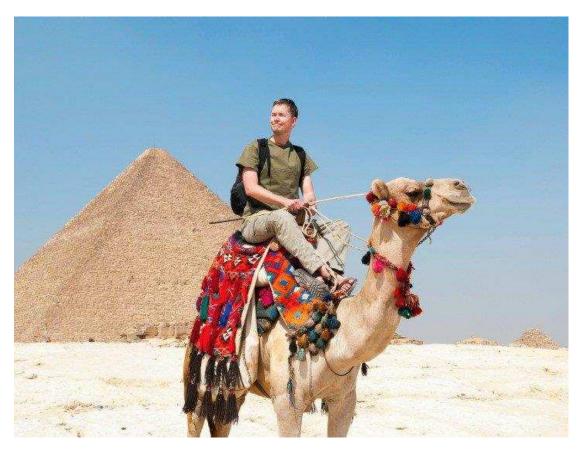
# Monograph On

# **Fungal Diseases of Camelidae**

A guide for postgraduate students



Ву

# Mohamed Kamal Refai Ahmed El-Naggar and Omar Tamam

2016

# Preface

# This Monograph is dedicated to

His Highness Sheikh Mohammed bin Rashid Al Maktoum,

who invited me to attend the first internal Camel Conference in Dubai m 1992 and handed me a wonderful hand watch with the name of the conference and head of a camel.

My presentation was appreciated, so that several daily newspapers announced a summary of it.



His Highness Sheikh Mohammed bin Rashid Al Maktoum

This Monograph is also dedicated to my son, **Prof. Dr.**Hosam Refai, Dean, Faculty of Tourism and Hotel
Management, Helwan University, who raised the level
of his faculty to the international standards, so that
postgraduate students from different countries,
including Germany, continue their studies in the faculty



Prof. Dr. Mohamed K. Refai, Cairo

August, 1, 2016

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https://www.academia.edu/manuals http://scholar.cu.edu.eg/?q=hanem/book/ https://www.researchgate.net/publication

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## Introduction

## Camelidae

(Bactrian camel, dromedary, guanaco, llama, vicuña, alpaca) Camelus bactrianus, C. dromedarius, Lama guanicoe, L. glama, L. pacos, Vicugna vicugna) http://placentation.ucsd.edu/camfs.htm

Camelidae are thought to have originated in North America (**Tibary, 1997, Nowak, 1999**) but disappeared there approximately 10,000 years ago. The Bactrian camel and the dromedary are the largest of these species, and they are confined to Asia/Africa. They hybridize freely to produce the "Tulu".

The large camels are widely used as pack animals, and for races, especially the dromedary. Hybrids of these two species are fertile. The wild Bactrian camel of the Gobi desert has been markedly reduced in numbers in recent years (**Hare**, **1997**). It is smaller than the Bactrian (two-humped) camel seen in zoos, and grayer.

With RFLPs of Bactrian camels, **Vidal-Rioja et al.** (1994) assessed their relation to the South American camelids. The common ancestor was estimated to have existed 5-10 MYA and some hybridization profiles were completely identical, others differed.

- The llama was believed to have differentiated from guanaco ancestors;
- **The vicuña** was found to be quite species-specific in these studies, perhaps further justifying its separation from *Lama*.
- The alpaca might have derived from hybridization between guanaco and vicuña.

#### 1. Bactrian camel:

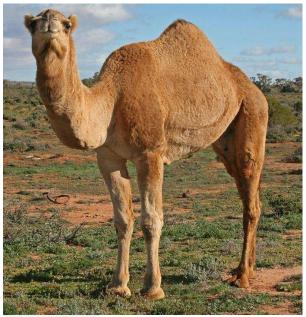


en.wikipedia.org

- adult weight is 300-690 kg;
- gestational length 360-440 days; usually a single young, very rarely twins;

- newborn weight is 37 kg; placental weight 4.2 kg;
- life expectancy is 50 years.

# 2. Dromedary:



en.wikipedia.org

- adult weight is 300-560 kg;
- gestational length 350-404 days; usually single offspring
- rare twins occur but they usually abort.
- newborn weight 26-45 kg;

# 3. Guanaco:



www.interestingfunfacts.com

- adult weight is 100-120 kg;
- gestational length is 345-360 days; single young weigh 8-15 kg;
- longevity is 28 years.

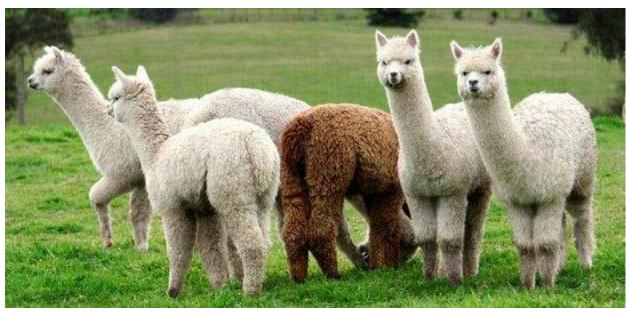
#### 4. Llama:



en.wikipedia.org

- adult weight is 130-155 kg;
- length of gestation is 342-355 days;
- life span is at least 20 years; twins occur occasionally, with rare free martinism.
- Neonatal weights are 8.6 14.9 kg (mean 11.98 kg).

# 5. Alpaca:



www.northwoodsalpacas.com

- adult weight is 55-65 kg;
- length of gestation is 342-345 days;
- neonatal weight is 8-9 kg; single young are born, but twins are often conceived.

#### 6. Vicugna:





en.wikipedia.org

Vicugna Baby by Josef Gelernter

- adult weight is 35-65 kg;
- gestational length is 330-350 days; single young weigh 4-6 kg;
- life expectancy is more than 28 years in captivity.

#### The history of the camel

The history of the camel is just as interesting as the animal itself. Evolving in North America, the camel apparently crossed the land bridge over the Bering Strait during prehistoric times. After a time, camels became domesticated, and now the only wild camels are a small population of 500-700 animals in the Gobi desert. Asia and Africa are home to domesticated camels only. Camels were introduced to Australia and a few feral animals exist in the wilds there. An experiment introducing the camel to the North American desert in the 1800's was unsuccessful, although occasional reports of a camel sighting are received.

While having a reputation for being an unpleasant animal, the camel is actually a friendly animal. The grunting and groaning when rising are similar to a grunt from us upon lifting a heavy weight. A distressed camel will spit a noxious stream of stomach contents, but generally a camel is a pleasant, hardworking, intelligent animal.

Throughout recorded history, the camel has been a helper to the desert dwellers. The camel assisted in providing transportation, shelter, fuel and food. The camel is able to carry loads as heavy as 900 pounds, although normally a camel will only carry a third of that. The camels hide provides tents for shelter, and the meat is said to be similar to veal, although a little tougher. The milk is actually more nutritious than cow's milk, and is used fresh as a drink, as well as being made into cheese. The camel's dung can be used as a fuel with no drying necessary.

#### **Camels in Egypt**

Ancient Egypt was a major trading power for most of its existence. Ancient Egyptians imported and exported a large variety of goods through a vast network



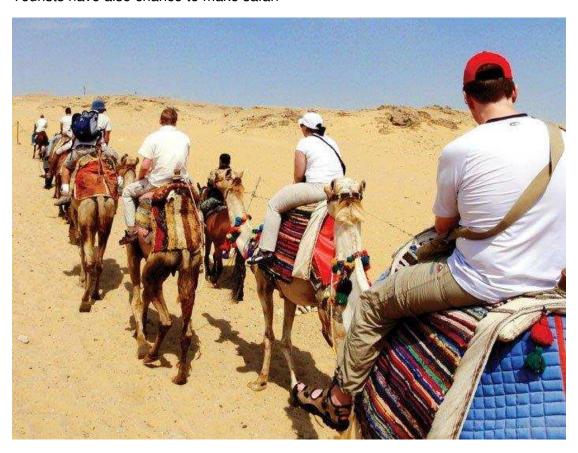
Ancient Egyptian www.emaze.com

Many nomadic tribes used the camels in the past, but as technology improves fewer people of Egypt use the camel. Now, the main purpose of camels is for tourist rides and racing. Gliding across the sands with that characteristic rocking gait, giving a romantic, exotic air to the pyramids of Egypt, the camel will forever be an important part of the way people imagine Egypt.

A camel ride is a must-do for every first-time Egypt traveler. Many people who visit the Giza plateau choose to go on a camel ride around the Pyramids. Yet a better idea is to explore the desert behind the Pyramids. Try to be there just before sunset to enjoy a magnificent view of the Pyramids, as well as the mystical and melodious Call to Prayer rising from hundreds of mosques at the same time.



Tourists have also chance to make safari





Sinai Camel Safari www.egypt-tours.org



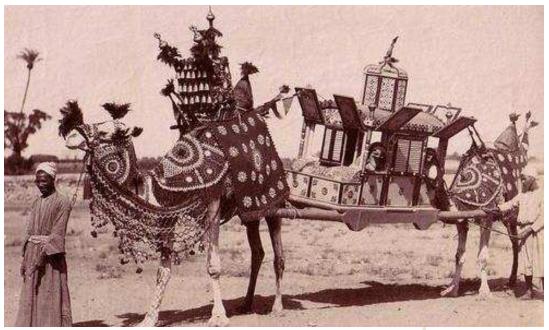
Camels on the Red Sea on the beach Canyon in Dahab. Camel Safari - one of the most popular attractions in Southern Sinai

# My story with camels

The most exciting for me the ceremony of (El Mahmal), the camels which carried the kiswa (The covering) of the Kaaba, which was made every year in Cairo and sent to Makkah. This I saw going through the streets in Cairo, before it was sent to Makkah, when I visited my grandfather (from mother), who lived in Cairo.



In my village, Badrashen, I was excited when I saw the Alhodaj on a camel carrying a bride to the marital home



Marriage Procession, Egypt, 19th Century (1870-80, Photograph Zangaki Brothers

Alhodaj was seen also every year at the 14<sup>th</sup> night of the Arabic month of Sha'ban, where the Egyptian celebrate this night of blessing, especially women and children who are waiting for Alhodaj of Sha'ban which brings the good news of approaching holy month. Ramadan



The ceremony of the middle of the Arabic month Shaaban

When I was 5 years old, I enjoyed hearing my grandfather (from father) endless and interesting stories with camels. He told me that he went to Makkah 3 times, starting each time by train to Suez, the by ship to Jeddah and then by camel caravan to Makkah.



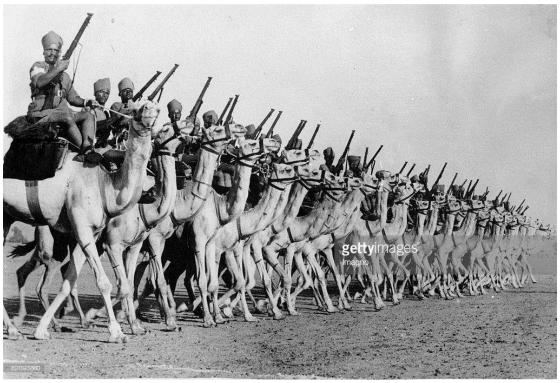
Hodaj Maka1910. lirak.free.fr

He participated in the army, first in the year 1918, among a group of Egyptian camel drivers who supported the British Army in Egypt during the First World War's Sinai and Palestine Campaign. The work done by the 170,000 men of the Corps helped British war operations in the Sinai desert and in Palestine and Syria by transporting supplies to the troops in extreme geographic and weather conditions.



Egyptian Camel Transport Corps crossing pontoon bridge at Ghoraniyeh March 1918

The second time was in 1937, one year before I was bone, in the special troops of the Egyptian army on their fast camels camel together with thousands of Egyptians.



Special troops of the Egyptian army on their fast camels ...www.gettyimages.com

When I was in India, attending the international buffalo conference in 1989 I saw the camel used for transport



When I was invited to attend the international camel conference in Dubai, in 1992, I saw for the first time in my life the camel racing



www.worldglobetrotters.com590 × 356Search by image

# 15. Ringworm

#### **Clinical symptoms**

- Young animals, which are more susceptible, have lesions similar to those seen in cattle on the head, the neck and shoulders, with a possible extension to the flanks and legs, leading sometimes to pyoderma and emaciation (**Khamiev**, 1981).
- The condition is characterized by circumscribed, crusty, hairless lesions, 1 to 2 cm in diameter, distributed over the head, neck, shoulder, limbs and flanks (**McGrane and Higgens**, 1985)
- Lesions were observed mainly on the head, neck and shoulder with frequent extension to the flanks and limbs .(Fadlelmula et al., 1994).
- The main clinical signs were characterized by the appearance of multiple lesions on the skin of different of the body. The lesions typically consisted of an area of alopecia and a prominent whitish asbestos-like accumulation of scales (Al-Ani et al., 1995)
- There are 2 clinical types of ringworm in camels (Wernery and Kaaden, 2002).
  - The first shows typical lesions that are gray-white in colour.may coalesce and mainly occur on the legs, neck and head of young animals.
  - The second is a more generalized infection on head, neck, limbs and flanks, whereby these lesions These lesions are characterized by small, round alopecic areas, which may initially be confused with mange.
- Lesions tend to be non-pruritic and alopecic, with thick crusts (**Cebra**, **2011**) on the head, the neck and shoulders, with a possible extension to the flanks and legs, leading sometimes to pyoderma and emaciation (**Chermette** *et al*, **2008**).

# Age and sex of infected camels

- Animal less than 1 year of age are at greater risk for dermatomycosis. Older animals with decreased immune function also may be at increased risk for generalized dermatomycosis (McPherson, 1957, Gupta and Singh, 1969 Scott, 1988).
- The disease was observed more frequently among young growing calves (1-2 years) than older animals but the prevalence among male and female animals was found to be similar.(Fadlelmula *et al.*, 1994).
- The age of the infected camels ranged from six months to 3 years (Al-Ani et al., 1995)
- Camel owners are familiar with ringworm, which occurs most commonly in camels under three years of age (McGrane and Higgens, 1985).

#### Season

• The peak incidence of the disease was found to be in Autumn and Winter (Fadlelmula *et al.*, 1994).

#### **Aetiology**

- The main cause of ringworm in camels is T. verrucosum and T. mentagrophytes
- T. verrucosum, T. mentagrophytes, T. schoenleinii. T. sakisovii, T. T. dankaliense. M. gypseum and M. canis were the dermatophytes reported as causes of ringworm in dromedary camels (Wernery and Kaaden, 2002).
- M. audouinii, M. canis, M. nanum, M. ferrugineum, T. verrucosum, T. mentagrophytes, T. schoenleinii, T. equinum, T. concentricum, T. tonsurans, T. violaceum, T. soudanense and T. rubrum are the causes of ringworm in camels in India (Tuteja et al. (2013.
- Ringworm caused primarily by Trichophyton schoenleinii is a common skin disease
  in Iraqi camels, especially in the young those under three years of age (Al-Ani et
  al,1995).
- Epidermophyton floccosum is reported as a main cause of ringworm in camels in India (**Tuteja** *et al.* (2013)
- Agents with changed names
  - o T. langeronii (Baudet,1930)
  - T. dankaliense (Dalling et al., 1966), the status has been questioned by Ainsworth and Austwick (1973), Accepted name: *Gymnascella dankaliensis* (*CYBERNOME*)
  - o T. camelus (Khamiev, 1980)
  - o T. sarkisovii, Current name: Trichophyton mentagrophytes (Mycobank)

#### **Diagnosis:**

#### **Direct microscopic examination**

Skin scrapings, hair or nail particles are placed on a clean slide with few drops of 10-25% KOH solution, or NaOH mixed with 5% glycerol, covered with a coverslip, heated gently and left for at least 30 minutes to 1 hour . The preparation is then pressed gently; the oozing fluid is dried and then examined microscopically for the presence of septate hyphae and arthroconidia characteristic of dermatophytes. Other formulation is 20% KOH-36% dimethyl sulfoxide and two techniques for fluorescence microscopy, the calcofluor white and the Congo red.

#### **Isolation of dermatophytes**

A part of the samples is embedded into Sabouraud dextrose agar with chloramphenicol and actidione in tubes using a mycological inoculation hook, preferably at the side of the slope and at different sites. It is better to use more than one tube for each sample. The tubes are then incubated at 30°C for 1-4 weeks.

## **Identification of dermatophytes**

☐ Identification characters include colony texture, pigmentation, growth rate and distinctive morphological structures such as macroconidia, microconidia, spirals, chlamydospores, etc.

# **Dermatophytes isolated from camels**

Dermatophytes	Authors
Trichophyton	
T. verrucosum	Curasson, 1947, Nasser, 1969, Torky and Hammad, 1981,
	Khamiev,1981,1982, 1983, El-Kader, 1985, Kuttin et al. (1986),
	El-Tamavy et al.,1988, Abou El-Yazeed, 1990, Mahmoud,
	1993, Fadelmula, 1994, Abou Zais, 1995, Gitao (1998a), Agab
	and Abbas (1999), Pal et al. (2000), Wisal et al. (2010), Abo El
	Foutah et al. (2012), Eissaa et al. (2013), Enany et al. (2013),
	Tuteja et al. (2013), Almuzaini et al. (2015), Baghza et al.
	(2016)
T. mentagrophytes	Refai and Miligy, 1968, Kuttin et al., 1977, Kuttin et al. (1986),
	Eltmawy, et al (1988) Mahmoud, 1993, Manefield and Tinson
	(1997), Wisal <i>et al.</i> (2010), Eissaa <i>et al.</i> (2013), Enany <i>et al.</i>
	(2013), Tuteja <i>et al.</i> (2013), Baghza <i>et al.</i> (2016)
T. schoenleinii	Kamel et al., 1977, Chatterjee et al., 1978, Al-Ani <i>et al.</i> ,1995,
	Al-Rawashdeh, et al (2000), Wisal et al. (2010), Tuteja et al.
	(2013), Baghza <i>et al.</i> (2016)
T. sarkisovii	Ivanova and Polyakov, 1983, Polyakov (1983), Ivanova (1987)
T. langeronii	Baudet (1930)
T. dankaliense	Dalling et al., 1966
T. camelus.	Khamiev,1980
T. tonsurans	Wisal et al. (2010), Tuteja et al. (2013), Baghza et al. (2016)
T. equinum,	Tuteja <i>et al.</i> (2013)
T. concentricum,	Tuteja <i>et al.</i> (2013)
T. violaceum,	Tuteja <i>et al.</i> (2013)
T. soudanense	Tuteja <i>et al.</i> (2013)
T. rubrum	Tuteja <i>et al.</i> (2013)
Microsporum	
M. gypseum	Boever and Rush, 1975, Kamel et al., 1977, <i>El-Kader</i> (1985),
	Fishman et al., 1987, Mancianti et al., 1988, Eltmawy, et al
	(1988), Mancianti et al. (1988, Gitao et al., 1998
M. canis	El-Kader, 1985, El-Tamavy et al., 1988, Abou El-Yazeed, 1990,
	Mahmoud, 1993, Fadelmula, 1994, Abou Zais, 1995, Eissaa et
	al. (2013), Tuteja et al. (2013), Baghza et al. (2016)
M. audouinii	Tuteja et al. (2013), Baghza et al. (2016)
M. nanum,	Tuteja et al. (2013
M. ferrugineum	Tuteja et al. (2013
Epidermophyton	
floccosum	Tuteja et al. (2013).

#### **Reports:**

Curasson (1920) reported that ringworm is common in the dromedary, and does not cause serious symptoms, or does it often become extensive. But the disease is readily communicable to man, and the author was able to demonstrate the fungus in the hair of the beard of several Moorish drivers, as well as in the hair of the dromedaries under their charge.

Castellani (1937) reported that from a piece of camel skin, submitted for examination by the Plant and Animal Products Department of the Imperial Institute, he isolated a species of Trichophyton forming rust- to orange-red cultures on glucose agar, peptonizing milk, liquefying gelatin, and serum, but elaborating neither acid nor gas from the various sugars and carbohydrates tested, with the possible exception of arbutin.

**Dalling (1966)** reported ringworm caused by **Trichophyton dankaliense** to be commonly found in northern Somalia

**Boever and <u>Rush (1975)</u>** reported the isolation of **Microsporum gypseum** from a zoo dromedary camel.

Chatterjee et al (1978) reported ringworm infection caused by T. schoenleinii in Indian camels

**Khamiev** (1980) reported a summer outbreak affecting all camels under 2 years old; 90% of cases are usually under 2 years old. Mycological examination of 200 skin scrapings yielded 90 cultures of **Trichophyton camelus**. Experiments showed that the fungus could survive at least 5 months in buildings.

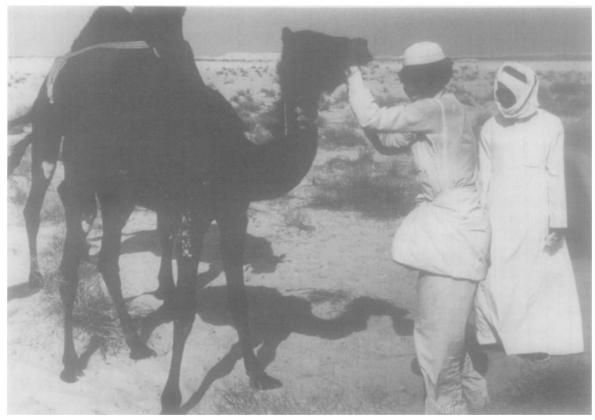
**Khamiev** (1981) mentioned that his observations over several years revealed a higher rate of Trichophyton ringworm in bactrian camels than dromedaries and a higher prevalence in females (77% of cases) than males (23%). The majority of camels aged 2-3 years had had 2-3 spells of the infection and, as a result of acquired immunity, the condition was not observed in animals aged over 4 years. Incubation lasted 8-30 days. The clinical form depended on age, duration of the condition and individual response.

**Khamiev** (1982;1983) recorded a higher prevalence of ringworm due to Trichophyton infection in Bacterian than in Dromedary camel and a higher prevalence in the she camel (77%) than males (23%). The incubation period of the disease was 8-30 days.

**Polyakov** (1983) mentioned that T. sarkovskii, sp. nov. was proposed and described in the course of a study on skin diseases of dromedary and Bactrian camels in the Kazakh SSR [USSR]. The difference between the antigen structure of this species and that of T. verrucosum was studied using the precipitation reaction and immunoelectrophoresis. One identical antigen was observed for the 2 spp. compared in cross reactions in the immunoelectrophoresis of the antiserum of T. sarkisovii and T. verrucosum. Specific components were observed in protein preparations of T. sarkisovii. T. sarkisovii was pathogenic on laboratory animals (rabbits and guinea pigs). The new species was also compared with T. schoenleinii and T. violaceum.

*El-Kader* (1985) examined 150 *camels* clinically in Assuit for skin lesions, 100 of them had clinical. picture of skin diseases. Hair and scales samples were taken from affected camels. Microscopical examination revealed that 34 samples were positive for mange, while 16 samples were positive for dermatomycosis. The cultural examination revealed the isolation of 11 was identified as **Trichophyton verrucosum**, 4 **Microsporum canis** and 3 **Microsporum gypseum** strains.

McGrane and Higgens (1985) mentioned that Camel owners are familiar with ringworm, which occurs most commonly in camels under three years of age. The condition is characterized by circumscribed, crusty, hairless lesions, 1 to 2 cm in diameter, distributed over the head, neck, shoulder, limbs and flanks. Trichophyton sp. is the usual causative fungus.



Ringworm on the legs of a camel in Saudi Arabia caused by Trichophyton sp. McGrane and Higgens (1985)

**Kuttin** *et al.* (1986) conducted a survey of ringworm in camels, that showed over 25% of young animals suffered from T. *verrucosum* infection, and fewer than 0.5% of the camels had T. *mentagrophytes*.

**Ivanova** (1987) obtained 270 isolates of **Trichophyton sarkisovii** were obtained from one-humped and two-humped camels in Kazakhstan, Uzbekistan and Turkmenia. The species differed from **T. verrucosum** in not requiring thiamine for growth in culture, and in growing more quickly, mainly on the surface of cultures. Chlamydospores measured 7-17 micro m against 6-9 micro m for T. verrucosum.

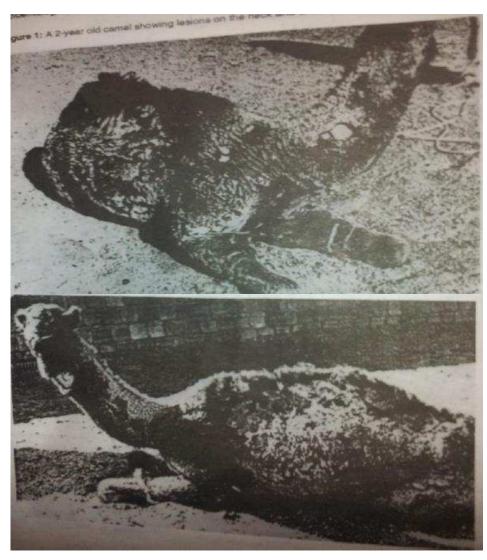
Eltmawy, et al (1988) reported 32 (16%) of dermatophytes isolated from camels 14 T. verrucosum, 8 T. mentagrophytes, 6 M. canis and 4 M. gypseum.

**Mancianti** *et al.* (1988) described dermatophytosis by **M. gypseum** in a camel (Camelus bactrianus) from a zoological garden (Pistoia, Italy). In vitro susceptibility of the dermatophyte to some antifungal agents was tested. The isolate was found to be susceptible to Griseofulvin, Clotimazole, Tioconazole and Econazole.

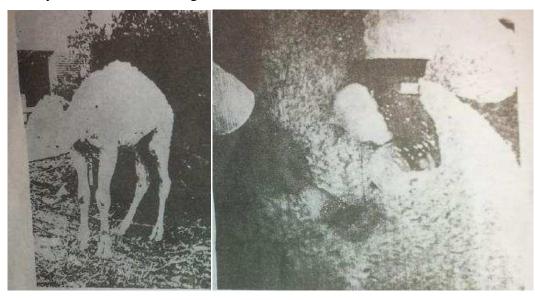
**Mahmoud** (1993) reported that among 75 camels showing skin lesions, 48% were positive for fungal infection. The younger individuals were more susceptible to this infection. Sixteen species belonging to nine genera of keratinophilic and cycloheximide-resistant fungi were recovered from diseased camels. Trichophyton, Microsporum and Chrysosporium were the most common genera. **T. verrucosum** appeared to be the main cause of ringworm in small camels while **T. mentagrophytes** infected older ones. Camel skin presents a suitable habitat for the growth of some dermatophytes and other potentially pathogenic fungi.

**Fadlelmula** *et al.* **(1994)** conducted a survey to study camel ringworm in Eastern Sudan. Ringworm was diagnosed in 217 out of 498 young camel calves less than two years old examined during a whole year (43.5%). The peak incidence of the disease was found to be in Autumn and Winter. The disease was observed more frequently among young growing calves (1-2 years) than older animals but the prevalence among male and female animals was found to be similar. Lesions were observed mainly on the head, neck and shoulder with frequent extension to the flanks and limbs. **Trichophyton verrucosum** was isolated in pure culture for the first time from camel ringworm in the Sudan. Histopathological findings of the natural disease are described. Epidemiology in Eastern Sudan is discussed.

Al-Ani et al. (1995) described Trichophyton schoenleinii infection in 80 camels in Iraq.. The age of the infected camels ranged from six months to 3 years. The main clinical signs were characterized by the appearance of multiple lesions on the skin of different of the body. The lesions typically consisted of an area of alopecia and a prominent whitish asbestos-like accumulation of scales. The causative agent Trichophyton schoenleinii was identified by the microscopic examination of stained direct smear and by culture. Experimental infection of a susceptible camel resulted in the development of clinical signs.



A 2-year old camel showing lesions on the neck and chest. Al-Ani et al. (1995)



A one-year old camel infected experimentally with Trichophyton schoenleinii, **Al-Ani** et al. (1995)





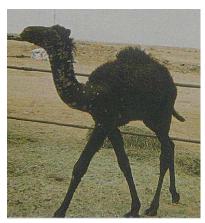
Histopathological section of the skin showing infected follicle, a10-days colony of Trichophyton schoenleinii on mycobiotic agar, **Al-Ani** et al. (1995)

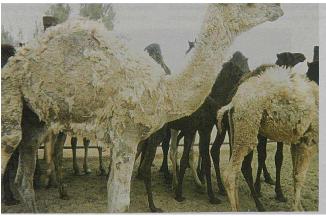
Manefield and Tinson (1997) reported that ringworm caused primarily by Trichophyton mentagrophytes is a common skin disease in Australian camels, especially in the young those under three years of age. The species found in Central Australia is contagious to humans. Skin lesions on camels are characteristically circular crusty areas of alopecia, which may coalesce. Veterinary investigations of skin lesions in 31 camels were conducted in Central Australia, which included laboratory testing. Trichophyton species was considered to have caused 15 of them. Chlorinated or iodine based topical treatments are effective

**Al-Ani** *et al.* (1998) designed an epidemiological study to study camel diseases based on systematic methods. A total of 369 living and 156 slaughtered camels of different sex, age and breeds in four different areas of Jordan were used. These areas include Al-Badia, Amman, Al-Ramtha and Jordan Valley. After a general clinical examination, blood, fecal and skin samples were taken. Among the common diseases and/or conditions were: 98% gastrointestinal parasites, 100% tick infestation, 83% mange, 44.2% hydated cyst, 33% trypanosomiasis, 33% nasal myiasis, and 21.79% plastic foreign bodies. Less commonly occurring diseases were mastitis, 3.5% pneumonia, **2.1% ringworm**, 1.2% liver abscess, 1.92% onchocerciasis and 1.9% infertility.

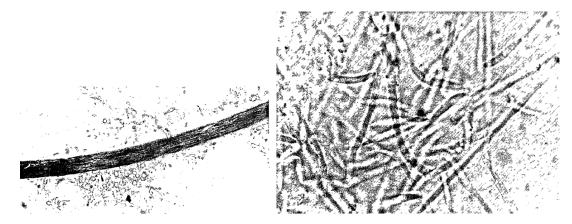
**Gitao** (1998a) reported that the incidence of D. congolensis was investigated in 3800 camels in 4 camel rearing areas in Kenya during dry and wet seasons. More camels were infected during the wet season (21.2%) than during the dry season (14.5%). Camel calves were more (23.1%) affected and had more severe and widespread lesions than adult camels (19%). There was no difference between the infection rates of males and females. One area near forest vegetation had a higher infection rate of camel calves than the other areas. 18 camels had a dual infection of D. congolensis and **T. verrucosum**. Mixed infections were found in calves during both dry and wet seasons. Infection with D. congolensis is a factor that affects camel production in Kenya.

**Gitao** *et al.* (1998b) described a mixed infection of D. congolensis and M. gypseum in camels reared on a dairy farm in Saudi Arabia. A total of 131 out of 559 camels (23.4%) were affected. Forty-eight camels less than one year of age had discrete, circumscribed, crusty, hairless lesions, found in particular on the neck and forelegs. Eighty-three camels of varying ages had extensive hair matting with crusty, hairless lesions, especially on the flanks. Camel calves and young camels demonstrated a relatively greater amount of skin lesions. D. congolensis and M. gypseum were diagnosed by direct microscopy, isolation and histopathology.



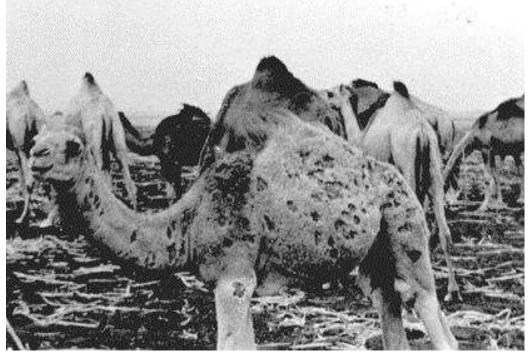


Discrete, circumscribed lesions on a camel calf belonging to the 'Majaheem' breed. Hair matting and crusty, hairless lesions on the flanks of a camel calf. **Gitao** *et al.* (1998)



Direct 10% potassium hydroxide (KOH) smear with septate mycelia and arthrospores around hair shaft (x 1,000). A lactophenol cotton blue mount of Microsporum gypseum culture featuring abundant macroconidia with 4-5 cells (x 1,000)  $Gitao\ et\ al.\ (1998)$ 

**Agab and Abbas** (1999) reported ringworm in 217 out of 3731 camels examined during the period March 1991-February 1992, of which 106 camels were diagnosed in summer, 61 in autumn and 50 in winter. Younger camels of up to three years of age were those affected most by ringworm (dermatophytosis), while older camels were rarely affected. *Trichophyton verrucosum* was isolated as the sole aetiologic agent.



A young camel calf affected by dermatophytosis (ringworm) Photo: H. agab

**Al-Rawashdeh**, *et al* (2000) isolated T. schoenlenii from generalized lesions from a group of young camels.

**Pal** *et al.* (2000) examined skin scrapings collected aseptically from cutaneous lesions on the face of a 6-month-old male camel and on the hand of a 29-year-old animal attendant, from Korea Republic, were examined by light microscopy after treatment with 10% KOH. Fungal hyphae were observed and cultures grew *T. verrucosum*. The camel was treated with application of a 2% iodine solution; the man was treated with 2% micronazole ointment

Wernery and Kaaden (2002) mentioned that T. verrucosum, T. mentagrophytes, T. schoenleinii. T. sakisovii, T. T. dankaliense. M. gypseum and M. canis were the dermatophytes reported as causes of ringworm in dromedary camels. There are 2 clinical types of ringworm in camels. The first shows typical lesions that are gray-white in colour.may coalesce and mainly occur on the legs, neck and head of young animals. The second is a more generalized infection on head, neck, limbs and flanks, whereby these lesions These lesions are characterized by small, round alopecic areas, which may initially be confused with mange.



Ringworm, generalized infection of the hind limb of a dromedary, Typical lesions of ringworm in a young dromedary. Wernery and Kaaden (2002)

**Abbas and Omer (2005)** reviewed the infectious diseases of the camel. Dermatophytosis or ringworm occurs commonly in young camels while camels above four years of age are apparently immune. The most commonly isolated dermatophytes are *Trichophyton spp.* and *Microsporum sp.* Fungal infections are more common during the cooler months, and three clinical forms have been described: superficial, follicular and generalized. The latter form is associated with loss of body condition and rather severe skin lesions, which are sometimes purulent

Wisal, et al. (2010) collected and examined 136 skin scrapings from camels suspected to be infected with dermatophytes from Alshowak (90 samples) and Alobied (46). Seventy seven Trichophyton verrucosum, 47 Trichophyton mentagrophytes, 9 Trichophyton schoenlenii and 3 Trichophyton tonsurans were isolated. Both female and male camels were susceptible and camels less than 3 year old were more susceptible to infection.

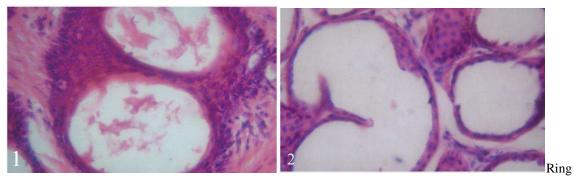


A camel from Alshowak areas, East Sudan showing localized lesions of ringworm on camel neck.

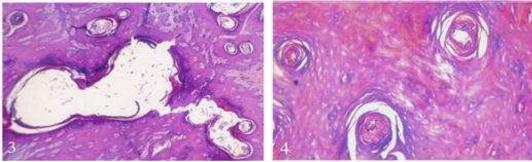


A camel from Elobied areas showing generalized lesions of ringworm giving moth-eaten appearance of wool. **Wisal**, *et al.* (2010)

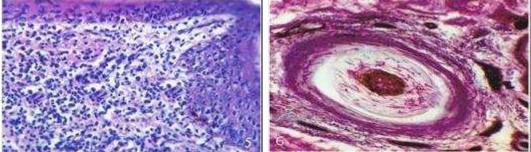
**Abo El Foutah** et al. (2012) carried out a study on 10 camels aged 3 -4 years old in Belies slaughtered house, 5 camels were apparently healthy (control group) and 5 camels sowing gross lesions of ringworm (diseased group) were used for isolation of etiological agent of ringworm as well as study the hemato-biochemical and pathological changes induced by ringworm in camels in Sharkia governorate. Mycological examination of skin scrapings revealed that affected camels were positive for **Trichophyton verrucosum**. Ringworm in camels induced significant decrease in total erythrocytic count, haemoglobin content and packed cell volume% associated significant increase leukocytic count, neutrophils and lymphocyte, AST, ALT, ALP, creatinine and urea In addition insignificant decrease in monocytes, eosinophils, basophiles, total protein, albumin and insignificant increase globulin. Camels suffering from ringworm revealed grossly white to brown area of scaly skin and circumscribed hairless areas. These lesions were noticed on the skin of the neck, muzzle, around the eyes, base of the eyes and limbs. Microscopically cystic dilatation of the hair follicles and sweat glands lined by atrophied lining epithelium, hyperkeratosis and acanthosis of epidermis, intra-epidermal pustules and acanthosis, aggregations of neutrophils and lymphocytes in the dermis were observed. Moreover basophilic granular material replaced the derma collagen and muscular fibers were also detected. It could be concluded that the ringworm in camels induce some adverse effect on haemato-biochemical parameters and pathological picture.



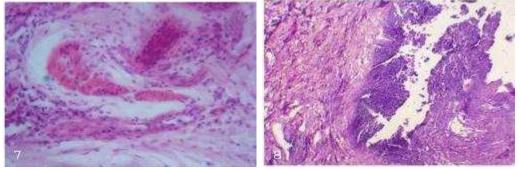
worm in camels, noticed destructed hair shaft, perifollculitis and cystic dilatation of the hair follicles (H&E X650). Ring worm in camels, noticed atrophied lining epithelium and cystic dilatation of sweat glands (H&E X400) **Abo El Foutah** *et al.* (2012)



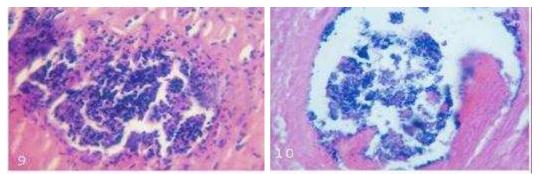
Ring worm in camels, noticed hyperkeratosis and acanthosis of epidermis (H&E X200). Hyperkeratosis of epidermis (H&E X200). **Abo El Foutah** *et al.* (2012)



Ring worm in camels, noticed intra epidermal pustules and acanthosis (H&E X400). Ring worm in camels, noticed tricophyton hyphae positive for PAS-reaction (H&E X400). **Abo El Foutah** *et al.* **(2012)** 



Ring worm in camels, noticed zenkers degeneration of muscle fiber and necrosis (H&E X1200) Ring worm in camels, noticed leukocytic aggregations and necrotic debris (H&E X200). **Abo El Foutah** *et al.* (2012)



Ring worm in camels, noticed aggregations of neutrophils and lymphocytes in the dermis of some cases (H&E X400). Ring worm in camels, noticed basophilic granular material replaced the dermal collagen and muscular fibers (H&E X400). **Abo El Foutah** *et al.* (2012)

**Eissaa** *et al.* (2013) investigated the causative fungal agents of dermatophytosis and the rate of distribution of disease among animals in El-Bieda-Libya. 185 samples of skin infection were collected from different animals (75 camels, 62 cattle, 35 sheep and goats, 10 dogs and 3 cats). These animals were clinically diagnosed as dermatophytosis. Results of the microscopical examination of 185 hair and scabs samples revealed the presence of characteristics arthrospores in 165 samples with incidence of (89.2%). On the other hand, results of cultivated samples on specific media showed that 85 were positive culture with incidence of (45.9%). Wide varieties of dermatophytes were isolated from animals, but few zoophilic species were responsible for the majority of the cases. The finding revealed the identification of 7 (8.2%) strains of **M. canis** and 78 (91.8%) strains of Trichophyton, which included 57 (67.1%) strains of **T. verrucosum** and 21 (24.7%) strains of **T. mentagrophytes**. From 75 Camels, T. verrucosum was isolated from 19 and T. mentagrophytes 10 cases.

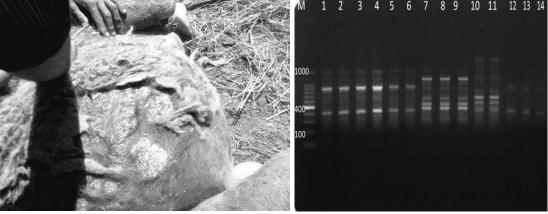
Enany et al. (2013) studied the phenotypic and genotypic identification of dermatophytes isolated from skin lesions of camels. In this study 70 specimens of hair and skin scrapings were taken from camels with skin lesions from El – Beheira Governorate ( Junbway / Kom Hamada / El – Dalngat), El- Giza Governorate (Briqash ) and El – Sharkia Governorate ( Belbeis ) for identification of common dermatophytes of camels using conventional techniques macromorphology , micromorphology and physiological test and compared with molecular method (polymerase chain reaction) using the short oligonucleotide (GACA)4 as a primer for identification of the tested dermatophyte isolates. ( GACA) 4 based PCR identified the tested isolates from camel skin lesions to the species level in full agreement with the culture-based method. The obtained dermatophytes isolates were identified by macromorphology and micromorphology into 22(73.33%) Trichophyton mentagrophytes, 5(16.66%) Trichophyton. verrucosum, 3(10%) mixed between T.mentagrophytes and T.verrucosum.



A young camel calf has Crusty and hairless lesions on the shoulder, A young camel calf have circumscribed, crusty and hairless lesions on the belly due to dermatophytes **Enany** et al. (2013)



Affected camel showed white hairless patches on different parts of the body. The lesions typically consisted of an area of alopecia. Affected camel showed heavy incrustation on different parts of the body and progressed down to belly and legs. **Enany** *et al.* (2013)



A prominent grayish white accumulation of scales on the belly difficult to pull out of the skin (thick and heavy encrustation) due to severe dermatophytosis. **Agarose gel electrophoresis for PCR using (GACA)4** M, molecular weight marker. Lanes (1-6) first profile of *T.mentagrophytes* Lanes (7-9) second profile of *T.mentagrophytes*. Lanes (10&11) third profile of *T. mentagrophytes* Lanes (12-14) *T. verrucosum* **Enany** *et al.* (2013)

Tuteja et al. (2013) mentioned that the species of common dermatophytes i.e. Microsporum and Trichophyton isolated from camel skin lesions in India included M. audouinii, M. canis, M. nanum, M. ferrugineum, T. verrucosum, T. mentagrophytes, T. schoenleinii, T. equinum, T. concentricum, T. tonsurans, T. violaceum, T. soudanense and T. rubrum. These fungi caused sporadic cases of skin infections in individually maintained camels as well as affecting many camels in the herds. These fungi create distinctive lesions of ringworm. *Epidermophyton floccosum* was isolated from skin infection in a herd of

camel at Charanwala (Bajju) with fast spreading lesions. Out of the 50 camels examined 16 were infected with such lesions. Lesions were peculiar as if hairs were burnt with fire leaving behind ash deposit over the skin. Lesions were observed throughout the body. All ages of the camel were affected but calves were more severely affected. The general dryness of the skin coat was more pronounced in such cases. During development of the lesions necrosis follows alopecia. It caused itching, uneasiness and resulted in weakness and debility of the animals





Epidermophyton initial lesions on thigh and flank, Epidermophyton mild lesions on the flank and neck **Tuteja** *et al.* (2013)





Epidermophyton fast spreading lesions with circular patches, Epidermophyton lesions giving just burning appearance **Tuteja** *et al.* (2013)



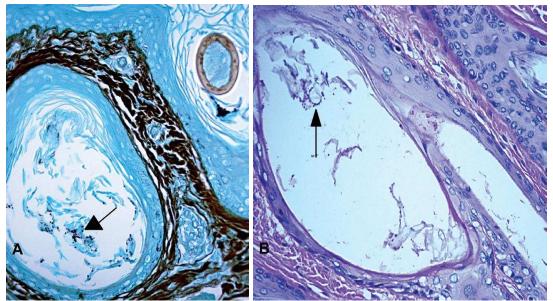


Epidermophyton lesions on the neck, Epidermophyton lesions on the forehead **Tuteja** *et al.* (2013)

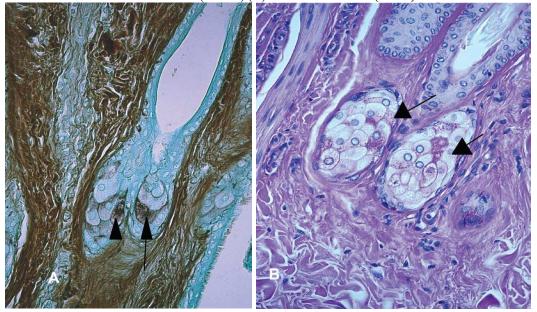
Al-Salihi et al. (2014) described the histopathological features of dermatomycosis and sarcoptic mange mixed infection accompanied with chronic granulomatous hidradinitis in five camel's skin samples collected from Al-Najaf slaughter house in Republic of Iraq. Direct examination of the skin scraping with 10% KOH revealed fungal organisms (mycelia and arthrospores) and mites that consistent with Sarcoptic spp (Sarcoptes scabiei var cameli) in the macerated debris. Histological examination of the skin sections revealed dermatitis characterized by: acanthosis with marked parakeratosis, hyperkeratosis and crust formation, rete-pegs, hyperplastic changes in sebaceous glands and hair follicles cells, granulomatous hidradinitis and infiltration with eosinophils, lymphocytes, macrophages and neutrophils. Sections stained with periodic acid-Schiff (PAS) and Gomori's methenamine silver (GMS) stain, revealed large numbers of fungal arthrospores and hyphae colored bright magenta with PAS and black with GMS. In conclusion, this study reported mite infestation that occurs concurrently with dermatomycosis accompanied with granulomatous hidradenitis in a species belonging to the Camelidae. Additional studies including better understanding of pathogenesis of mixed infection and its effects on the skin immunity will be desirable for further understanding of those skin diseases in camels.



A skin scraping after treatment with 10% KOH shows: A) Sheet of arthroconidia X10 (arrows); B) Fungal hyphae in the infected hair (arrows) X 2 **Al-Salihi** *et al.* (2014)



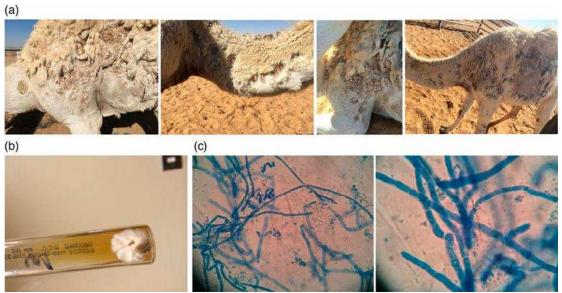
Refractile arthrospores and hyphae appear: bright magenta with Periodic Acid-Schiff stain (A) and black with Gomori's Methenamine Silver stain (arrows) (B). **Al-Salihi** et al. (2014)



Hyperplastic changes in sebaceous glands which were filled with spores that appeared black (arrows) with Gomori's methenamine silver X10. (A) and sebaceous glands filled with bright magenta spores (arrows) (Periodic Acid-Schiff) X40 (B). **Al-Salihi** *et al.* **(2014)** 

**Almuzaini** et al. (2015) studied dermatophytosis in a private farm of dromedary camels at Qassim Region, Central of Saudi Arabia. The prevalence of dermatophytosis in camels was 11.5% and significantly differed among different age groups ( $p \le .0001$  and odds ratio = 14.61), with higher prevalence among camels younger than three years (22.10%). Clinical signs of ringworm recorded in this study were non-pruriginous dry circumscribed discrete, crusty hairless lesion distributed over the head, neck, shoulder, limbs and flanks. Mycological examination revealed **Trichophyton verrucosum** from the clinically diseased camels (n = 23). Significant improvement in the terms of rapid recovery was recorded in camels receiving topical application of 10% iodine ointment for three weeks in addition to intramuscular injection of vitamin A (400,000

IU/animal) on alternate days for three times and mineral mixture supplementation as dietary additives for three weeks compared to the group that was treated using iodine ointment for three weeks alone.



(a) Cutaneous lesions, (b) Colonies on SDA and (c) Distorted hyphae with chlamydospores in chains confirming T. verrucosum. **Almuzaini** *et al.* (2015)

.Sabra and Al-Harbi (2015) conducted a study on 15 camel farms (1187 camels and 45 farm workers) at Taif area, KSA. The farm workers were (42.2, 31.1 and 26.7%) with nationality (Somalis, Sudanese and others). Camels were infected by superficial skin mycosis as 19.2%; also farm workers were infected as 24.4% by zoonotic superficial skin mycosis. Eleven farm workers infected had given 37 specimens from their lesions area, which resulted in (29.7, 18.9, 18.9, 16.2 and 16.2%) respectively from sites of (upper limbs, lower limbs, body, heads and faces) of them. The results of dermatophytes isolation and identification were (34.4, 26.6, 17.2, 12.5 and 9.4%) respectively from upper limbs included (Tinea unguium and Tinea manuum), lower limbs (Tinea unguium, Tinea pedis, Trichophyton verrucosum and Trichophyton rubrum), body (Tinea corporis, Tinea cruris, Trichophyton verrucosum and Trichophyton rubrum), faces (Tinea barbae and Tinea faciei) and heads (Tinea capitis). Tinea species were higher than Trichophyton species with values of (92.2 and 7.8%) respectively. The results of Tinea species were (28.1, 17.2, 10.9, 10.9, 9.4, 7.8, 4.7 and 3.1%) for (Tinea unguium, Tinea manuum, Tinea pedis, Tinea corporis, Tinea capitis, Tinea barbae, and Tinea cruris) respectively, while Trichophyton species were (4.7 and 3.1%) for (Trichophyton verrucosum and Trichophyton rubrum) respectively. The conclusion intends to recommend many improvements from veterinary medicine site which in-need for farm workers in the protection of them against occupational diseases for farm field.

**Volpato** *et al.* (2015) focused on the traditional knowledge of camel diseases and their treatments among Sahrawi nomads, detailing how this knowledge is powerfully reflected on pastoral adaptation strategies to the ecological system of Western Sahara. Methods: Between 2005 and 2010, free listing exercise on camel diseases with 46 Sahrawi nomads and refugees, semi-structured interviews with 36 knowledgeable informants about camel diseases

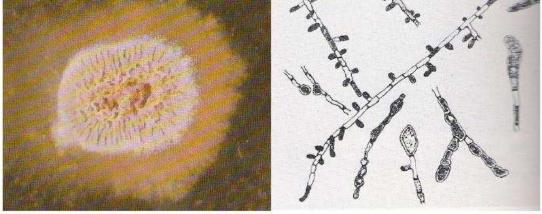
and associated treatments, and a voucher specimen collection of the plants and products cited were conducted in the territories administered by the Saharawi Arab Democratic Republic, Western Sahara. Analytical methods included standard ethno biological, ethnobotanical and cultural consensus analyses. Results: In total, 42 camel diseases were free listed by informants, of which sarcoptic mange (jrab) is the most reported camel disease, mentioned by more than 82 % of the informants, while dermatomycosis (legrad) is ranking as second and reported by three-quarters of the informants

**AL-Bassam. and Mahmud** (2016). presented a case report on dermatophytosis in a three years old dromedary camel. Case history, clinical signs response to treatment and laboratory confirmation of infection are included; with a short review of literatures concerning reports on dermatophytosis in camels all around the world.

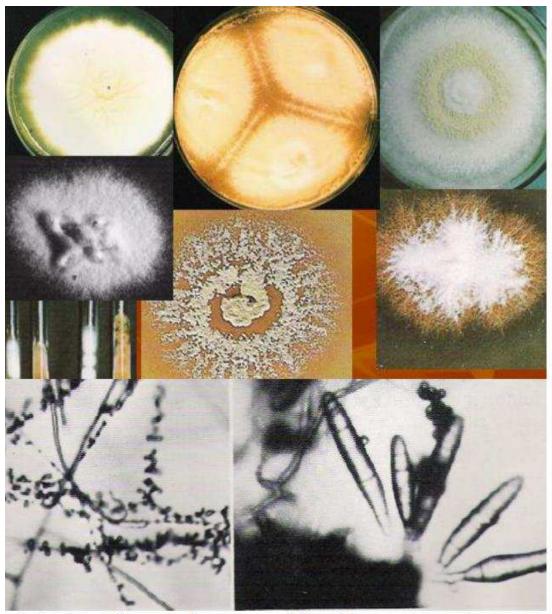
Baghza et al. (2016) isolated and identified dermatophytes from 165 suspected camels in Dhamar area, Yemen. Skin scrapings were collected from ringworm lesions of the study animals. They were microscopically examined using potassium hydroxide (KOH) preparation. Specimens that showed a positive result were then cultured on Sabouraud's Dextrose Agar (SDA) for identification. A total of 159 (96.4%) of the suspected camels were found to be positive for fungal infection during the direct KOH examination. The infection was significantly higher among young animals of  $\leq 12$  months (94.3%,  $\chi^2 = 73$ , P < 0.05). Majority of the cultured specimens showed positive growth (93.1%,  $\chi^2 = 118$ , P < 0.05). The overall rate of dermatophyte infection was 83.11%. The frequency of Trichophyton and Microsporum genera were 89.4% and 10.6% of the isolated genera, respectively... Trichophyton schoenlenii showed the highest frequency (49.6%) followed by *Trichophyton verrucosum* (14.6%), *T. mentagrophytes* (13%) and **Trichophyton** tonsurans (12.2%). On the other hand, Microsporum audouinii and Microsporum canis represented 5.7% and 4.9% of the identified species.

## Morphology of dermatophytes isolated from camels

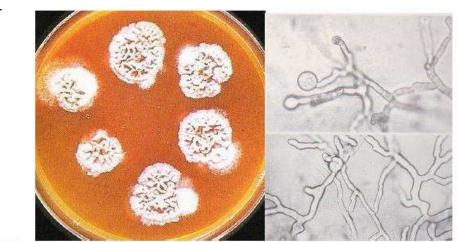
# 1. Trichophyton species



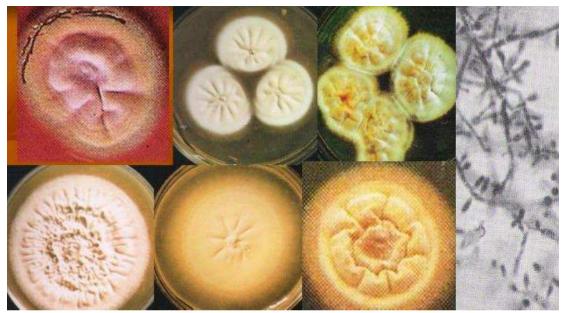
Colony, microconidia and chlamydospres of T. verrucosum



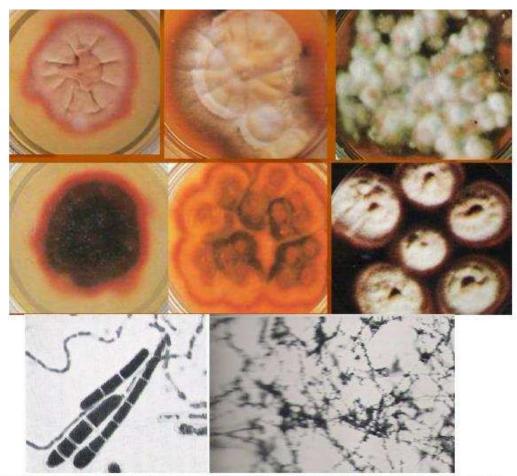
Colonies, micro- and macroconidia of T. mentagrophytes



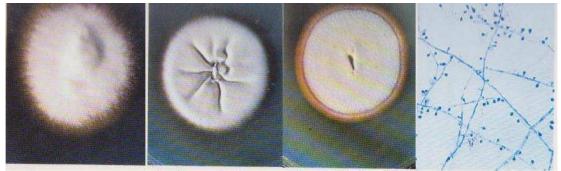
Colonies and favic chandliers characteristic of T. schoenleinii



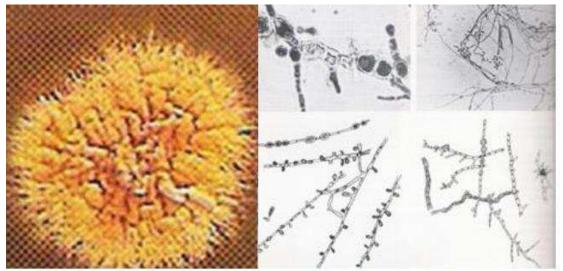
Colonies and microconidia of Trichophyton tonsurans



Colonies, macro- and microconidia of Trichophyton rubrum



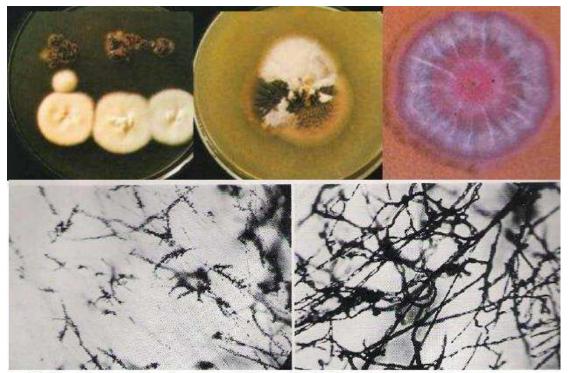
Colonies and microconidia of Trichophyton equinum



Colony and microconidia of Trichophyton soudanense



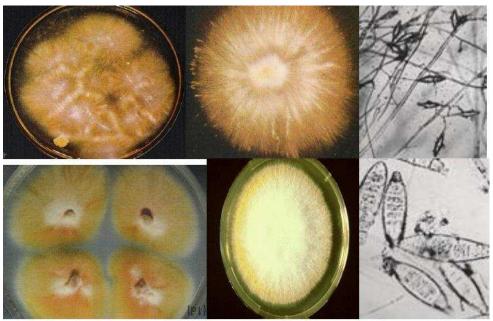
Colonies and sterile hyphae of T. concentricum



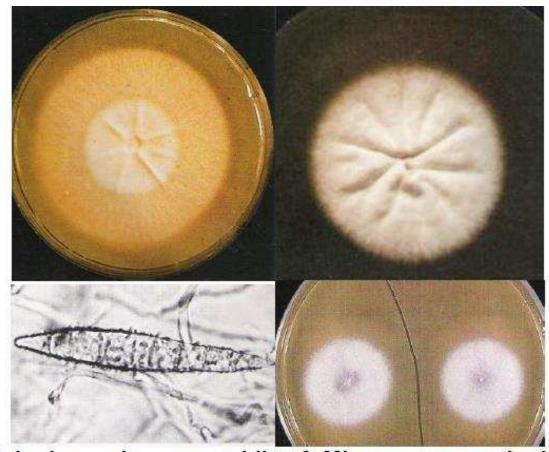
Colonies and microconidia of Trichophyton violaceum



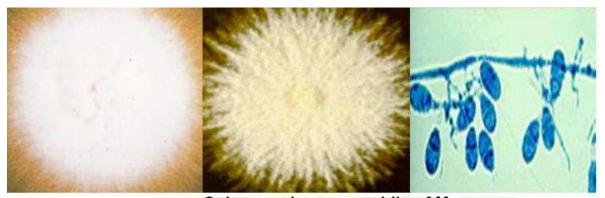
Colonies and macroconidia of Microsporum gypseum



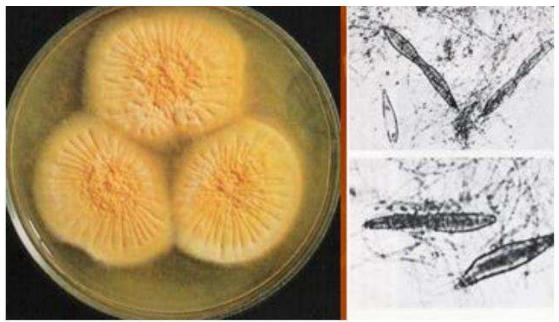
Colonies and macroconidia of Microsporum canis



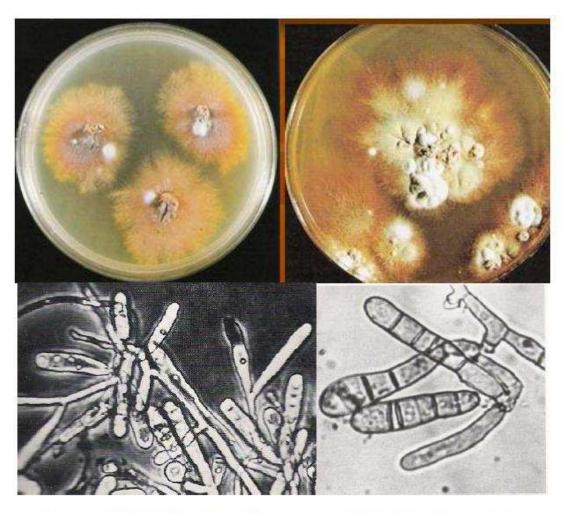
Colonies and macroconidia of Microsporum audouinii



Colony and macroconidia of M. nanum



Colonies and macroconidia of Microsporum ferrugineum



Colonies and club-shaped macroconidia of E. floccosum

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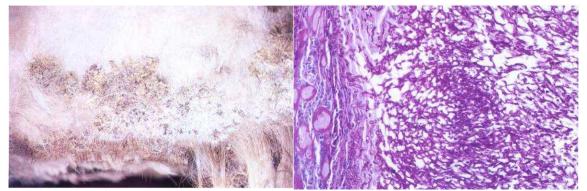
# 2. Candidosis

Candidosis is a fungal disease affecting the mucous membranes and the skin and may cause infection of any organ or system or systemic infections. It is distributed worldwide in a variety of animals and is most commonly caused by *Candida albicans* but other Candida species and other yeasts have been also reported in animals. In camels, Candida species have been incriminated as the causative agents of infections of the skin, gastrointestinal and genital tracts, mastitis, eye infection as well as systemic infection.

#### **Reports:**

#### 1. Candidosis of the skin

Wernery et al. (2002) diagnosed a skin lesion caused by C. albicans. The 6-week-old camel calf had developed thick crusts near the hump in which hyphae were demonstrated with PAS stain.



Thickened crusts near the hump of a dromedary calf caused by C. *albicans* hyphae from the skin of a camel calf. **Wernery** *et al.* (2002)

Pal et al. (2007) reported a case of otitis in camel due to C. albicans.

Lamm et al. (2009) reported an 8-year-old castrated male llama was evaluated because of skin disease that had waxed and waned in severity over the last few years. The animal was treated topically by 20% zinc oxide ointment for more than one year with no apparent effect. The crusts were firm and moist and could be manipulated away from the skin. The underlying skin was moist, red and had a foul odour. Within the affected area there were scattered small pustules. Punch biopsy specimens were examined histologically and microbiologically. The diagnosis was Fungal dermatitis in a camel caused by Candida albicans



Skin lesion showing coalescing crusts over the muzzle of a llama, Lamm et al. (2009)

**Tuteja** *et al* (2010, 2012) reported that, lesions of candidosis of the skin in camels are initially observed on the back near the hump; later on the lesions extend towards the abdomen and may cover the whole body. Lesions are initially round in shape and measure less than one centimeter in size which may enlarge to more than 10 centimeters in size and may coalesce.



Initial stage of lesions of candidiasis Initiation of bleeding from lesions in candidosis **Tuteja** et al (2010, 2012),



Skin candidosis in calf herd Tuteja et al (2010, 2012),



heavily infected camel calf Tuteja et al (2010, 2012),

**Abdalla1 and M Salim (2014)** carried out a study to determine the ability of fungi to be one of the causative agents of **contagious skin necrosis** of camel. A total of 40 pus samples from suspected contagious skin necrosis lesions were collected with sterile absorbent swabs and were labelled with date of collection, sex, age and location. Samples were collected from Gadarif state during winter and summer. Swab from contagious skin necrosis lesion was cultured by dipping it into brain heart infusion broth and incubated for 1-2 days at 37°C. The isolate was sub cultured on Sabouraud's dextrose agar and incubated at 37°C for 1-2 days. Identification of moulds was done by rate of growth, texture and the pigmentation of cultures was used, (Laura, et al, 1998). From forty swabs of camel infected with contagious skin necrosis, eleven mould isolates of three genera were recovered on Sabouraud's dextrose agar, Aspergillus niger (4 isolates), Aspergillus flavus (2 isolates), Aspergillus terreus (1

isolate), penicillum spp (1 isolate) and Scopulariopsis brevicaulis (3 isolates). Three Candida spp were isolated and were identified as **Candida parapsillosis**, **Candida gulliermondii and Candida zeylanoides**.

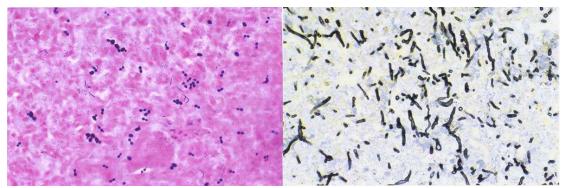
#### 2. Gastrointestinal candidosis

**Hajsig** *et al.* (1985) reported a neonate llama that had developed yellowish diarrhoea; despite antibiotic treatment and electrolyte therapy died 5 days later. On necropsy, the walls of C1 and C2 were thickened and edematous. A white-grayish pseudomembrane several millimeters thick was diagnosed. Microscopically, the epithelium of the mucous membranes was necrotic and invaded by masses of pseudohyphae and budding yeast cells.

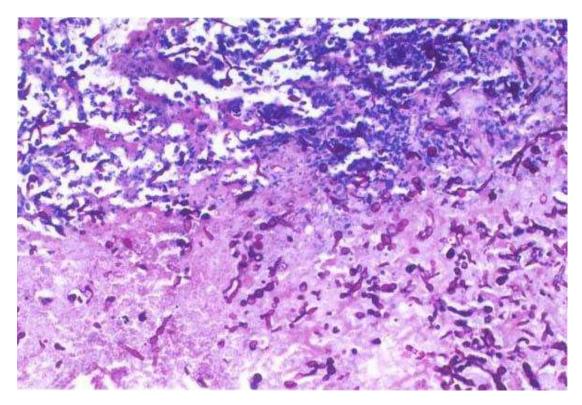
Wernery et al. (2002) reported candidosis in 8 to 48 hour-old dromedary calves in the UAE. These calves developed yellowish diarrhea. On necropsy, yellow pseudomembranes were found in the small intestines. There was no milk in their digestive system, but variable amounts of sand and water were seen in the abomasum. Smears taken during necropsy from the intestinal mucosa showed C. albicans and C. perfringens organisms. Microscopic investigation showed necrosis of the mucous membranes invaded by yeast cells that were limited to the epithelial tissue. The dromedary calves had also developed a colisepticemia and some of them a C. perfringens enterotoxaemia. The authors could prove that the calves possessed very low levels of copper and therefore had ingested sand with which they took up clostridial spores. In adult camels that had been treated with antibiotics over a long period, multiple ulcers have been observed in the abomasum, invaded by masses of C. albicans organisms. The same authors have also diagnosed a skin lesion caused by C. albicans. The 6-week-old camel calf had developed thick crusts near the hump in which hyphae were demonstrated with PAS stain.



Yellow pseudomembrane of the small intestine of a camel calf with candidiasis, Multiple ulcers in the abomasum of a dromedary



Direct smear from the intestine of a camel calf with candidiasis showing C. albicans budding yeast cells and C. perfringens rods

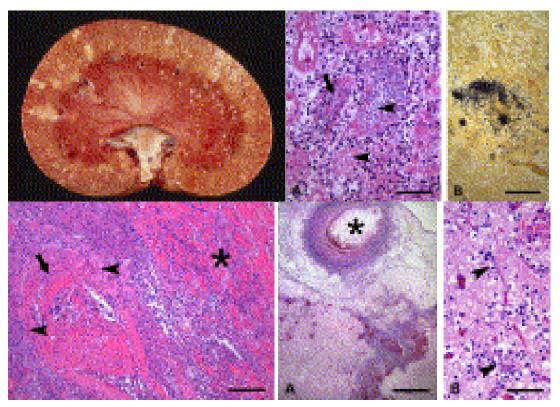


C. albicans invasion of the ulcers

# 3. Systemic candidosis

Kramer et al. (2008) diagnosed systemic Candida albicans infection in two adult alpaca stallions originating from different herds. Case 1 had a history of chronic dermatitis with unknown aetiology that had been treated long-term with glucocorticoids. Case 2 had suffered from transient facial paralysis and psoroptic mange of the external ear. Both animals died suddenly after recovering from their initial disorders. Necropsy examination of case 1 revealed multifocal erosive dermatitis, thoracic and abdominal serofibrinous effusions, and multiple suppurative foci in lung, myocardium, kidney, pancreas and brain. Case 2 had multiple ulcers of the third gastric compartment and focal suppurative nephritis. Additionally, moderate depletion of lymphoid organs was observed in both animals. Histologically, suppurative to necrotizing inflammation with necrotizing vasculitis was present in the grossly affected organs of both animals. Yeast, pseudohyphae and branching

hyphae were present within these lesions and C. albicans was isolated from lesional tissue of both animals. The primary site of Candida invasion was not determined in case 1, but the most likely portal of entry in case 2 was the gastric ulcers. Depletion of lymphoid tissue suggested a possible underlying immune suppression in both animals.



Kramer *et al.* (2008)

#### 4. Candida mastitis

Bakeer et al. (1994) reported that 1.1% of she-camel mastitis cases were caused by fungi.

**Salwa** (1995) reported mycotic she-camel mastitis in Sudan by who stated that the percentage of the disease was only 0.6%.

**Mohammed** (1996) reported that the over-all incidence of fungal isolation in apparently healthy camel, sub-clinical and clinical cases was 10 (55.55), 7 (38.8%) and 1(5.55%) respectively.

**Abdulkadhim** (2012) studied the fungal sub-clinical mastitic she camels and determined the factors that influenced it, (7.3) % was the percent of Candida sp. Infection was restricted to the age (11-15) years.

**Pal** (2015) carried out an investigation to elucidate the role of fungi in mastitis of one humped camel (*Camellus dromendarius*) in Ethiopia by employing standard mycological techniques. *Candida albicans*, a medically important yeast, was identified as cause of mastitis in one of the 25 lactating camels. The yeast was isolated in pure and heavy growth

from the mastitic milk of a 6-year-old she-camel on Pal's sunflower seed medium. There was no growth of bacteria on nutrient agar, and blood agar. The morphology of the fungal isolate was attempted in "Narayan" stain. As far as could be ascertained, this seems to be the first laboratory confirmed case of mycotic mastitis due to *Candida albicans* in a she-camel from Ethiopia. It is emphasized that future investigation of mastitic milk should be attempted to elucidate the role of yeasts and moulds in clinical and sub-clinical mastitis in dairy animals. As Pal's sunflower seed medium is very economical, and easy to prepare, its wider application in microbiology and public health laboratories for the study of fungi is recommended.

Yam et al. (2015) identified the yeasts in samples of the Iranian traditional fermented camel milk. Yeast species were identified including Pichia. anomala, Pichia. jadinii, Debaryomyces. hansenii, Pichia. guilliermondii, Kluyvermyces. marxianus, Candida. fermentati, Pichia. ciferrii, Torulospora. delbrueckii, Candida. versatilis, Kluyvermyces. lactis, Candida. kefir, Saccharomyces. pastorianus, Saccharomyces. serevisiae, Candida. friedrichii, Kluyvermyces. polysporus, Rhodotorula. musilaginosa, Candida. lipolytica and Candida. lusitaniae. All of them could assimilate the glucose and liquefacted the gelatin, but could not production starch, tolerated 1% acetic acid, growth in the presence of Nacl 16% (except Debaryomyces. hansenii), anassimilateed the nitrate (except Rhodotorula. musilaginosa). Within the yeasts species, Kluyvermyces. lactis (8.57%) and Kluyvermyces. marxianus (8.57%) were the predominant.

### 5. Candida and other yeast flora in genital tract

Shokri et al. (2010) performed a study to identify yeast flora and to determine the number of colony forming units (CFUs) in genital tract of healthy female dromedary camels, establishing their connection in both mated and unmated conditions. The samples were taken from different parts of genital tract including vestibule, vagina, cervix, uterine body, and uterine horns of 50 camels using sterilized cotton swabs. They were cultured onto Sabouraud glucose agar containing chloramphenicol and incubated at 30 degrees C for 7-10 days. A total of 454 yeast colonies were obtained from genital tract. Yeast isolates belonged to 8 genera: Candida (73.1%), Trichosporon (10.1%), Geotrichum (7.5%), Kluyveromyces (3.5%), Rhodotorula (2.4%), Aureobasidium (1.4%), Cryptococcus (1.1%) and Prototheca (0.8%). Among different Candida species, C. zeylanoides was the most common isolated species, representing significant difference with other Candida species (P<0.05). The mean number of yeasts found in the vestibule (46%) was significantly higher than the results obtained from other parts (P<0.05). In addition, the mean value of CFUs from unmated females (71.1%) was significantly higher than mated females (P<0.05). The results showed that C. zeylanoides was a common component of healthy camel females' genital mycoflora and the number of yeasts varied between mated and unmated females.

**Shokri** (2011) carried out a study to isolate Candida flora from healthy female dromedary camel's genitalia and to determine the prevalence of different Candida species based on mate and age conditions. The specimens were collected from various sites of genital tract including vestibule, vagina, cervix, uterine body and uterine horns of 40 camels. These were grown on Sabouraud dextrose agar and incubated at 30 degrees C for 7-10 days. A total of 332 Candida yeasts were obtained from the genital tract. Candida zeylanoides (27.7%) was the most prevalent Candida species, followed by C. tropicalis (19%), C. krusei (14.2%), C. albicans (12.6%) and C. rugosa (12.1%). The mean number of Candida isolates found in the vestibule (40.9%) was significantly higher than the results obtained from other parts

(P<0.05). In addition, the mean value of CFUs from unmated females (68.3%) was significantly higher than mated females (P<0.05). The results showed that C. zeylanoides was a common component of healthy female dromedary camels genitalia and the number of Candida species varied between mated and unmated females.

Shokri (2012) determined the genetic diversity and antifungal susceptibility of Candida zeylanoides strains. A total of 14 C. zeylanoides strains isolated from the genital tract of female camels (Camelus dromedarius) were characterised by disc diffusion antifungal susceptibility test and random amplified polymorphic DNA (RAPD) technique. The inhibition zones were ranged from 20±2.6 to 30±3.6 mm for nystatin, 18±1.8 to 44±5.2 mm for ketoconazole and 8±0.3 to 32±3.5 mm for fluconazole. In addition, Zataria multiflora (Z. multiflora) and Pulicaria gnaphalodes (P. gnaphalodes) essential oils had a potent activity against C. zeylanoides with inhibition zones ranging from 50±2.8 to 70±5.2 mm and 22±1.0 to 56±4.8 mm, respectively. At 81% similarity, three distinct groups were observed for the 14 strains phenotypically identified as C. zeylanoides; the first group composed of one isolate, the second group composed of two isolates and the third group as the largest group composed of 11 isolates. The values of 35.7%, 85.7% and 100% of C. zeylanoides genotypes were susceptible to fluconazole, ketoconazole and nystatin, respectively. The results showed that RAPD fingerprinting with primers M13 and Candida differentiation unit (CDU) has presented phenotypically similar but genetically distinct organisms among C. zeylanoides strains.

Sharifzadeh et al. (2015) performed a study to investigate the enzymatic activity of different Candida species and their antifungal susceptibility patterns. The study involved a total of 83 isolates of Candida from the genital tract of the female Camelus dromedarius. After species identification, the isolates were analyzed for the production/activity of phospholipase, proteinase and haemolysin. In addition, the agar disc diffusion method was performed on the basis of CLSI guidelines M44-A2 protocol for antifungal susceptibility testing. All the isolates were able to produce phospholipase, proteinase and haemolysin. A total of 35.48%, 87.09% and 64.51% of C. albicans isolates exhibited very high phospholipase, proteinase and haemolytic activities, respectively, whereas very high phospholipase, proteinase and haemolytic activities were determined in 5.76%, 23.07% and 45.16% of non-C. albicans isolates respectively. Overall, 61 (73.5%) of Candida isolates were susceptible to fluconazole, 70 (84.3%) susceptible to clotrimazole, 82 (98.8%) susceptible to voriconazole, 76 (91.6%) susceptible to itraconazole, 75 (90.4%) susceptible to ketoconazole, 83 (100%) susceptible to amphotericin B, 81 (97.6%) susceptible to nystatin and 36 (43.4%) susceptible to flucytosine. Candida isolates showed higher haemolytic activity than that of other secreted hydrolases among vaginal Candida species. In addition, amphotericin B was the most in vitro effective antifungal drug and flucytosine had the poorest activity under such conditions.

# **6.** Candida and other fungi in the eye

**Khosravi** *et al.* (2009) carried out a study to isolate and identify the eye and nose fungal flora from healthy dromedary camels living in Iran during January and May 2009. The samples were taken using premoistened swabs from the right and/or left eye (n=63) and nose (n=69) of camels, seeded onto Sabouraud glucose agar and incubated at 30 C over a period of 7-10 days. Moulds identification was achieved to the genus level and yeast colonies were

identified for macro and micro-morphologic and physiological characteristics. A total of 162 and 646 fungal isolates were obtained from eye and nose samples, respectively. The most predominant fungal isolates were Cladosporium (38.2%) and **Candida krusei** (34.9%) in the eye and Cladosporium (32.1%), **C. tropicalis** (30.9%) and **C. glabrata** (29.2%) in the nose of animals (P<0.05). The yeasts were associated with moulds. Also, 22 certain pathogens identified as Nocardia asteroides and Actionmyces bovis, which was not commonly related to fungal flora of animals' nose were also found. Our results showed that Cladosporium and Candida species were the most frequent fungal isolates obtained from eye and nose of camel

# 7. Candida albicans in the nasopharyngeal cavity

**Nawal** *et al.* (1991). isolated Candida albicans from the nasopharyngeal cavity of apparently healthy camels in Assiut.

Osman et al. (2003) isolated Candida albicans from the nasopharyngeal cavity of apparently healthy camels at Shalateen, Halaieb and Abou-Ramad areas.

**Mahmoud** *et al.* (2005) isolated Candida albicans from both pneumonic lung samples of slaughtered camels and nasopharyngeal swabs collected from diseased camels in at Shalateen, Halaieb and Abou-Ramad suffering from different respiratory manifestations.

## **Aetiology:**

C. albicans is the most abundant and significant species. Other Candida species such as C. tropicalis, C. glabrata, C. parapsilosis, C. krusei and C. zeylanoides

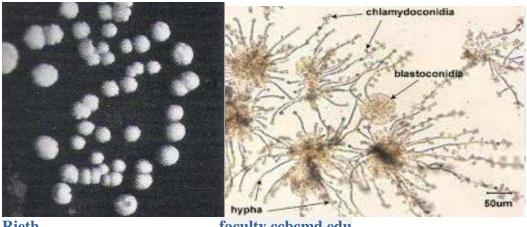
# Description of Candida species reported in camels

# 1. Candida albicans (Robin) Berkhout 1923

#### **Synonyms**

- =Blastomyces albicans Brownlie: 425-431 (1920)
- =Candida biliaria Bat. & J.S. Silveira, Hospital Rio de Janeiro 56 (2): 295 (1959)
- =Candida claussenii Lodder & Kreger, The Yeasts: a taxonomic study: 578 (1952)
- =Candida desidiosa Cif. & Redaelli, Archiv für Mikrobiologie 6: 65 (1935)
- =Candida genitalis Bat. & Silveira, Public Inst Micol Uni do Recife 170: 11 (1962)
- =Candida intestinalis Bat. & J.S. Silveira, Hosp Rio de Janeiro 56 (2): 293 (1959)
- =Candida langeronii Dietrichson, Ann Parasitol Hum Comparée 29: 479 (1954)
- =Candida mycotoruloidea Redaelli & Cif., Archiv für Mikrobiologie 6: 50 (1935)
- =Candida nouvelii Saëz, Bull Société Mycologique de France 89 (1): 82 (1973)
- =Candida truncata Vanbreus., Arch Belge de Derm et Syphil 4: 307-313 (1948)
- =Endomyces albicans Okabe, Zbl Bakt, Parasit Infek, 1.Erste Abt: 181-187 (1929)
- =Monilia alba Castell. & Chalm., Manual of Tropical Medicine: 1089 (1919)
- =Monilia albicans Plaut (1919) [MB#479429]

MorphologyOn Sabouraud's dextrose agar colonies are white to cream coloured, smooth, glabrous and yeast-like in appearance. Microscopic morphology shows spherical to subspherical budding yeast-like cells or blastoconidia, 2.0-7.0 x 3.0-8.5 um in size.



faculty.ccbcmd.edu Rieth

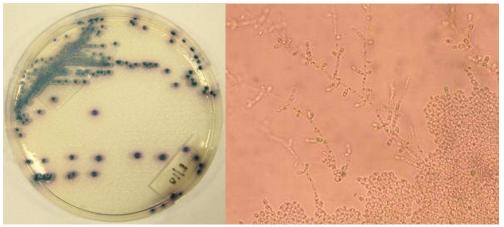
#### **Physiological Tests:**

Germ Tube test + within 3 hours. Hydrolysis of Urea +,Growth on Cycloheximide medium +. Growth at 37C +, fermentation: Glucose +; Maltose +, Galactose +/-; Trehalose+/-, Sucrose (some strains +); Lactose -. Assimilation: Glucose +; Maltose +; Galactose +; Trehalose +; Sucrose (some negative); D-Xylose +; Soluble Starch +; D-Mannitol +; D-Glucitol (Delayed), Melezitose +/-; Glycerol +/-; Succinic acid +/-; L-Arabinose +/-; L-Sorbose +/-; D-Ribose (some positive); Citric acid +/-; DL-Lactic acid +/-. Potassium nitrate -; Lactose -; Ribito-

# 2. Candida tropicalis (Castell.) Berkhout, De schimmelgeslachten Monilia, Oidium, Oospora en Torula: 44 (1923)

- =Atelosaccharomyces tropicalis (Castell.) Mello, Arq Hig Patol Exóti6: 263 (1918)
- =Candida albicans var. tropicalis (Castell.) Cif., Man Micol Medica 2: 252 (1960)
- =Candida tropicalis var. tropicalis
- =Castellania tropicalis (Castell.) C.W. Dodge, Medi Mycol. 258 (1935)
- =Endomyces tropicalis (Castell.) Castell., Zbl. Bakt. ParasitKde, Abt. 1: 236 (1911)
- =Monilia tropicalis (Castell.) Castell. & Chalm., Manual Trop Med 1086 (1919)
- =Myceloblastanon tropicale (Castell.) M. Ota, Jap. J. Dermatol. Urol.: 178 (1927)
- =Mycotorula tropicalis (Castell.) Cif. & Redaelli, (1943)
- =Oidium tropicale Castell., Philippine J Sci Section B Medical Science 5 (2): 202 (1910)
- =Procandida tropicalis (Castell.) E.K. Novák & Zsolt, Acta Bot Sci Hung 7: 133 (1961)

Colonies (YPGA) cream-coloured, off-white, soft, smooth and creamy or wrinkled near the margin. Microscopy. Budding cells (RA) ellipsoidal. Pseudomycelium abundant, consisting of long, poorly branched elements, often narrowed towards a sterile apex; conidia arranged in small groups around the middle of each cellular element. Differential diagnosis. Species signature: fermentation of maltose, +, and assimilation: galactose +, lactose, raffinose, 1rhamnose, meso-erythritol, myo-inositol, d-tryptophan (N), w/o biotin, growth at 40°C

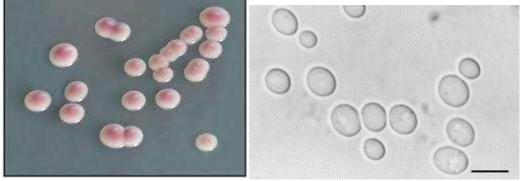


CHROMAgar<sup>TM</sup> image of Candida tropicaliswww.life-worldwide.org

3. Candida glabrata (H.W. Anderson) S.A. Mey. & Yarrow, International Journal of Systematic Bacteriology 28: 612 (1978)

≡Cryptococcus glabratus H.W. Anderson, J Infectious Diseases 21: 379 (1917) ≡Torulopsis glabrata Lodder & N.F. de Vries, Mycopa 1 (2): 102 (1938) =Torulopsis stercoralis Uden

Colonies on Glucose Peptone Agar at 25°C: after 3 days cream-coloured, smooth, dull, regular in shape, spherical, domed. Yeast-like cells are generally ovoid, single or budding 2·0-4·0 x 3·0-5·5 µm. Cultures on Corn Meal Agar: ovoid, budding cells only. No pseudomycelium (chains of elongated yeast-like cells) produced. Germ Tube Test: negative. Fermentation of Carbohydrates: Glucose + Sucrose - Maltose - Lactose - Galactose - Raffinose - Trehalose. Assimilation of Organic Compounds: Glucose + Sucrose - Maltose - Lactose - Galactose - Raffinose - Trehalose +Cellobiose - Inositol - Melezitose - Melibiose - Mannitol - L-Sorbose - D-Xylose - L-Arabinose - D-Arabinose - D-Ribose - L-Rhamnose - Glycerol v Erythritol - Ribitol - Galactitol - D-Glucitol - Salicin - DL-Lactic Acid - Succinic Acid - Citric Acid - Soluble Starch -. Assimilation of Inorganic Compounds: Nitrate -. Ability to split urea: -.

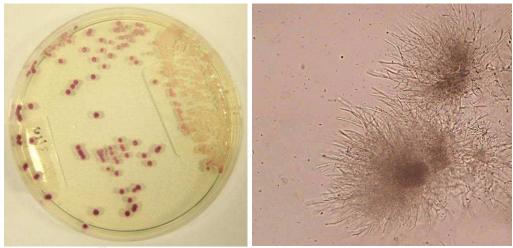


C. glabrata on HardyCHROM Candida www.hardydiagnostics.com

**4.** Candida parapsilosis (Ashford) Langeron & Talice, Annales de Parasitologie Humaine Comparée 10: 54 (1932)

- =Candida parapsilosis var. parapsilosis, Ann Parasitol Hum Comparée 10: 1 (1932)
- =Monilia parapsilosis Ashford, Amer J Tropical Medicine 8: 518 (1928)
- =Mycocandida parapsilosis (Ashford) C.W. Dodge, Medical Mycology. 294 (1935)
- =Mycotorula parapsilopsis (Ashford) Cif. & Redaelli (1943)
- =Mycotorula parapsilosis (Ashford) Cif. & Redaelli, Atti dell'Istituto Botanico della Università e Laboratorio Crittogamico di Pavia 3 (1): 47 (1943)

Colonies (YPGA) cream-coloured to yellowish, glistening and soft, mostly smooth or partly or entirely wrinkled. Pseudomycelium (RA) present, mostly abundant, consisting of branched chains of elongate cells in more or less christmastree-like arrangement, lateral branches gradually becoming shorter towards the hyphal apex. Differential diagnosis. Species signature: fermentation of glucose +, and assimilation: cellobiose, raffinose, melebiose, melezitose +, soluble starch, d-xylose +, salicin, arbutin, 5-keto-d-gluconate (but may be slowly positive), nitrate, growth at  $37\text{Å}_{s}\text{C}$  +, d-tryptophan (N), w/o thiamine +. Physiologically



C. parapsilosis on CHROMAgar<sup>TM</sup> www.life-worldwide.org , www.medical-labs.net

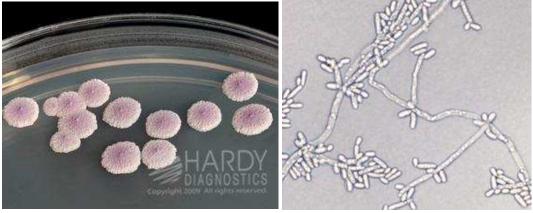
# 5. Candida krusei (Castell.) Berkhout, De schimmelgeslachten Monilia, Oidium, Oospora en Torula: 44 (1923)

#### Synonyms:

- =Candida krusei var. krusei
- =Endomyces krusei (Castell.) Castell., British Medical Journal 2: 1210 (1912)
- =Geotrichoides krusei (Castell.) Langeron & Talice, Ann Parasitol Hum Compa 10: 67 (1932)
- =Monilia krusei (Castell.) Castell. & Chalm., Manual of Tropical Medicine: 826 (1913)
- =Myceloblastanon krusei (Castell.) M. Ota, Jap. J. Dermatol. Urol.: 178 (1928) =
- =Mycotoruloides krusei (Castell.) Langeron & Guerra, Ann Parasitol Hum Comp10 (1932)
- =Saccharomyces krusei Castell., Journal of Tropical Medicine and Hygiene 11 (1908)
- =Trichosporon krusei (Castell.) Cif. & Redaelli, Archiv für Mikrobiologie 6: 19 (1935)

Colonies on Glucose Peptone Agar incubated at 25°C: after 3 days cream-coloured, smooth, dull with 'ground glass'appearance. After 7 days the colonies are flat-topped with a broad mycelial edge (the entire colony is 'hat-shaped'). Yeast-like cells oval to elongate to long rectangular 2,0-5,5 x 4,0-15,0 µm, single, budding and in short chains. After 7 days branched chains of elongated cells (15-25 µm long) are produced. Dalmau Plate Cultures on Corn Meal Agar: extensive long, branched chains of elongated cells are produced after 3

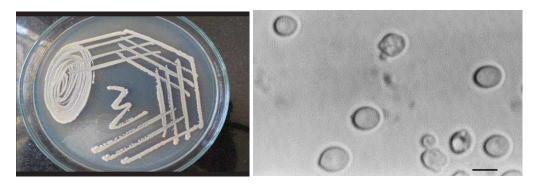
days.Globose to ovoid, thin-walled spores are produced singly, in pairs or clusters mainly at the junctions of the elongated cells. Germ Tube Test: negative.Fermentation of Carbohydrates: Glucose + Sucrose - Maltose - Lactose - Galactose - Raffinose - Trehalose - Assimilation of Organic Compounds Glucose + Sucrose - Maltose - Lactose - Galactose - Raffinose - Trehalose - Cellobiose - Inositol - Melezitose - Melibiose - Mannitol - L-Sorbose v D-Xylose - L-Arabinose - D-Arabinose - D-Ribose - L-Rhamnose - Glycerol + Erythritol - Ribitol - Galactitol - D-Glucitol - Salicin - DL-Lactic Acid + Succinic Acid + CitricAcid v Soluble Starch -.Assimilation of Inorganic Compounds: Nitrate -. Ability to split urea: variable.



Candida krusei catalog.hardydiagnostics.com, s3.amazonaws.com

- **6.** Candida rugosa (H.W. Anderson) Diddens & Lodder, Die anaskosporogenen Hefen, II Hälfte: 280 (1942)
- ≡Mycoderma rugosum H.W. Anderson, J Infectious Diseases 21: 341-385 (1917)
- ≡Candida rugosa var. rugosa, Die anaskosporogenen Hefen, II Hälfte: 280 (1942)
- ≡Candida rugosa var. elegans Dietrichson, Ann Parasitol Hum Comp 29: 485 (1954)
- ≡Azymocandida rugosa E.K. Novák & Zsolt, Acta Bot Acad Sci Hung 7: 134 (1961)
- =Endomyces rugosus Castell., British Medical Journal 2: 1209 (1912)
- =Torula rugosa Saito, J Japanese Botany 1: 49 (1922)
- =Trichosporon rugosum (Castell.) M. Ota (1926)
- =Candida rugosa var. elegans Dietrichson, Ann Parasitol Hum Comp 29: 485 (1954)

Growth in glucose-yeast extract-peptone broth: After 3 days at  $25^{\circ}$ C, the cells are ovoid, ellipsoidal to cylindrical,  $(2.4\text{-}6.4) \times (3.2\text{-}8.0) \, \mu\text{m}$ , single, in pairs, and cluster, multilateral budding. Growth on glucose-yeast extract-peptone agar: Aerobic growth is white to cream, butyrous, colonies. Dalmau plate culture on corn meal agar: After 7 days at  $25^{\circ}$ C, primary pseudohyphae are found. Formation of ascospores: Ascospores are not formed.

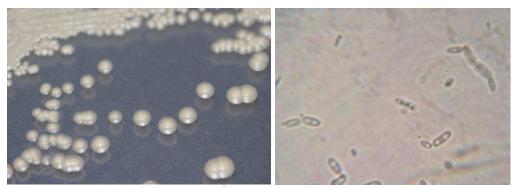


C. rugosa on CHROM agar www.jdrntruhs.org, www.bcrc.firdi.org.tw

# 7. Candida zeylanoides (Castell.) Langeron & Guerra, Annales de Parasitologie Humaine Comparée 16 (5):

- ≡Monilia zeylanoides Castell., J. Tropical Med. Hygiene 23: 17-22 (1920)
- ≡Mycotorula zeylanoides (Castell.) Cif. & Redaelli, Archiv Mikrobiol 6: 43 (1935)
- ≡Parendomyces zeylanoides (Castell.) C.W. Dodge, Med.Mycol . 240 (1935) ≡Candida zeylanoides var. zeylanoides, Annal Parasitol Hum Comp 16 ,501 (1938)
- =Cryptococcus uvae Pollacci & Nann., Atti Reale Accad. Fisiocrit. Siena: 636 (1926)
- =Blastodendrion canis von Szilvinyi, Zblt Bakt Parasitenk Abt 2 89: 297 (1934)
- =Monilia parazeylanoides Castell., J Tropical Med Hygiene 40: 293-307 (1937)
- =Monilia zeylanoides var. zeylanoides (1937)
- =Candida iberica C. Ramírez & A.E. González, Canad. J. Microbiol 18: 1778 (1972)
- =Candida krissii Goto & Iizuka, J General Appl Microbiol Tokyo 20 (5): 313 (1974)

Colonies on Glucose Peptone Agar at 25°C: after 3 days cream to yellowish-cream coloured, dull, smooth, regular in shape, spherical, domed, occasionally with a narrow border on prolonged incubation. Yeast-like cells globose to ovoid, single orbudding 3,0- 5,5 x 4,5-9,0 µmDalmau Plate Cultures on Corn Meal Agar: globose to ovoid, budding cells with pseudomycelium (chains of elongated yeast-like cells) also produced. Clusters of globose to ovoid thin-walled spores (often referred to as blastospores) are producedalong the branched chains of cells becoming less abundant towards proximal ends of the chains.Germ Tube Test: negative. Fermentation of Carbohydrates: Glucose - (+ weak) Sucrose - Maltose - Lactose - Galactose - Raffinose - Trehalose - (+ slow). Assimilation of Organic Compounds: Glucose + Sucrose - Maltose - Lactose - Galactose v Raffinose - Trehalose + Cellobiose v Inositol - Melezitose - Melibiose - Mannitol + L-Sorbose v D-Xylose - L-Arabinose - D-Arabinose - D-Ribose - L-Rhamnose - Glycerol + Erythritol - Ribitol v Galactitol - D-Glucitol + Salicin v DL-Lactic Acid - Succinic Acid + CitricAcid + Soluble Starch -. Assimilation of Inorganic Compounds: Nitrate -.



Candida zeylanoides morphology., s3.amazonaws.com

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# 3. Cryptococcosis

Cryptococcosis is a fungal disease caused by C. neoformans and C. gattii), an ubiquitous, saprophytic, round, basidiomycetous yeast (5 to 10  $\mu$ m) with a large heteropolysaccharide capsule (1 to 30  $\mu$ m) that does not take up common cytologic stains. The capsule forms a clear halo when stained with India ink. Cryptococcosis is relatively common in cats, but it has been also reported in most other species of domesticated animals including dogs, ferrets, guinea pigs, horses, donkeys, cattle, sheep, goats, water buffalo, pigs and South American camelids (llamas, alpacas and vicunas).

## **Description of Cryptococcus species reported in camelids**

# 1. Cryptococcus neoformans (San Felice) Vuillemin, 1901

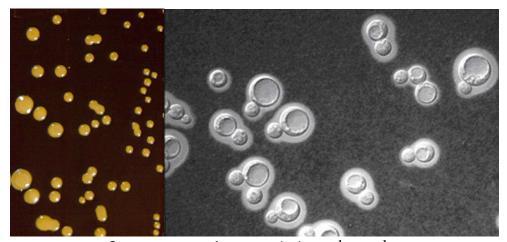
#### Synonyms:

- 1. Saccharomyces neoformans San Felice, Annali Ig. Sperim.: 241 (1895)
- 2. Torula neoformans (San Felice) J.D. Weis, Journal of Medical Research 7 (1902)
- 3. Blastomyces neoformans (Vuill.) Arzt, Archiv Dermatolo und Syphilis 145: 311 (1924)
- 4. *Debaryomyces neoformans* (San Felice) Redaelli, Cif. & Giordano, Boll. Sez. Ital. Soc. Int. Microbiol.: 24 (1937)
- 5. Lipomyces neoformans (San Felice) Cif., Manuale de Micologica Medica 2: 214 (1960)
- 6. Torulopsis neoformans var. sheppei A. Giord.
- 7. Saccharomyces hominis Costantin, Bulle. Soc. Mycol. de France 17: 145-148 (1901)
- 8. *Cryptococcus guilliermondii* Beauverie & Lesieur, Journal de Physiologie et Pathologie Général 14 (1912)
- 9. *Torula histolytica* J.L. Stoddart & Cutler, Studies from the Rockefeller Institute for Medical Research (1916)

- 10. Torulopsis neoformans var. neoformans (1931)
- 11. Cryptococcus neoformans var. grubii Franzot et al., J. Clin. Microbiol. 37: 839 (1999)

#### Morphology

Colonies of <u>Cryptococcus neoformans</u> are fast growing, soft, glistening to dull, smooth, usually mucoid, and cream to slightly pink or yellowish brown in color. The growth rate is somewhat slower than <u>Candida</u> and usually takes 48 to 72 h. It grows well at 25°C as well as 37°C. Ability to grow at 37°C is one of the features that differentiates <u>Cryptococcus neoformans</u> from other <u>Cryptococcus spp.</u> However, temperature-sensitive mutants that fail to grow at 37°C in vitro may also be observed. At 39-40°C, the growth of <u>Cryptococcus neoformans</u> starts to slow down.



Cryptococcus neoformans colonies and capsules

#### Micromorphology

On cornmeal tween 80 agar, <u>Cryptococcus neoformans</u> produces round, budding yeast cells. No true hyphae are visible. Pseudohyphae are usually absent or rudimentary. The capsule is best visible in India ink preparations. The thickness of the capsule is both strain-related and varies depending on the environmental conditions. Upon growth in 1% peptone solution, production of capsule is enhanced

#### Physiological data

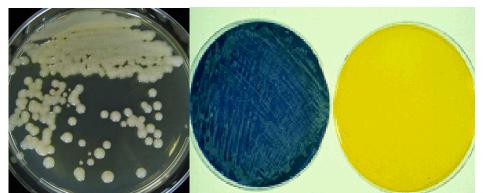
C1 D-Glucose+	C20 Melezitose+	C39 Succinated
C2 D-Galactose+	C21 Inulind	C40 Citrate-
C3 L-Sorbose+	C22 Starch+	C43 Propane 1,2 diold
C4 D-Glucosamine+	C23 Glycerol-	C44 Butane 2,3 diol-
C5 D-Ribose+	C24 Erythritol+	C45 Quinic acid-
C6 D-Xylose+	C25 Ribitol+	C46 D-glucarate+
C7 L-Arabinose+	C26 Xylitol+	C47 D-Galactonated
C8 D-Arabinose+	C27 L-Arabinitol+	N1 Nitrate-
C9 L-Rhamnose+	C28 D-Glucitol+	N2 Nitrite-
C10 Sucrose+	O3 Acetic acid 1%-	N3 Ethylamine+
C11 Maltose+	C29 D-Mannitol+	N4 L-Lysine+
C12 a,a-Trehalose+	C30 Galactitol+	N5 Cadaverine-
C13 Me a-D-Glucoside+	C31 myo-Inositol+	N6 Creatine-
C14 Cellobiose+	C32 D-Glucono-1,5-lactone+	N7 Creatinine+
C15 Salicin+	C33 2-Keto-D-Gluconate+	N8 Glucosamine-
C16 Arbutin+	C35 D-Gluconate-	N9 Imidazole-
C17 Melibiose-	C36 D-Glucuronate+	N10 D-Tryptophan-

C18 Lactose-	C37 D-Galacturonate+	V1 w/o vitamins-
C19 Raffinose+	C38 DL-Lactate	O1 Cycloheximide 0.01%-

# 2. Cryptococcus gattii (Vanbreusghem & Takashio) Kwon-Chung & Boekhout, Taxon 51 (4): 806 (2002)

#### **Synonyms:**

- Cryptococcus neoformans var. gattii Vanbreuseghem& Takashio, Annal. de la Soci. Belge de Méd.Trop. 50 (6): 701 (1970)
- Cryptococcus neoformans var. gattii Vanbreuseghem & Takashio ex De Vroey & Gatti, Mycoses 32 (12): 675 (1989)
- Cryptococcus bacillisporus Kwon-Chung & J.E. Benn., Intern.J.Syste. Bacteriol.28: 618
  (1978)
  - o Cryptococcus neoformans var. shanghaiensis W.Q. Liao et al., Chinese Med. J.: 287 (1983)



Cryptococcus gattii colonies. YPGA\*, 25°C, 5 days www.pf.chiba-u.ac.jp, CGB agar turns blue for Cryptococcus gattii, gattii www.mycology.adelaide.edu.au

### Physiological data

C1 D-Glucose+	C22 Starch+	C44 Butane 2,3 diol-
C2 D-Galactose+	C23 Glycerold	C45 Quinic acid-
C3 L-Sorbose+	C24 Erythritold	C46 D-glucarate+
C4 D-Glucosamined	C25 Ribitol+	C47 D-Galactonated
C5 D-Ribose+	C26 Xylitol+	N1 Nitrate-
C6 D-Xylose+	C27 L-Arabinitol+	N2 Nitrite-
C7 L-Arabinose+	C28 D-Glucitol+	N3 Ethylamine+
C8 D-Arabinose+	C29 D-Mannitol+	N4 L-Lysine-, d, w
C9 L-Rhamnose+	C30 Galactitol+	N5 Cadaverine-
C10 Sucrose+	C31 myo-Inositol+	N6 Creatine-
C11 Maltose+	C32 D-Glucono-1,5-	N7 Creatinine+
C12 a,a-Trehalose+	lactone+	N8 Glucosamine-
C13 Me a-D-Glucoside+	C33 2-Keto-D-Gluconate+	N9 Imidazole-
C14 Cellobiose+	C35 D-Gluconate+	N10 D-Tryptophan-

C15 Salicind	C36 D-Glucuronate+	V1 w/o vitamins-	
C16 Arbutin+	C37 D-Galacturonate+	V2 w/o myo-Inositol+	
C17 Melibiose-	C38 DL-Lactate-	V3 w/o Pantothenate+	
C18 Lactose-	C39 Succinate+	V4 w/o Biotin+	
C19 Raffinose+	C40 Citrate+	V5 w/o Thiamin-	
C20 Melezitose+	C43 Propane 1,2 diol-	V6 w/o Biotin & Thiamin	
C21 Inulind			

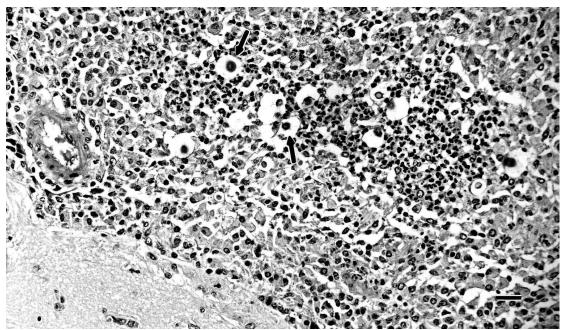
# **Reports:**

**Griner (1983)** reported a vicuna in which a concurrent cryptococcal pneumonia was detected at necropsy.

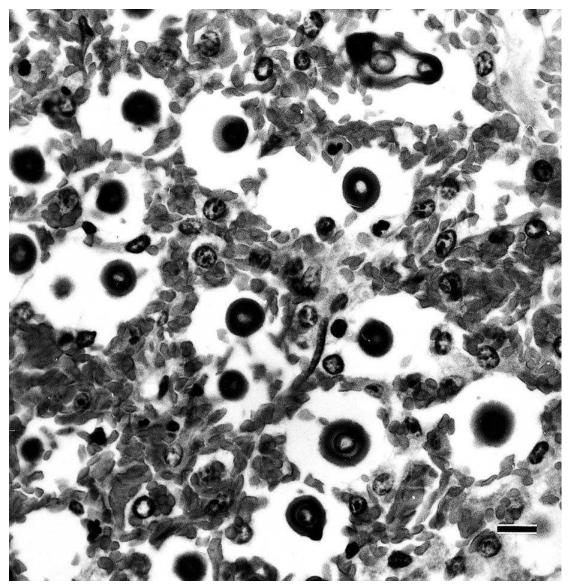
Goodchild et al. (1996) reported an 8-year-old female alpaca with a stiff hind-limb gait and purulent vaginal discharge. Therapeutic measures, including broad-spectrum intravenous antibiotics, were unsuccessful and the animal was euthanatized. No respiratory lesions were identified, but microscopic changes in the brain were similar to those found in the present case. Culture of cerebrospinal fluid (CSF) from the alpaca yielded C. neoformans var. gatti

**Ramadan** *et al.* (1998) reported Cryptococcosis in a camel (Camelus dromedarius) in **Saudi Arabia**. The tissue reaction in cryptococcosis varied depending on the organ affected. Two basic histological patterns are described: gelatinous and granulomatous. The granulomatous lesion consisted of histocytes, giant cells and lymphocytes. The pathology in this camel was modified by the secondary bacterial infection.

Bildfell et al. (2002) described cryptococcal infection of multiple tissues in a llama. A pastured 17-year-old male llama, found in lateral recumbency, was undergoing continuous tonic clonic convulsions of all limbs with periodic spasms of the head and neck. The animal was hypothermic and unresponsive to stimuli. A complete neurologic examination could not be performed due to the clinical status of the animal. The llama was in good body condition with no history of serious health problems and no known exposure to toxins. The owners had noted this llama to be mildly anorexic during the preceding 3 days but had not attempted any therapy. A health program for the 16 animals on the premises included periodic deworming, vaccination, and health checks. Differential diagnoses at the time of presentation included various neurologic diseases such as trauma, organophosphate toxicity, hypomagnesemia, visceral larval migrans, equine herpes virus type 1 (EHV1) encephalomyelitis, rabies, and bacterial infections of the CNS such as listeriosis or brain abscessation. After considering the prognosis, the owner requested that the llama be euthanatized without further clinical workup. Tissues affected included the brain, spinal cord, lung, and kidney. The character of the leukocytic response varied from minimal to pyogranulomatous meningitis with intralesional yeast that were bordered by a non-staining halo.



Cerebrocortical meninges; llama. Pyogranulomatous meningitis with intralesional yeast that are bordered by a non-staining halo. Two of these organisms are identified with arrows. HE stain. Bar 40 m. **Bildfell** *et al.* (2002)



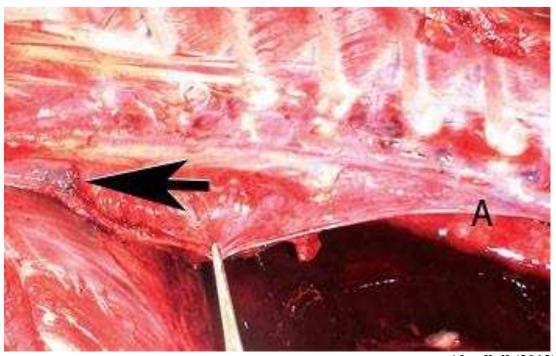
Meninges of lumbar spinal cord; llama. Micrograph of the hemorrhagic focus noted grossly in distal lumbar spinal cord. Note budding Cryptococcus sp. in upper right quadrant and the paucity of leukocytes in this field. HE stain. Bar 20 m. **Bildfell** *et al.* (2002)

Aboellail (2012) reported Cryptococcus gattii infection in a six-year-old black male alpaca. The alpaca had traveled to Northern Colorado from Oregon several months prior to the onset of clinical disease, which started as skin eruptions on the lips. Papules soon ulcerated forming multifocal and coalescing ulcerative dermatitis that did not respond to antibiotic treatment for more than two months. Progressive respiratory disease ended up in complete obliteration of the left lung, which made the referring DVM suspect a neoplastic disease. Four months into the clinical disease, the animal succumbed to worsening respiratory distress with pericardial and pleural fluid associated with chronic weight loss. The animal was submitted to the CSU VDL in November 2010 for necropsy. On necropsy, the left lung was diffusely consolidated with a large, blood-filled cystic cavity present in the middle of the cardiac lobe that was firmly adhered to the costal pleura. Tracheobronchial and mediastinal lymph nodes were markedly enlarged and on cut surface were diffusely gelatinous with taut capsules. Also, discrete lymph nodes were visible along the ventral surface of the thoracic aorta. Histological examination of the lungs, aforementioned lymph nodes and skin revealed

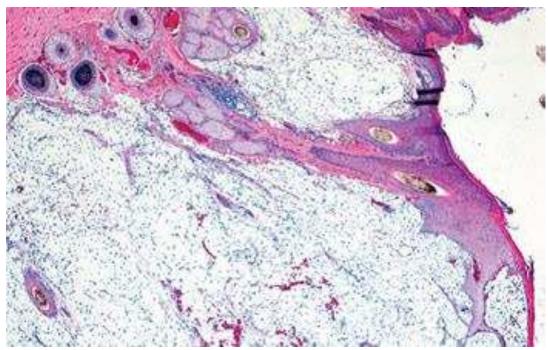
mats of yeas-like organisms organized as soap bubble replacing most of the parenchyma in affected organs, including the skin. Little to no inflammation was associated with these fungal mats. The lungs were submitted to CDC for genotyping, which confirmed the identity of the fungal organisms to be Gaia, the common isolate from the Pacific Northwest.



Papular to ulcerative chelitis evident on both lips of a six-year-old black alpaca infected with Crypto gattii. **Aboellail (2012)** 



Enlarged gelatinous lymph nodes (arrow) along the ventral surface of the aorta (A). Aboellail (2012)



Markedly enlarged skin by soap-bubble fungal mats separating the adnexa, characteristic of Cryptococcus gattii as identified in a Colorado alpaca. Aboellail (2012)

Kotler (2015) reported An 18-year old Alpaca gelding that was examined for acute onset paresis and incoordination. Other than a depressed mentation and a wide-based stance, no other abnormalities were present on the physical exam. Incoordination resulting in collapse occurred when ambulation was attempted. Initial treatment consisted of administration of vitamin B1 (thiamine), flunixin meglumine, and procaine penicillin. With no improvement in condition and owing to the time of year (end of August), normal llama plasma with a high West Nile virus antibody titer was administered the following day along with continuing medications administered at the initial treatment. By the third day, the alpaca began head pressing and humming with teeth grinding. He had also become sternally recumbent but was still able to eat and drink normally. All of the medications from the previous day were continued. By the fourth day, eating and drinking were no longer observed and the gelding would rarely lift his head and neck off the ground. Although the alpaca had been immunized against rabies two years prior to presentation, a vector animal positive for rabies had been diagnosed recently within the vicinity. Outcome Because of his deteriorating condition and the potential zoonotic risk to the owners, the alpaca was euthanized. At necropsy, no gross lesions were evident. Rabies virus fluorescent antibody test of the brain stem was negative. On histopathology, although the cerebral cortex was normal, the leptomeninges of the thalamus and hippocampus contained small numbers of lymphocytes, plasma cells, and macrophages containing brown granular pigment (hemosiderin). The cerebellar meninges also contained small numbers of lymphocytes and infrequent plasma cells. However, within the spinal cord, the leptomeninges were severely expanded by a marked infiltrate of lymphocytes, macrophages, and few neutrophils. Fungal yeast measuring approximately 12um in width with a broad clear capsule (consistent with Cryptococcus gattii) was present. The white matter also displayed severe axon sheath swelling and axonophagia. All other organs, including the lungs, were within normal limits.

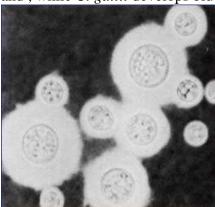
# Diagnosis of cryptococcosis

### Direct microscopic examination of India ink preparation

Preliminary diagnosis of cryptococcal infection is made by direct microscopic examination of India ink preparations of samples

#### Isolation and identification

Definitive diagnosis is confirmed by the culture of specimens, often the cerebrospinal fluid (CSF) or blood, and sometimes in respiratory secretions. Cryptococcus neoformans and C. gattii grow well at 37<sub>o</sub>C. On Sabouraud dextrose agar colonies appear soft, creamy, and opaque in 3-5 days, then colonies become mucoid and creamy to tan Cryptococcus colonies are brown on bird seed agar, modified tobacco and Eucalyptus leave extract agar as well as on Pal 's medium. Other yeasts develop white to creamy colonies. On canavanine glycine bromthymol blue (CGB) medium, Cryptococcus neoformans develop non-coloured colonies and, while C. gattii develops blue colonies.







India ink preparation

bird seed agar

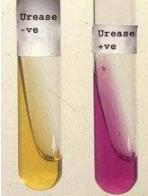
CGB medium

#### **Biochemical identification**

Cryptococcus neoformans and C. gattii do not ferment sugars, but assimilate several sugars such as glucose, galactose, sucrose, maltose and inositol, but not lactose or nitrate and hydrolyses urea.

#### Serotyping of Cryptococcus neoformans and C. gattii

To determine the antigenic formulas of *Cryptococcus* species, equal volumes of factor serum and heat-killed cell suspension are mixed on a glass slide and rotated for 5 min, and then the results of agglutination are observed. The formation of aggregates within 5 min is considered positive. Smaller clumps are recorded as weakly positive. PSS is used for a negative control.





Urease test

serotyping kit

#### **Molecular typing**

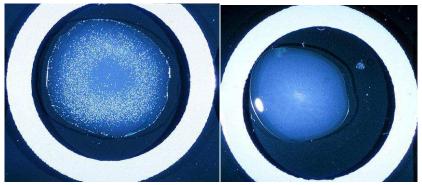
Numerous molecular techniques have been applied to subtype *C. neoformans* and *C. gattii* strains, only three methods were proved to produce comparable results: PCR Fingerprinting, AFLP, and MLST. M13 PCR Fingerprinting and *URA5* RFLP:

#### Serological diagnosis of cryptococcosis

Cryptococcal antigen from cerebrospinal fluid is the best test for diagnosis of cryptococcal meningitis in terms of sensitivity. Rapid diagnostic methods to detect cryptococcal antigen by latex agglutination test, lateral flow immunochromatographic assay (LFA), or enzyme immunoassay (EIA).

#### Rapid latex agglutination

This qualitative and semi quantitative test detects capsular polysaccharide antigens of *Cryptococcus neoformans* in serum and cerebrospinal fluid. It utilizes latex particles coated with anticryptococcal globulin. This latex reacts with the cryptococcal polysaccharide antigen, causing a visible agglutination.



Positive latex test Negative latex test

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# 4. Aspergillosis

Aspergillosis in camels was reported to be caused mainly by Aspergillus fumigatus and in some cases by Aspergillus niger, Aspergillus flavus and Aspergillus terreus. The disease was reported as:

- Pulmonary aspergillosis Bhatia et al. (1983)
- Invasive aspergillosis in alpacas was reported by Severo et al. (1989)
- Fatal pulmonary aspergillosis in camels was reported by El-Khouly et al. (1992)
- Fatal pulmonary aspergillosis in camels was reported by Al Hizab (2014)
- Systemic aspergillosis with pneumonia and extensive haemorrhages in the heart, pleura, mediastenal lymph nodes and omasal and abomasal mucosa and bloody diarrhea. Vomiting and nervous signs preceded death reported by Abbas and Ali (1992)
- **Pyogranulomatous pneumonia** with bronchiectasis *Aspergillus niger* was reported in an alpaca (Muntz, 1999)
- Aspergillosis granulomas some 5cm in diameter in a breeding dromedary was reported by **Wernery and Kaaden (2002**
- Aspergillus fumigatus rumenitis in a guanaco suffering from impaction of the stomachs due to an inflamed diverticle obstructing the duodenum was reported by Wernery and Kaaden (2002
- Systemic aspergillosis with subsequent abortion in alpaca was reported by Hughes and Mueller (2008)
- **Bilateral chorioretinitis** secondary to disseminated aspergillosis in an alpaca was reported by **Pickett** *et al.* (1985)
- Fungal keratitis was reported in alpacas by . Ledbetter et al. (2013)
- Abortion in camels was reported by **Dehkordi** et al. (2012)
- Contagious skin necrosis in camels due Aspergillus niger, Aspergillus flavus and Aspergillus terreus to was reported by Abdalla1 and M Salim (2014)

#### **Diagnosis**

- Antemortem diagnosis of aspergillosis can be very difficult since the signs of disease mimic
  those of many other illnesses, especially in the chronic form. Cases of aspergillosis are often
  suspected based on postmortem findings
- Direct Microscopy (Wet Smear Examination)
  - o by preparing a wet smear. in a drop of 20% potassium hydroxide, lactophenol cotton blue.and Calcofluor or Parker ink and/or Gram stain.
- Histopathological Examinations
  - o tissue samples (lungs, trachea, as well as other organs) fixed in 10% neutral buffered formalin are processed and embedded in paraffin blocks
  - o Aspergillus hyphae are stained poorly in H and E stained sections.
  - O Differential stains such as Periodic acid-Schiff (PAS), Bauer's and Gridley's stains differentiate and easily identify the hyphae and mycelia.
  - Special stains for fungus Grocott's and Gomori Methanamine Silver stain should be employed to detect the presence of fungal hyphae

#### • Culture

- Small pieces of lesions aseptically removed are placed onto plates or slants containing malt agar, Sabouraud's glucose agar or antibiotics and incubated at 37°C for 24 hours.
- Species of Aspergillus can be identified by observing the characteristic conidial head and colony

#### • Immunohistochemistry

- o Immunohistochemistry with monoclonal or polyclonal antibodies can be used
- Serology
  - A number of serological test have been applied in the diagnosis of aspergillosis. It includes counter
    - immunoelectrophoresis (CIE),
    - agar gel immunodiffusion (AGI)
    - enzyme-linked immunosorbent assays (ELISA).
- Polymerase chain reaction assays (PCR)

#### **Treatment**

• Drugs used have included thiabendazole, flucytosine, and amphotericin B, but very little is known about their effect on camelids.

#### **Reports:**

**Shigidi** (1973) isolated Aspergillus species from nasal swabs. Lungs and lymph nodes from apparently healthy camels in Sudan.

**Bhatia** *et al.* (1983) reported pulmonary aspergillosis in a 9-year-old camel from India. Several nodules were found in the lung surrounded by dark colored consolidated pulmonary tissue containing semisolid caseous necrotic material. Numerous abscesses were also scattered over the lung parenchyma. A necrotizing suppurative pneumonia was diagnosed and branching, septate fungal elements that resembled *Aspergillus* species were seen.

**Pickett** *et al.* (1985) reported a case of bilateral chorioretinitis secondary to disseminated aspergillosis in in a young female alpaca (Lama pacos) captured in the wild in Chile and delivered to the Milwaukee County Zoo. Initial physical examination indicated blindness and a slight non-differentiated head tilt, with occasional circling. The case was diagnosed only histopathologically.

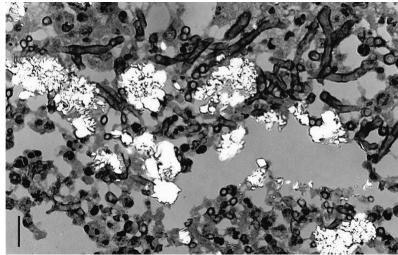
Severo et al.(1989) described an invasive form of aspergillosis in an alpaca (Lama pacos) with dissemination causing small abscesses and multifocal areas of necrosis in lung, heart, spleen and kidneys. large numbers of branching, septate fungal hyphae were detected in the necrotic retina, ciliary body and posterior lens capsule of one eye. This caused blindness associated with head tilt and intermittent circling. The morphology of the hyphae seen in histology sections was compatible with an Aspergillus species, but no cultivation of the fungus was attempted.

**Nawal** *et al.* **(1991)** A. niger, A. fumigatus and A. flavus, A. from the nasopharyngeal cavity of apparently healthy camels in Assiut.

Abbas and Ali (1992) reported that aspergillosis affected 70 camels and 40 camels died after a brief illness characterized by pyrexia, lachrymation, oedema of the throat and a bloody diarrhea. Vomiting and nervous signs preceded death. Post mortem findings included pneumonia and extensive haemorrhages in the heart, pleura, mediastenal lymph nodes and omasal and abomasal mucosa. Depressed retinol values were reported from some of these camels, but it is not known whether the deficiency predisposed the camels to the poisoning or the result of it.

El-Khouly et al. (1992) reported on death in racing camels (Camelus dromedarius) associated with a specific disease syndrome. Clinical signs included pyrexia, coughing, lachrymation, oedema of the throat and submandibular region and enlargement of submandibular lymph nodes. In terminal cases nervous signs were present and sometimes there was bloody diarrhoea and vomiting. Of 480 camels at least 70 animals were affected with the disease and about 40 died. Morbidity and mortality was greater in camels recently imported. Consistent necropsy findings were extensive petechial and ecchymotic haemorrhage beneath the epicardium, endocardium and visceral pleura and in the mediastinal lymph nodes, and haemorrhagic oedema of the pharyngeal and laryngeal areas. Haemorrhages occurred more variably in abdominal organs and on the omasal and abomasal mucosa. Bronchopneumonia, omasitis and abomasitis were observed on microscopic examination, together with liver and kidney lesions of presumed toxic origin. Fungal hyphae and, occasionally, the characteristic conidial morphology of Aspergillus fumigatus were seen in sections and direct smears from lesions in the respiratory and alimentary tracts. A fumigatus was cultured from trachea, bronchi, bronchioles, lung tissue, heart blood, omasum, abomasum, ileum and submandibular lymph nodes. Whether the role of Aspergillus in the overall syndrome is primary or secondary has not been established; no other potential aetiological agent has been identified.

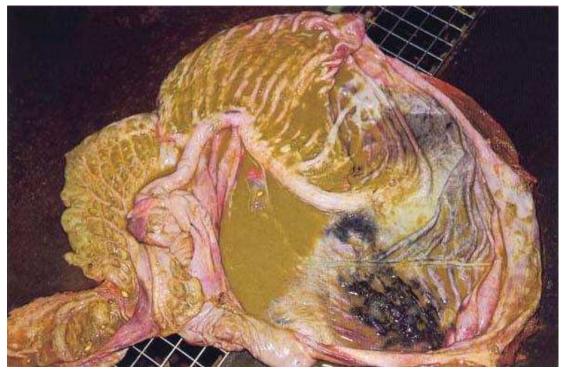
Muntz (1999) reported an aging lactating alpaca with sternal recumbency. The alpaca was euthanized due to poor prognosis 5 weeks after initial presentation. Gross postmortem examination revealed purulent material in the pulmonary airways. Histologic examination of the lungs revealed an extensive pyogranulomatous pneumonia with bronchiectasis. There were abundant fungal hyphae and high numbers of associated oxalate crystals, which were presumed to have been produced by the fungus. Low numbers of yeast cells were also present. Microbiological culture of tissues on horse blood agar and Sabouraud's agar identified the fungus to be Aspergillus niger. There was also moderate growth of Candida albicans. Calcium oxalate crystals in cytologic and histologic preparations can suggest an underlying Aspergillus infection. This is the first reported veterinary case of pneumonia due to Aspergillus niger infection and the associated production of oxalate crystals.



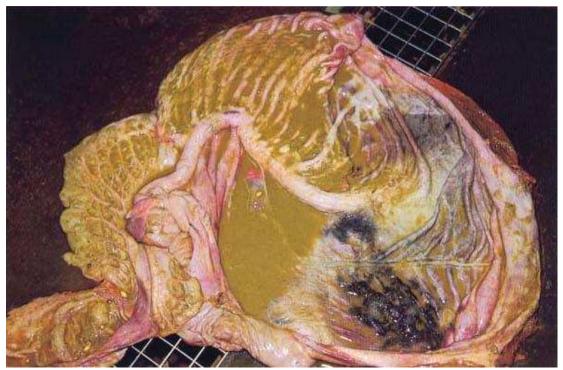
Lung; alpaca. Fungal hyphae and oxalate crystals viewed under partially polarized light. HE. Bar \_ 20 \_m.

**EL-Naggar** *et al.* **(2002)** detected antibodies to Aspergillus fumigatus in serum samples collected from 200 camels at Shalateen, Abou-Ramad and Halaieb triangle, in the rates of 2-5% by IHA and ELISA, respectively

Wernery and Kaaden (2002) reported Aspergillosis granulomas some 5cm in diameter in a breeding dromedary in the UAE which had suffered from generalized camel pox for several weeks and which was treated with tetracyclines for some time. They also diagnosed *Aspergillus fumigatus* rumenitis in the UAE in a guanaco suffering from impaction of the stomachs due to an inflamed diverticle obstructing the duodenum



Aspergillus spp. Granuloma in the lung of a breeding drornedary Wernery and Kaaden (2002)

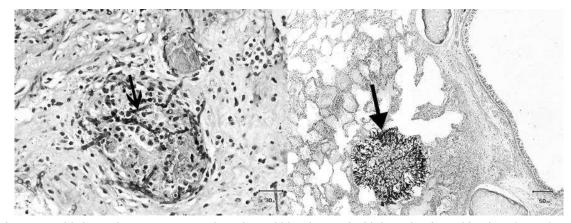


Aspergillus fumigatus rumenitis in a guanaco indicated by the black area Wernery and Kaaden (2002)

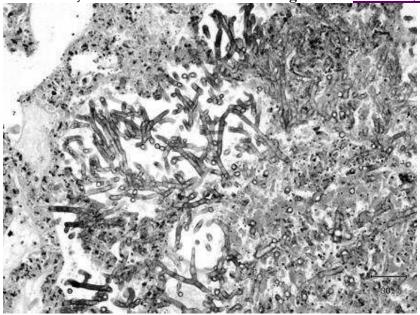
Osman et al. (2003) isolated A. fumigatus, A. terreus. A. flavus, A. parasiticus and A. niger from the nasopharyngeal cavity of apparently healthy camels at Shalateen, Halaieb and Abou-Ramad areas

Mahmoud *et al.* (2005) isolated A. fumigatus, A. terreus and A. niger from both pneumonic lung samples of slaughtered camels and nasopharyngeal swabs collected from diseased camels at Shalateen, Halaieb and Abou-Ramad suffering from different respiratory manifestations.

Hughes and Mueller (2008) described abortion in a 4-year-old female alpaca at approximately 245 days in gestation. The patient had developed watery diarrhea 24 hr after abortion and exhibited bruxism, progressive depression, and ultimately collapse. On presentation to the referral institute, the alpaca was unable to stand and exhibited severe tachypnea, dyspnea, and tachycardia. The patient was considered to be moribund and was euthanized. Macroscopic postmortem assessment and subsequent histological investigations revealed multifocal ulceration of the third gastric compartment, with vascular mycotic invasion and disseminated multifocal embolic mycotic pneumonia. Real-time polymerase chain reaction targeting the large ribosomal subunit (28S) performed on paraffin-embedded lung tissue successfully amplified 28S RNA specific for Aspergillus species. The primary cause of the depression and collapse of this animal was considered to be a combination of respiratory compromise due to severe embolic mycotic pneumonia and endotoxic shock associated with the ulcerative gastritis of the third gastric compartment. To the authors' knowledge, this is the first report of ulcerative gastritis of the third gastric compartment histologically illustrated as a portal of entry for Aspergillus species causing embolic mycotic pneumonia in an alpaca.



Submucosa third gastric compartment; thrombosed blood vessel with invasive fungal hyphae (arrow). Gomori methenamine-silver counterstained with hematoxylin and eosin stain. Bar =  $30 \mu m$  Lung; large aggregate of fungal hyphae (arrow) adjacent to a bronchus. Gomori methenamine-silver counter-stained with hematoxylin and eosin stain. Bar =  $50 \mu m$  Hughes and Mueller (2008)



Lung; fungal hyphae consistent with *Aspergillus* spp. Note that the hyphae are septate and exhibit acute, dichotomous branching. Hematoxylin and eosin stain. Bar =  $30 \mu m$  **Hughes and Mueller (2008)** 

**Zakia** *et al.* (2000) diagnosed concurrent infection with aspergillosis and pneumoconiosis in a lung of she camel slaughtered at El-Gadarif abattoir, Eastern state, Sudan. Lesions of Aspergillus fumigatus (AF) alone or concomitant infection with dust-laden macrophages (DLM) revealed mixed necrotising and purulent exudate. Multifocal aggregates of DLM were seen in peribronchiolar, perialveolar and periarterial connective tissue with or without inflammatory reaction. Fungal hyphae morphologically identical to A.F. hyphae were seen histologically in lung sections and confirmed by isolation and identification of AF.

Na'ma and Hussain (2011). conducted a study to discover the immune status against Aspergillus fumigatus in camels. 90 camels were inspected and the blood was sampled from herds in Thi Qar governorate. Camels ranked in different ages, 1-10 years, and both male and female were included. The study dialed with camels suffering respiratory disorder. Serum was examined by passive hemagglutination test using the antigen of Aspergillus fumigatus isolated and prepared by (1). 16 camels were positive which present 17.8%, this result

informs an important role of Aspergillus fumigatus as a cause of respiratory tract disorder in Iraqi camels.

Abo-Elnaga and Osman (2012) carried out a study on 175 camels with the aim of identifying bacterial, parasitic and fungal species involved in lung lesions of camel's slaughtered at Matrouh main abattoirs. All slaughtered camels were originated from northwestern coast. A total of 175 lungs were inspected during the study, of which 50 (28.6%) possessed pneumonic lesions. Bacterial and mycotic growth was observed from 45 (25.7%) of the pneumonic lung samples. A total of 70 bacterial and mycotic species were isolated and identified. These included staphylococcus aureus (28.6%), Bacillus species (21.4%), Klebsiella species (10%) Escherichia coli (8.6%), Corynebacterium pyogenes (5.7%), Streptococcus pyogenes (2.9%), Streptococcus pneumoniae (1.8%), Pasteurella multocida (2.9%), Manhemia haemolytica (1.4%), Actinomyces pyogenes (1.4%), Aspergillus fumigatus (5.7%), Aspergillus flavus (1.4%) and Candida albicans (1.4%). Serological detection of Aspergillus fumigatus antibodies using IHA was found to be (14%).

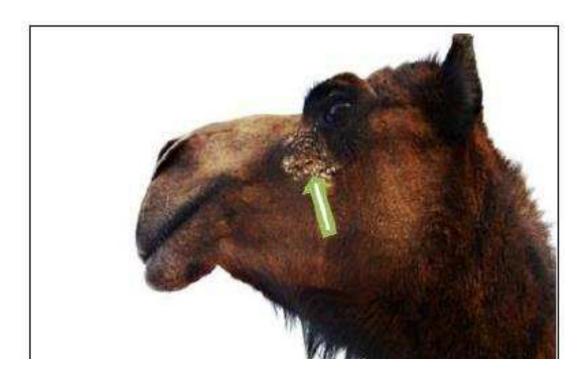
**Dehkordi** *et al.* (2012) performed research for detection of Aspergillus species (A. fumigatus, A. flavus, A. niger and A. terreus) in aborted bovine, ovine, caprine and camel foeti by real-time PCR in Iran. After modification of real-time PCR on abomasal contents, from the total number of 970 samples, 141 (14.53%) gave positive results for Aspergillus species. Of them, 62 (17.71%), 33 (14.04%), 27 (12.05%) and 19 (11.8%) positive specimens were detected in bovine, ovine, caprine and camel foeti respectively. Statistical analysis showed significant differences (P<0.05) between bovine and camel and bovine and caprine aborted foeti. Aspergillus abortion was the most prevalent in cattle whereas camels tended to be the most resistant.

Ledbetter et al. (2013) evaluated medical records of alpacas evaluated by the ophthalmology service of a veterinary teaching hospital to identify animals with a clinical diagnosis of fungal keratitis and positive results for fungal culture of a corneal sample between 2003 and 2012. Signalment and historical, clinical, and microbiological details were recorded. Results of cytologic, histopathologic, and in vivo confocal microscopic corneal examinations were collected when available. Fungal keratitis was diagnosed in 11 of 169 (6.5%) alpacas that underwent ophthalmologic examination by the ophthalmology service during the study period. Ten of the 11 alpacas were evaluated in the summer or fall months. Corneal lesions included stromal ulcer, stromal abscess, corneal perforation, and nonulcerative keratitis. Aspergillus fumigatus and Fusarium solani were the most frequently cultured fungi. Fungi were also identified through corneal cytologic examination, histologic examination, or in vivo confocal microscopy in 9 alpacas. Historically, 2 alpacas were evaluated following external ocular trauma and 1 following corneal foreign body removal. Nine alpacas had received topical treatment with antimicrobials and 2 had antimicrobialcorticosteroid combinations administered topically prior to referral. Nine of 10 alpacas for which follow-up information was available were successfully treated, with globe and vision retention.

Abdalla1 and M Salim (2014) carried out a study to determine the ability of fungi to be one of the causative agent of contagious skin necrosis skin disease of camel. Methods: A total of

40 pus samples from suspected contagious skin necrosis lesions were collected with sterile absorbent swabs and were labelled with date of collection, sex, age and location. Samples were collected from Gadarif state during winter and summer. Swab from contagious skin necrosis lesion was cultured by dipping it into brain heart infusion broth and incubated for 1-2 days at 37°C. The isolate was sub cultured on Sabouraud's dextrose agar and incubated at 37°C for 1-2 days. Identification of mould was done by rate of growth, texture and the pigmentation of cultures was used, (Laura, et al, 1998). Results: From forty swabs of camel infected with contagious skin necrosis, eleven mould isolates of three genera were recovered from forty samples on Sabouraud's dextrose agar, Aspergillus niger (4 isolates), Aspergillus flavus (2 isolates), Aspergillus terreus (1 isolate), penicillum spp (1 isolate) and Scopulariopsis brevicaulis (3 isolates). Three Candida spp were isolated and were identified as Candida parapsillosis, Candida gulliermondii and Candida zeylanoides. It was concluded that, Aspergillus spp can be causative agent of contagious skin necrosis skin disease of camel.

Al Hizab (2014) conducted a study in Eastern Province in the Kingdom of Saudi Arabia on the mortality among camels (Camelus dromedarius) in Hardd. Clinical signs were anorexia, depression, nasal discharges, serous lacrimation and enlargement of submandibular lymph nodes. Moreover, there was bloody diarrhea. Total of (n = 150) camels, out of which (n = 70) animals showed the clinical disease and among (n = 19) of them were dying within three days after the appearance of the first clinical sign. Consistent necropsy findings were hemorrhagic abomasal folds, massive hemorrhage within the small intestine associated with severely congested mesenteric lymph nodes. Epicardial and subendocardial hemorrhages were also observed. The pharyngeal and laryngeal areas were congested and contained serosanguinous fluid. Aspergillus fumigatus was cultured and isolated from trachea, lung, omasum and intestine. No other potential microorganisms or toxic agents have been identified.



Aspergillosis in camels, ocular discharge (arrow); Al Hizab (2014)



Aspergillosis in camels, enlargement of submandibular lymph node (arrow); Al Hizab (2014)



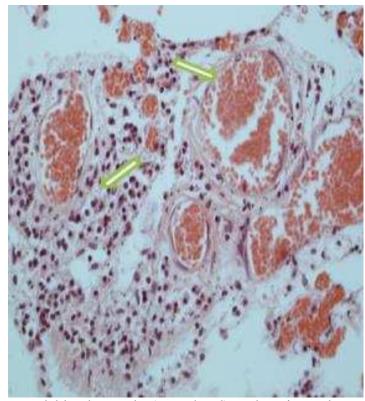


a. Congested and haemorrhagic laryngeal (arrowhead) and pharyngeal mucosa b. .Petechial and ecchymotic haemorrhage along the lung surface (arrow), Al Hizab (2014)





Liver showing massive areas of haemorrhage (arrow). HE x400; Al Hizab (2014)



lung showing congested blood vessels (arrowhead) and perivascular cuffing (arrow). HE x400. Al Hizab (2014)

# Description of Aspergillus species isolated from cases of camelids aspergillosis

#### i. Aspergillus fumigatus Fresenius, 1863.

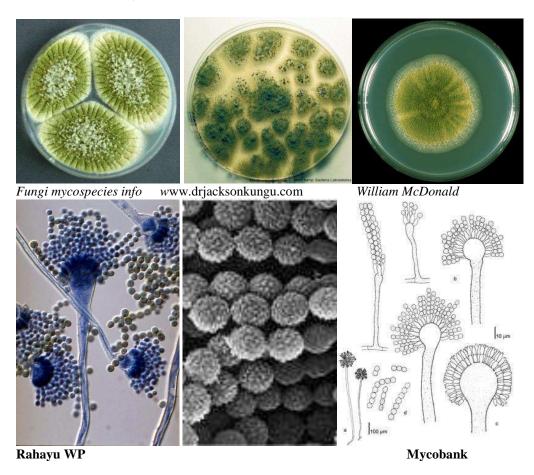
Colony diam (7 d): CYA25: 21-67 mm; MEA25: 25-69 mm; YES25: 48-74 mm; OA25: 34-62 mm, CYA37: 60-75 mm, CREA: poor growth, no or very weak acid production. Colour: greyish turquoise or dark turquoise to dark green to dull green. Reverse colour (CYA): creamy, yellow to orange. Colony texture: velutinous, st. floccose. Conidial head: columnar. Conidiation: abundant, rarely less abundant. Stipe:  $50-350 \times 3.5-10 \,\mu\text{m}$ . Vesicle diam, shape:  $10-26 \,\mu\text{m}$ , pyriform to subclavate, sometimes subglobose, but rarely globose. Conidia length, shape, surface texture:  $2-3.5(-6) \,\mu\text{m}$ , globose to ellipsoidal, smooth to finely rough



Aspergillus fumigatus, Mycoba

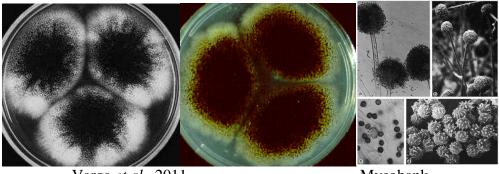
#### ii. Aspergillus flavus Link, 1809

A. flavus is known as a velvety, yellow to green or brown mould with a goldish to red-brown reverse. On Czapek dox agar, colonies are granular, flat, often with radial grooves, yellow at first but quickly becoming bright to dark yellow-green with age. Conidial heads are typically radiate, mostly 300-400 um in diameter, later splitting to form loose columns .The conidiophores are variable in length, rough, pitted and spiny. They may be either uniseriate or biseriate. They cover the entire vesicle, and phialides point out in all directions. Conidia are globose to subglobose, conspicuously echinulate, varying from 3.5 to 4.5 mm in diameter. Based on the characteristics of the sclerotia produced, A. flavus isolates can be divided into two phenotypic types. The S strain produces numerous small sclerotia (average diameter ,400 mm).



### iii. Aspergillus niger van Tieghem 1867

On Czapek dox agar, colonies consist of a compact white or yellow basal felt covered by a dense layer of dark-brown to black conidial heads. Conidial heads are large (up to 3 mm x 15-20 um in diameter), globose, dark brown, becoming radiate and tending to split into several loose columns with age. Conidiophores are smooth-walled, hyaline or turning dark towards the vesicle. Conidial heads are biseriate with the phialides borne on brown, often septate metulae. Conidia are globose to subglobose (3.5-5.0 um in diameter), dark brown to black and rough-walled.

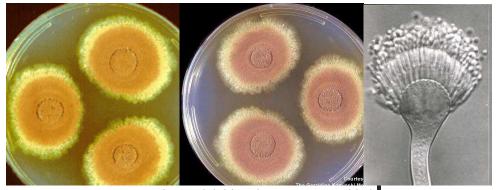


Varga et al., 2011

Mycobank

#### iv. Aspergillus terreus Thom, (1918)

Colonies on potato dextrose agar at  $25^{\circ}$ C are beige to buff to cinnamon. Reverse is yellow and yellow soluble pigments are frequently present. Moderate to rapid growth rate. Colonies become finely granular with conidial production. Hyphae are septate and hyaline. Conidial heads are biseriate (containing metula that support phialides) and columnar (conidia form in long columns from the upper portion of the vesicle). Conidiophores are smooth-walled and hyaline, 70 to  $300\mu$ m long, terminating in mostly globose vesicles. Conidia are small (2-2.5  $\mu$ m), globose, and smooth. Globose, sessile, hyaline accessory conidia (2-6  $\mu$ m) frequently produced on submerged hyphae.



A. terreus mycology.adelaide.edu.au www.mold.ph Mycobank

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#### 5. Penicillosis

Infections with *Penicillium* spp are rare in domestic animals. In dogs, infections of the nasal cavity, lungs, lymph nodes, and bones have been reported. Nasal disease is most common and behaves similar to nasal aspergillosis. In cats, the fungus has been isolated from the nasal cavity, orbital cellulitis and sinusitis, and lungs. It has also been reported to cause systemic disease in captive toucanets (*P griseofulvum*) and bamboo rats (*P marneffei*) in

southeast Asia. *Penicillium* spp are widely distributed in nature and are found in soils, grains, and various foods and feeds.

This is the only report found concerning fatal Penicillium infection in a camel.

#### **Diagnosis**

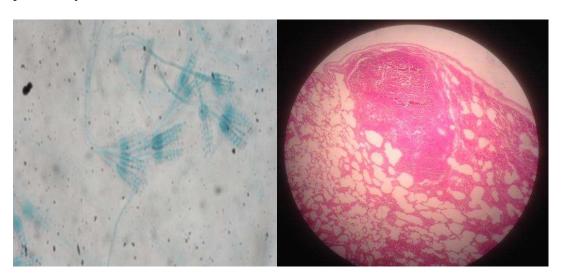
Diagnosis is based on fungal culture, character of the lesions, presence of fungal hyphae, and a positive agar-gel double-diffusion test. Cultural isolation of a *Penicillium* sp must be accompanied by demonstration of tissue invasion by the fungus for confirmation.

#### **Reports:**

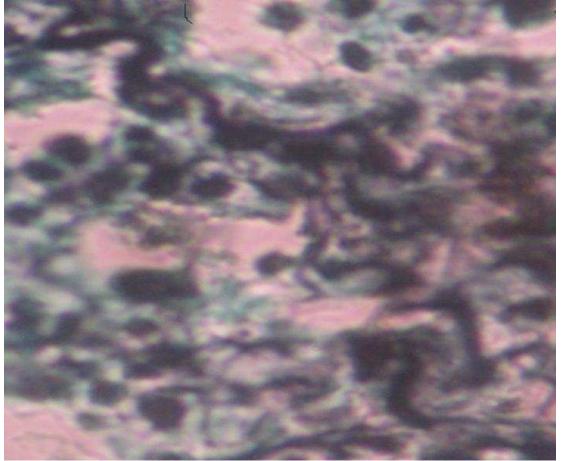
Nawal et al. (1991) isolated Penicillium species from the nasopharyngeal cavity of apparently healthy camels in Assiut.

Mahmoud *et al.* (2005) isolated Penicillium species from the nasopharyngea Iswabs collected from diseased camels in at Shalateen, Halaieb and Abou-Ramad suffering from different respiratory manifestations.

Suleiman *et al.* (2015) conducted a study to investigate the cause of death associated with lung abscesses of dead dromedary camels. Lung biopsy was examined by culturing onto Sabouraud's dextrose agar (SDA) media supplemented with chloramphenicol. Colony developed was microscopically examined using lactophenol cotton blue. Slide culture was performed to identify the isolated fungus. Histopathological study of lung biopsy using Haematoxylin and Eosin (H&E) and Methenamine silver (Grocott's modification) (GMS) stains was carried to detect the pathological feature and fungal hyphae in lung tissue. The isolate was initially identified by conventional morphological analysis; and confirmed by molecular analysis after amplification of the β-tubulin gene, by polymerase chain reaction (PCR) and sequencing of the amplicons. *Penicillium citrinum* was isolated from lung biopsy of sacrificed dromedary camels. Molecular technique confirmed its identification. Histology of nodular lung lesion showing granulomatous inflammation (H&E) and fungal mycelia on GMS. The study revealed the first case of *P. citrinum* isolation from the lung of camel with pulmonary disease.



Slide culture showing hyphae of *P. citrinum* with matulae bearing spherical conidia on lactophenol cotton blue, Magnification  $400\times$ , Histological sections of the lung showing granulomatous lesion (H&E  $40\times$ )



GMS staining of lung tissue showing fungal hyphae. Magnification,  $100 \times$ 

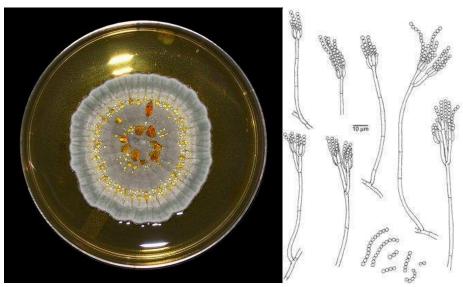
#### **Description of the aetiology**

# 1. Penicillium citrinum Thom, U.S.D.A. Bureau of Animal Industry Bulletin 118: 61 (1910)

- =Penicillium citrinum var. pseudopaxilli Martínez & Ramírez
- =Citromyces subtilis Bainier & Sartory, Bulletin de la Société Mycol de France 28: 46 (1912)
- =Penicillium aurifluum Biourge, La Cellule 33: 250 (1923)
- =Penicillium phaeojanthinellum Biourge, La Cellule 33: 289 (1923)
- =Penicillium sartoryi Thom, The Penicillia: 233 (1930)
- =Penicillium sartorii Thom (1930)
- =Penicillium botryosum Bat. & H. Maia, Anais da Sociedade de Biologia de

Colony characteristics. Colonies (CzA) with slow to moderate growth, velutinous to floccose; mycelium white to greyish-orange. Conidial masses greyish-turquoise; frequently a pale yellow to reddish-brown soluble pigment is produced. Exudate on MEA greyish-blue.Microscopy. Conidiophore stipes smooth-walled, 100-300 µm long; penicilli

biverticillate. Metulae 12-15  $\mu$ m long, divergent, in whorls of 3-5. Phialides flask-shaped, 7-12  $\mu$ m long. Conidia spherical to subspherical, smooth-walled or finely roughened, 2.2-3.0  $\mu$ m diam



P. citrinum thunderhouse4-yuri.blogspot.com

#### **References:**

- Mahmoud Mona A, Wafa A. Osman, , A.L EL-Naggar and M. A. Balata (2005). Prevalence of respiratory diseases in camels at Shalateen, Halaieb and Abou-Ramad. 2d Int.Sci. Conf., April 26-28, Guena & Luxor, Egypt
- Nawal. G, .Laila S. Ahmed.Seham M. Ali. Elyas A. H., Nashed. S. M. and Amer.A.A. (1991) Myco- and microflora of the nasal cavity of apparently healthy camels. Assiut Vet. Med. J. 24,48, 125-130
- 3. Penicilliosis, Merck Vet. Manual. http://www.merckvetmanual.com/mvm/generalized\_conditions/fungal\_infections/penicilliosis.html
- 4. Suleiman, E. A., Salma Bushra Elmagboal<sup>2</sup>, Fahad Elghazali<sup>2</sup>, Manal Hassan Salih and Omer Mohamed Ahmed Fatal Pulmonary Penicillosis in Camels (*Camelus dromedarius*). British Microbiology Research Journal, 7, 3, 118-125,2015

## 6. Phaeohyphomycosis

**Synonyms:** Cerebral chromomycosis, chromoblastomycosis, cladosporiosis, phaeomycotic cyst, phaeosporotrichosis, subcutaneous mycotic cyst

Phaeohyphomycosis is a name applied to diseases caused by various species of dematiaceous fungi, which are frequently found in soil, water, and decaying vegetable matter. Infections usually follow contamination of injured tissue by the fungi, causing the formation of single or multiple ulcerated nodules in the regions of the head and neck and, occasionally, lymphangitis and regional lymphadenopathy. The fungal filaments are not organized into

granules, as is the case with mycetomas, but are dispersed singly or in small groups in the lesions. The individual diseases vary in severity and clinical course, depending on the fungus involved, the tissues that are affected and the host. They usually involve immunocompromised individuals and animals, including horses

Phaeohyphomycosis includes cutaneous, subcutaneous, and systemic diseases that affect humans and animals. The diseases are usually associated with immunocompromised hosts. Subcutaneous phaeohyphomycosis occurs sporadically in horses, cats and cattle. Infection results from introduction of saprophytic fungi into the skin. Lesions present as single or multiple subcutaneous nodules, which are most common on the distal extremities (frequently accompanied by osteomyelitis) or face.

#### **Reports:**

**Tuteja** *et al* (2010) described alternariosis in camels.. Lesions of the disease were observed throughout the body including the lips and udder. Lesions initially start as small raised areas which gives roughness of the affected skin then there appears a slight whiteness at the top of the raised area. Lesions may enlarge to more than 10 centimeter in size, enlargement of the lesions occur in centrifugal manner and later the lesions may coalesce. During development of the lesions necrosis follows alopecia. The general presentation is a regular and circular alopecia. The disease occur more frequently in semi-arid than arid region of the state. Lesions of the disease are found anywhere on the body including the lips and udder. This disease may be quite confusing with other dermatophyte infections. Repeated culture of skin scrapings lead to isolation of *Alternaria alternata*. As ethno veterinary treatment either sulphur in mustard oil or leather ash in ghee (butter fat in India) is applied by the farmers. After removal of skin scrapings, topical application of an ointment containing sulphur, salicylic acid and mustard oil



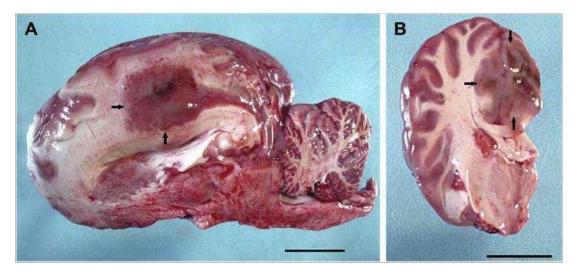
Lesions of alternariosis on abdomen, Lesions of alternariosis on thighs Tuteja et al (2010)



Lesions of alternariosis on neck, Lesions of alternariosis udder Tuteja et al (2010)

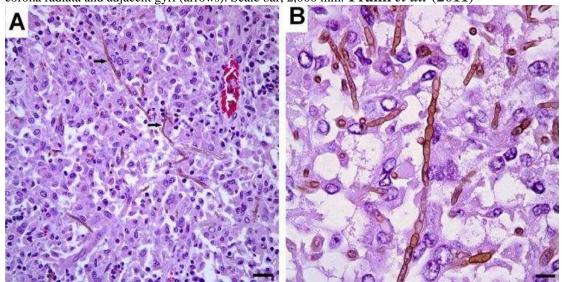
**AlKanzee** *et al.* (2011) isolated Alternaria spp and Alternaria species from cases of contagious skin necrosis (CSN) (Naeita, Alara) in camel (Camelus dromedarius) in Hail Region

Frank et al. (2011) diagnosed cerebral phaeohyphomycosis, caused by Cladophialophora bantiana, in a Huacaya alpaca (Vicugna pacos). An 8-year-old, intact male Huacaya alpaca from a farm in Indiana was found dead at pasture and submitted to the Animal Disease Diagnostic Laboratory at Purdue University for necropsy examination. Gross lesions were limited to the cerebrum, which had a  $2 \times 2.5 \times 5$  cm, well-demarcated, firm, mottled, pale grey to brown-red mass with a granular texture, centered on the left and right cingulate gyri. Microscopically, granulomatous and necrotizing meningoencephalitis with intralesional pigmented fungal hyphae effaced the cerebral grey and white matter of the cingulate gyri. The hyphae were 4–6  $\mu$ m in diameter and septate, with non-parallel walls and occasional branching. Polymerase chain reaction for the internal transcribed spacer-1 of the nuclear small-subunit ribosomal RNA genes was performed on extracts from formalin-fixed and paraffin wax-embedded sections of cerebrum. Nucleotide sequence analysis of the amplified fragment identified the fungal agent as C. bantiana. This is the first report of cerebral phaeohyphomycosis attributable to C. bantiana in a camelid.



Brain of a ruminant, huacaya alpaca (*Vicugna pacos*) with cerebral phaeohyphomycosis due to *Cladophialophora bantiana*. (Reproduced from reference 13 with permission of Elsevier.) (A) A parasagittal section of the brain demonstrates a 2.5- by 2-cm, fairly well-demarcated, slightly firm, red to gray mass dorsal

to the corpus callosum and extending from the level of the post-cruciate gyrus caudally to the level of the occipital lobe (arrows). Scale bar, 2,000 mm. (B) A cross section of the left cerebral hemisphere at the level of the thalamus shows that the mass effaces the neuroparenchyma of the cingulate gyrus and extends into the corona radiata and adjacent gyri (arrows). Scale bar, 2,000 mm. **Frank** *et al.* (2011)



Brain of a ruminant, huacaya alpaca (*Vicugna pacos*), with cerebral phaeohyphomycosis due to *Cladophialophora bantiana*. (Reproduced from reference 13 with permission of Elsevier.) (A) A photomicrograph of the cerebral granuloma shows that sheets of epithelioid macrophages, multinucleated giant cells, and rare neutrophils surround intracellular and extracellular pigmented fungal hyphae (arrows). Hematoxylin and eosin staining was used. Scale bar, 0.025 mm. (B) A histopathological section of brain shows brown septate hyphae with nonparallel walls, which are 4 by 6 mm. Hematoxylin and eosin staining was used. Scale bar, 0.01 mm. **Frank** *et al.* (2011)

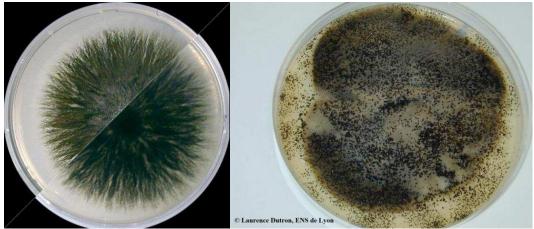
#### **Description of the agent**

#### Alternaria alternata (Fr.) Keissl. (1912)

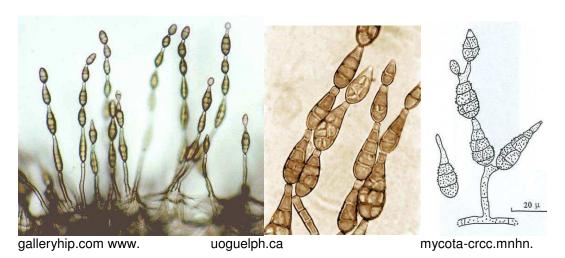
#### **Synonyms**:

- =Alternaria tenuis Nees 1917
- =Macrosporium fasciculatum Cooke & Ellis (1817),
- =Torula alternata Fr. (1832),
- =Alternaria fasciculata Jones & Grout (1897),
- =Alternaria rugosa McAlpine (1896)

Alternaria species grow rapidly producing flat, downy to woolly colonies, covered by grayish, short, aerial hyphae. The surface is greyish white at the beginning which later darkens and becomes greenish black or olive brown with a light border. Microscopically, the fungus develops septate, brown hyphae. Conidiophores are also septate and brown in colour, occasionally producing a zigzag appearance. They bear simple or branched large conidia, which have both transverse and longitudinal septations (muriform conidia). They are dark in colour, elongated and found in chains. The conidia may be observed singly or in acropetal chains and may produce germ tubes. They are ovoid to obclavate, darkly pigmented, muriform, smooth or roughened. The end of the conidium nearest the conidiophore is round while it tapers towards the apex.



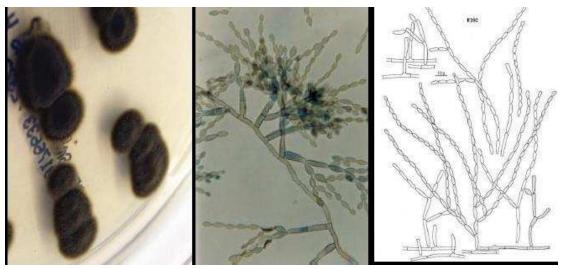
Alternaria alternata...www.inspq.qc.ca biologie.ens-lyon.fr



## Cladophialophora bantiana de Hoog, Kwon-Chung & McGinnis, (1995) Synonyms:

- =Torula bantiana Sacc., in Saccardo, (1912)
- =Cladosporium bantianum (Sacc.) Borelli, (1960)
- =Xylohypha bantiana (Sacc.) McGinnis, Borelli, Padhye & Ajello, (1986)
- =Cladosporium trichoides Emmons Binford, Thompson & Gorham, (1952)
- =Cladosporium bantianum (Sacc.) Borelli, (1960)
- =Cladosporium trichoides var. chlamydosporum Kwon-Chung, (1978)

The colony is black with a velvety texture or dark grey in colour, depending on the type of agar medium it is grown on. It grows slowly under temperatures ranging from 14-42 °C with optimal growth around 30 °C. It can be distinguished from other species of the genus Cladophialophora by the presence of urease activity. Microscopically, the fungus produces predominantly hyphal growth both in vivo and in vitro, that consists of dark coloured largely unbranched, wavy chains of conidia, individually 5–10  $\mu$ m in length. The dark colour is due to the presence of the dark pigment melanin.



Cladophialophora bantiana jcm.asm.org

## 7. Scopulariopsis infection

**Tuteja** *et al* (2013) described cases of skin infection in camels caused by *Scopulariopsis brevicaulis*. The infection occurred after heavy rains in the year 2010, leading to high humidity in the environment. In a herd of 147 camels, several hyperkeratotic nodules were detected on the back of few animals. Lesions were generalized in distribution but occurred particularly on the abdomen. Lesions were observed more under the hairy portion of the skin. Cutting the hairs revealed more clear visibility of the lesions. After about 15 days there occurred incrustation of the nodules, which gave appearance of patchy skin necrosis. These lesions measured up to five centimetre in



Scopulariopsis hyperkeratotic nodules Scopulariopsis lesions of large patchy skin necrosis **Tuteja** *et al* (2010)

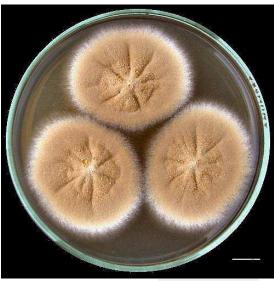
Abdalla and M Salim (2014) isolated scopulariopsis brevicaulis from cases of contagious skin necrosis, a skin disease of camels

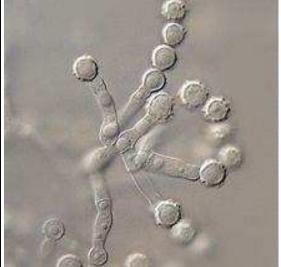
#### **Description of the agent**

# Scopulariopsis brevicaulis (Sacc.) Bainier, Bulletin de la Société Mycologique de France 23: 99 (1907)

- ≡Penicillium brevicaule Sacc., Michelia 2 (8): 547 (1882)
- =Monilia penicillioides Delacr., Bulletin de la Société Mycologique de France 13: 114 (1897)
- =Penicillium brevicaule var. hominis Brumpt & Langeron, Précis de parasitologie: 838 (1910)
- =Acaulium insectivorum Sopp, Skrifter udgivne af Videnskabs-Selskabet i Christiania. Mathematisk-Naturvidenskabelig Klasse 11: 60 (1912)

Colony characteristics. Colonies (MEA 2%) expanding, whitish, powdery to felty, soon becoming avellaneous or pinkish-brown; reverse cream-coloured to brownish. Microscopy. Conidiogenous cells single or in small brush-like groups on undifferentiated hyphae, cylindrical with slightly swollen base, 9-25 x 2.5-3.5  $\mu$ m, with equally wide annellated zone of variable length. Conidia subhyaline, spherical to obovoidal or bullet-shaped, with truncate base, 5-8 x 5-7  $\mu$ m, mostly rough-walled.





Scopulariopsis brevicaulis, en.wikipedia.org

#### **References:**

- 1. Frank C, Vemulapalli R, Lin T: 2011, Cerebral phaeohyphomycosis due to *Cladophialophora bantiana* in a Huacaya alpaca (*Vicugna pacos*). J Comp Pathol145:410–413.
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- **3.** Tuteja. F.C., S.K. Ghorui and S.D. Narnaware. Journal of Camel Practice and Research 17 (2): 225-228, December 2010 Cutaneous alternariosis in dromedary camel

4.

### 8. Paecilomycosis

**Tuteja** *et al.* (2012) reported a male calf of Jaisalmeri breed that was born to a completely agalactic dam. Since calf could not get colostrum, so the camel milk feeding through nipple bottle was started on day first, as a usual practice adopted for such calves on the farm. On the 3rd day calf stopped feeding and suffered from respiratory insufficiency with deep laboured respirations along with abdominal movements and fever of 103°F. The calf was treated with antibiotics, analgesics, corticosteroids and fluid therapy but the calf died on 6th day. Postmortem examination revealed that whole of the lung was black in colour with papules and patchy fibrinous deposit on the surface along with emphysema. Mycological examination revealed for the isolation and identification of Paecilomyces lilacinus

#### **Description of the agent**

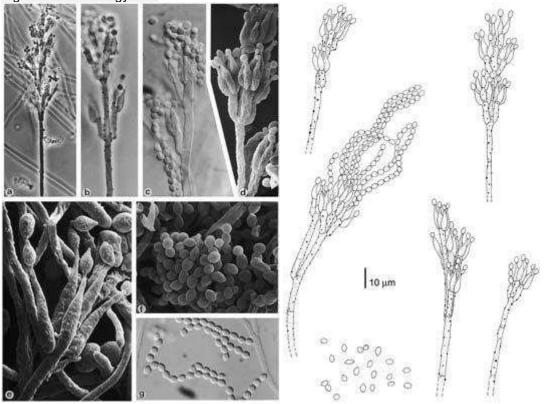
#### Paecilomyces lilacinus (Thom) Samson, Studies in Mycology 6: 58 (1974)

- ≡Penicillium lilacinum Thom, Bull. Bur. Anim. Ind. U.S. Dep. Agric.: 73 (1910)
- ≡Purpureocillium lilacinum (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson, FEMS Microbiology Letters 321: 144 (2011)
- =Penicillium amethystinum Wehmer
- =Spicaria rubidopurpurea Aoki, Bull. Imp. Seri cult. Exp. Sta. Japan: 419-441 (1941)

Colonies (MEA 2%) growing rapidly, floccose, vinaceous to violet. Microscopy. Conidiophores erect, 400-600  $\mu$ m in length, mostly arising from submerged hyphae, occasionally forming tufts up to 2 mm high, bearing branches with densely clustered phialides; conidiophore stipes 3-4  $\mu$ m wide, yellow to purple, rough-walled. Phialides consisting of a swollen basal part, tapering into a thin neck. Conidia ellipsoidal to fusiform, smooth-walled to slightly roughened, hyaline, purple in mass, 2.5-3.0 x 2.0-2.2  $\mu$ m, in divergent chains.



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Mycobank

## **Reference:**

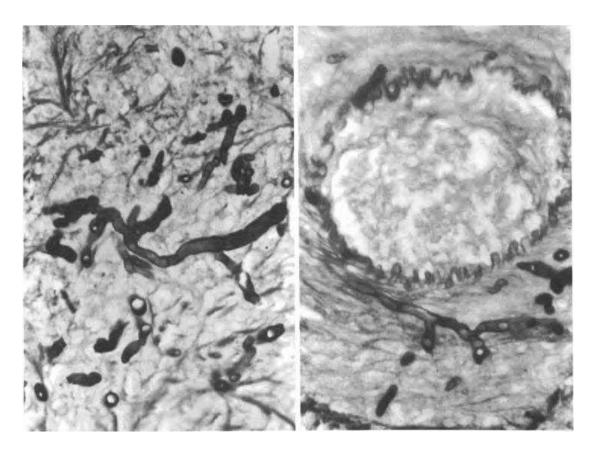
Tuteja , F.C., N.V. Patil, S.D. Narnaware, S.S. Dahiya, G. Nagarajan, G. Sivakumar and K. Nath. Mortality of a neonatal camel calf due to paecilomycosis infection of the lungs . Veterinary Practitioner 13 (2)319-312, December 2012

## 9. Zygomycosis

- Zygomycosis is a unifying term that includes a diverse group of mycotic diseases
  caused by fungi of the class Zygomycetes. The pathogenic zygomycetes are grouped
  in the orders Entomophthorales and Mucorales; disease in animals is caused by
  members of the genera Basidiobolus and Conidiobolus (entomophthoramycosis) and
  Ahsidia, Mortierella, Mucor, and Rhizopus (mucormycosis).
- Members of the order Mucorales cause disseminated disease with hyphal angioinvasion, and members of the order Entomophthorales cause localized subcutaneous granulomas characterized by marked deformation of infected tissues.

#### **Reports:**

SATIR et al. (1993) described two cases of phycomycosis of the abomasum in dromedary camels. In one of the cases there was evidence of spread to the liver. It was believed that this was the first report of phycomycosis in Camelidae. Case 1: A 10 year old dromedary was brought from Sultanate of Oman when it was 3 years old. It was healthy until February 1984 when it started to lose appetite, it developed intermittent temperature (37 O - 37.5 "C) that would continue for 2 - 3 days and subside spontaneously. The animal was grinding its teeth and reluctant to lie down despite profound weakness. A laparotomy for reticular foreign bodies was negative. The animal died one month after the laparotomy. Case 2: A 15 year old female dromedary camel was noticed to be lagging behind the herd, with loss of appetite. The condition progressed slowly over one year and eventually the camel stopped chewing the cudd and started grinding its teeth. It was running a temperature of 38.5 "C and started to vomit. Pathology Postmortem examination was limited and both animals showed similar appearances. There was emaciation and gelatinization of the carcasses. The rumen and intestine were nearly empty and distended with gas. There was ascites in case I. The abomasum showed multiple necrotic ulcers with irregular edges that varied from 0.5 to 4 cms. The wall was markedly thickened and the cut surface showed multiple soft areas of confluent necrosis alternating with firm gritty fibrosis involving the full thickness of the abomasum. Case I showed multiple areas of necrosis involving most of the liver with liquification. Other organs were macroscopically normal. Only the abomasum from both cases was available for histological examination. Sections of representative blocks were stained with haematoxylin and eosin (H&E). Reticulin, PAS, Grocott methenamine silver and Ziehl-Neelsen stains were done as required.



Mutliple irregular non-septate fungal hyphae in the necrotic areas. Some in cross section. (Grocott's silver **x** 400 Thrombosed artery showing fungal hyphae in the wall. (Grocott's silver **x** 400) **SATIR** *et al.* (1993)

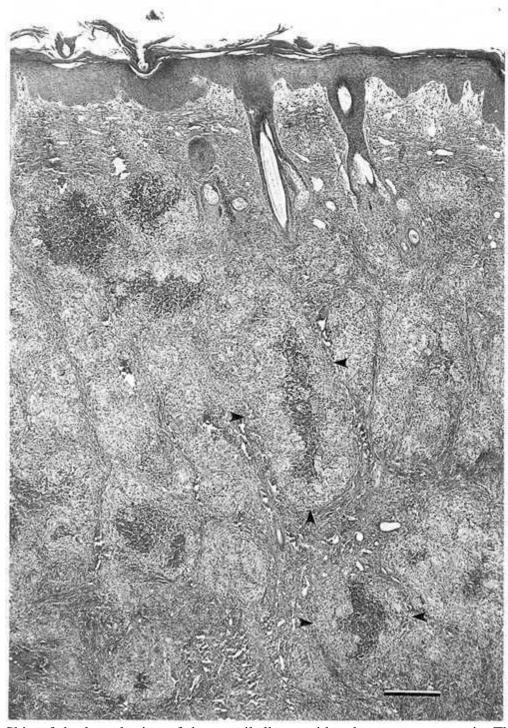
French and Ashworth (1994) described a case of deformative and obstructive nasal entomophthoramycosis (zygomycosis) caused by Conidiobolus coronatus in a llama (Larna glama) and compare d the disease in llamas with that described in the horse. A 3-year-old intact female llama was presented to the University of Illinois Veterinary Teaching Hospital with a 2-year history of sneezing and a 6-month history of nasal discharge, swollen nares, weight loss, and lethargy. Affected skin, extending rostrad from the incisive notch and covering the muzzle and upper lip, was firm, nodular, mildly hyperemic, and covered by multifocal crusts, with some focal erosions and ulcerations. The nares contained a greenbrown exudate. The lesion did not respond to treatment with topical Panalog and cleansing with Betadine. The llama developed dyspnea and stridor attributed to partial stenosis of the external nares caused by swelling and accumulation of exudate and nasal discharge. Minimal exercise resulted in open-mouth breathing. The llama was nonresponsive to treatment and was euthanatized because of its deteriorating condition. No other lesions were noted at necropsy. Sections of skin from the lateral wing of the right nostril were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 pm, and stained with hematoxylin and eosin (HE). Microscopically, the deep dermis contained multiple distinct and coalescing granulomas surrounded by fibrovascular connective tissue. Granulomas consisted of a central necrotic core of cell debris, intensely eosinophilic material, and degenerate and nondegenerate eosinophils and neutrophils that were surrounded by large numbers of

epithelioid macrophages and variable numbers of multinucleate giant cells. Giant cells were more prominent in less organized granulomas. Large numbers of eosinophils and fewer neutrophils, lymphocytes, and plasma cells were admixed with the macrophages in the surrounding connective tissue. Perivascular accumulations of small to moderate numbers of lymphocytes and plasma cells were present in the lesion. Within the core of the granulomas and within multinucleate giant cells were 6.0-12.5-pm round and elongated channels or clear spaces. In a few granulomas, a cuff of brightly eosinophilic granular material (eosinophil cytoplasmic debris) surrounded fungal elements. Some of the hyphae had slightly refractile walls. Grocott's methenamine silver stain demonstrated a fungus characterized by short randomly branched 6.0-1 5.0-pm-diameter infrequently septate hyphae Hyphal walls were nonparallel, and hyphal segments often were barrel-shaped.

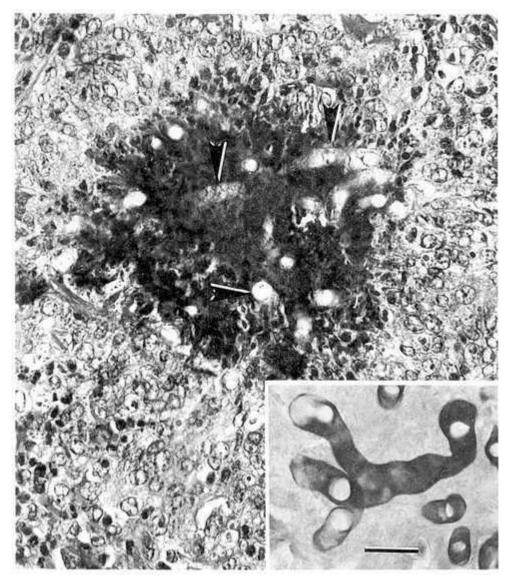
Culture of the affected tissue on Sabouraud's agar resulted in the isolation of a slightly elevated, pale yellow-white, glabrous circular fungal colony with evidence of radially striated folding. Growth on agar was superficial and did not extend into the agar. The colonies developed a white frosting of surface hyphae and short conidiospores. Coincident with this color change, forcibly ejected conidia collected on the lids of petri dishes. Conidia were round and smooth walled, 36-45 pm in diameter, with a conspicuous papillary protuberence. Various stages of conidia maturation were observed on the nonnutritive petri dish lid, such as hyphal germination, reproduction by replication, and thin-walled, villose "resting" conidia, which are characteristic of *C. coronatus* 



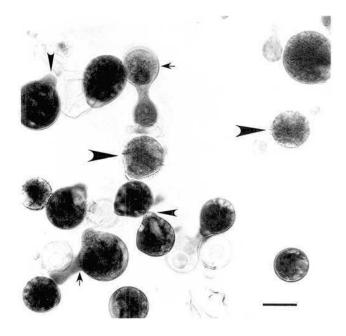
Nostrils; llama with subcutaneous mycosis. The nostrils are partially deformed and have multifocal alopecia, ulceration, and crust formation (small arrowheads). The nares (arrows) are partially occluded by an exudate, debris, and nasal discharge. **French and Ashworth (1994)** 



Skin of the lateral wing of the nostril; llama with subcutaneous mycosis. The deep dermis has multiple discrete and coalescing granulomas (arrowheads). HE. Bar = 250 pm. French and Ashworth (1994)



Skin of the lateral wing of the nostril; llama with subcutaneous mycosis. A granuloma contains an eosinophilic coagulum and cell debris. Within the granuloma, there are nonstaining "shadows" of Conidiobolus sp. hyphae (arrowheads). HE. Bar = 32 pm. Inset: Branching C. coronatus hypha. Grocott's methenamine silver. Bar = 15 pm.



Fungal culture, conidiospores; Conidiobolus coronatus. Primary and discharged globose conidia. Some conidia have a papillum (small arrowheads), and others are relatively thin-walled villose conidia (large arrowheads), characteristic of C. coronatus. Also present are stages of hyphal germination and sporulation by replication (arrows). New methylene blue. Bar = 30 pm.

**Fowler** (1998) isolated *Rhizopus* spp.from a llama that suffered from a disseminated, multisystemic infection in association with a facial paralysis of cranial nerve. During the course of the disease, swallowing became impossible and the llama began to lose weight. An endoscopic examination of the nasal cavity revealed a black membrane with white patches with filamentous growth on the surface of a necrotic rhinitis, the

meninges on the ventral aspect of the brain were inflamed, and granulomas were present in the area of the cranial nerves.

**Liebknecht** (2013) reported a camel bakterian camel, 5 years of age, came to treatment because of chronic rumen bloat that was repeated three times during the month, with a history of long anorexia (more than 3 weeks), intense abdominal wall with weak rumination (4-5 strokes per minute), tympanic sound on percussion scar. General blood analysis have shown significant leukocytosis. In the overall analysis of urine ketone bodies were found. When conducting abdominal ultrasound cavity revealed the presence of fibrous effusion in the peritoneum in the ventral abdominal wall. Based on the history and laboratory studies the case was diagnosed as tympany chronic scar, Hoflunda syndrome, peritonitis, secondary ketosis. Symptomatic therapy

Was in the form of ceftriaxone 20 mg / kg intravenously, dyufalayt 100 ml / 50 kg intravenously, dexamethasone 0.5 mg / kg intravenously, sodium bicarbonate 200 g oral glucose 500 ml of 40%. 10 days after the start of treatment, the animal slightly improved, but rumen motility remained unchanged. leukocytosis decreased. Within 30 days after receipt, the camel fell suddenly with symptoms of acute respiratory failure, which developed as a result of hemorrhagic pneumonia and pulmonary edema, acute frothy bloat, peritonitis and severe general intoxication. Histological examination revealed: congestion of the microvasculature, degenerative changes in the liver and the development of cirrhosis, with overgrowth of the hyphae of Mucor species in adipose tissue.



Palpation of the abdominal wall.

Intravenous infusion Liebknecht (2013)



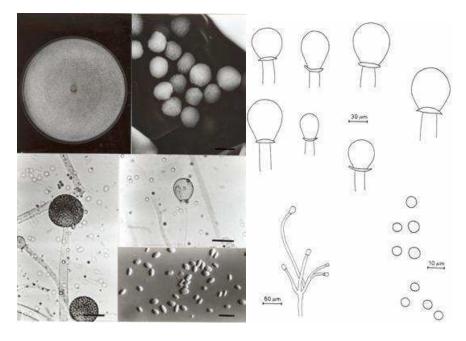
Ulceration of the intestinal wall. The deposition of fibrin on the serous membranes **Liebknecht (2013)** 

#### Description of some agents of zygomycosis

#### 1. Mucor racemosus Fresen., Beiträge zur Mykologie 1: 12 (1850)

≡Mucor oudemansii Vánová, Ceská Mykologie 45 (1-2): 25 (1991) [MB#127967] =Mucor racemosus f. racemosus =Mucor racemosus f. brunneus Morini, Malpighia 10: 88 (1896) [MB#494408] =Mucor dimorphosporus Lendn., Matériaux pour la Flore Cryptogamique Suisse 3 (1): 93 (1908) =Mucor christianensis Hagem, Annales Mycologici 8 (3): 268 (1910) [MB#198906] =Mucor varians Povah, Bulletin of the Torrey Botanical Club 44: 297 (1917) [MB#155181] =Mucor pispekii Naumov, Encyclopédie Mycologique 9: 47 (1939)

Colonies (MEA) expanding, pale greyish-brown. Sporangiophores hyaline, up to 20 mm high, 14-17 ?m wide, sympodially and monopodially branched, the short monopodial branches often being recurved. Sporangia brownish, up to 80 (-90) ?m diam; columellae subspherical to pyriform, often with truncate bases, light brown, with collars. Sporangiospores smooth-walled, spherical to broadly ellipsoidal, up to 8-10 ?m diam. Chlamydospores mostly occurring in sporangiophores. Zygospores up to 110 ?m diam, with short spines, brown.

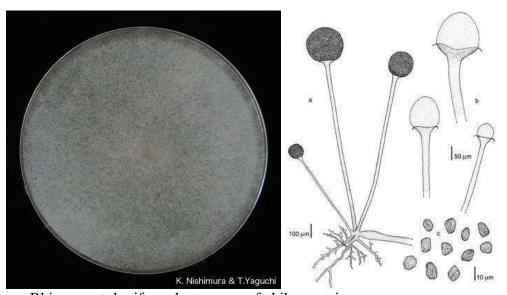


Mucor racemosus Mycobank

## 2. Rhizopus stolonifer (Ehrenb.) Vuill., Revue Mycologique Toulouse 24: 54 (1902)

≡Mucor stolonifer Ehrenb., Sylvae mycologicae Berolinenses: 25 (1818)

Colonies (MEA) expanding, whitish. Sporangiophores with 1-3 together (occasionally more), brownish, up to 2 mm high and 20 ?m wide; rhizoids well developed, profusely branched. Sporangia black, spherical, up to 275 ?m diam; columellae conical, up to 140 ?m high. Sporangiospores angular-spherical to ellipsoidal, up to 13 ?m in length, striate. Chlamydospores absent. Zygospores. Zygospores black, warted, up to 200 ?m diam, between unequal suspensors. Heterothallic. Maximum growth temperature 30-32°C.



Rhizopus stolonifer colony-www.pf.chiba-u.ac.jp

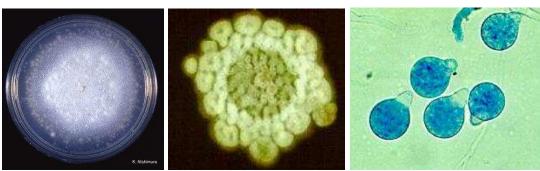
## 3. Conidiobolus coronatus (Costantin) Batko, Entomophaga 2: 129 (1964)

#### **Synonyms:**

≡Boudierella coronata Costantin, Bulletin de la Société Mycologique de France 13: 40 (1897) ≡Delacroixia coronata (Costantin) Sacc. & P. Syd., Sylloge Fungorum 14: 457 (1899) ≡Entomophthora coronata (Costantin) Kevorkian, J. Agriculture University of Puerto Rico 21 (2): 191 (1937) =Conidiobolus villosus G.W. Martin, Botanical Gazette Crawfordsville 80 (3): 311 (1925)

Colonies of *Conidiobolus coronatus* are fast growing, flat, waxy, creamy white to tan with age. As a colony becomes older it obtains powdery texture and the lid of the petri dish becomes covered with sticky conidia. The colonies grow quickly and can reach a diameter of 6 cm in 48 hours.

Conidiobolus coronatus has wide vegetative coenocytic hyphae that are 6 to 15 µm in size. The conidiophores are 8-12 µm in width by 60-90 µm in length and they produce primary conidia that are 25 to 45 µm in diameter, spherical, single-celled and have a nipple-like structure at the base that assists in discharging conidia. The villose conidia's spikes are 10 to 15 µm in length. Hyphae 6-15 µm diam. Conidiophores 60-90 µm high, slightly tapering towards the tip. Primary conidia, about 40 µm diam, with prominent, papillate base, in older cultures forming hair-like appendages (villose conidia); conidia forcibly discharged, replicative microconidia often produced, passively discharged.



Conidiobolus coronatus *colony on potato dextrose* Spherical conidia with prominent papillae from Mycology Online *Chiba Univ. Res. Center for Pathogenic Fungi and Microbial Toxicoses* 

#### **Reference:**

- 1. Fowler, M.E. **1998.** Medicine and surgery of South American Camelids. Iowa State University Press, Ames.
- 2. French, R. A. and C. D. Ashworth Zygomycosis Caused by Conidiobolus coronatus in a Llama (Lama glama). Vet Pathol 31:120-122 (1994)
- 3. Liebknecht, K. Clinical case of zygomycosis in camels. UDC 619 : 616. http://min.usaca.ru/uploads/article/attachment/121/01
- 4. SATIR. A. A., M. I. ABU BAKR, A. ABALKHAIL, A. E. ABDEI. GHAFFAR', and A. E. BABIKER'. Phycomycosis of the Abomasum in Camelus Dromedarius. J. Vet. Mcd. A 40,672-675 (1993)

### 10. Pythiosis

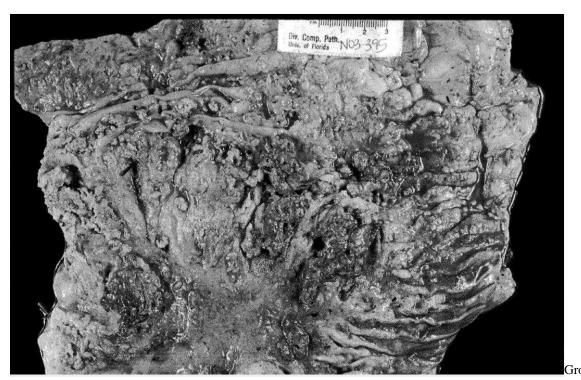
Pythiosis is an emerging tropical disease caused by *Pythium insidiosum*. *Pythium* is a genus of parasitic oomycetes. They are commonly called water moulds. The genus *Pythium* consists of about 200 species and are common pathogens causing disease in plants and fish. Pythium insidiosum is the only species which causes infection in animals. Pythium insidiosum, the etiological agent of pythiosis insidiosii, causes lifethreatening infections in animals. The disease most commonly infects horses and dogs, but can also infect cats, cattle, equines, camels, captive polar bears and humans

Pythium insidiosum, like other Pythium spp, need wet environments to carry out their life cycle in nature. Pythiosis occurs primarily in the fall and early winter after warm summer months, especially after periods of high precipitation. Animals exposed to warm, standing water are more likely to encounter the infectious zoospores and may have an increased risk for the disease; however, the infection can be acquired after contacting moist soil and grass. It is suspected that the invading zoospores enter an animal through open wounds (http://pythiosis.com).

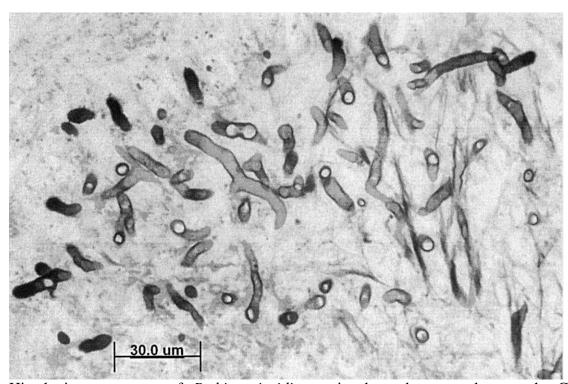
- Pythiosis has been reported in both humans and animals causing cutaneous, subcutaneous, intestinal and less frequently vascular disseminated infections (Krajaejun et al., 2006)
- In the USA, pythiosis is more prevalent in dogs and horses inhabiting the coastal states of the Gulf of Mexico, but has also been reported in Georgia, Illinois, Indiana, Kansas, Kentucky, Missouri, New Jersey, North and South Carolina, Tennessee, Virginia, and Wisconsin. More recently the disease has also been diagnosed in dogs and horses in Arizona, California, and New Mexico (White et al., 2008)
- Sporadic cases of bovine pythiosis have also been described in cattle in the USA, Venezuela and Brazil (Mille et al., 1985, Santurio et al., 1998, Pérez et al., 2005)
- Cutaneous and subcutaneous cases of the disease have been diagnosed in captive animals (Grooters, 2003)
- Pythiosis on the face of a camel (*Camelus dromedaries*) was recently described in an animal living in captivity in a Florida, USA zoo (**Wellehan** et al., 2004)
- The first reported case of vulvar pythiosis in camels (*C. dromedarius*) living on a private Tennessee wildlife animal farm (**Videla** *et al.* (2011).

#### **Reports:**

Wellehan et al. (2004) examined a 4.5-yr-old male dromedary camel (Camelus dromedarius) for a mass on the right side of the face. A complete blood count and blood chemistry revealed anemia and hypoproteinemia. Radiographs did not reveal bony involvement. The mass was resected and Pythium insidiosum was cultured. The camel was treated with an experimental immunotherapeutic vaccine and with sodium iodide and ceftiofur. The camel began to lose weight postoperatively and died 6 mo later. At necropsy, the camel was found to have gastritis of the thrid compartment of the stomach with intralesional hyphae of this oomycete



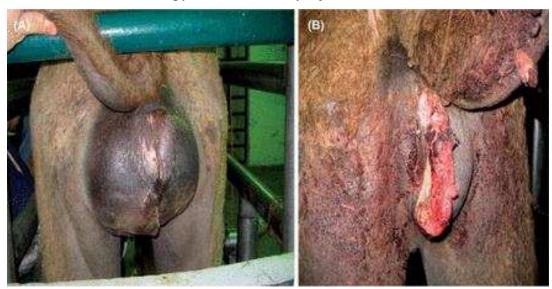
appearance of the third compartment of the dromedary camel's stomach at necropsy. Bar 5 3 cm. Wellehan et al. (2004)

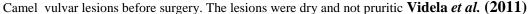


Histologic appearance of *Pythium insidiosum* in dromedary camel stomach. Gomori methenamine silver staining. 8- to 12-mm-diameter, rarely branching and infrequently septate hyphae can be seen within a granuloma. Bar 5 30 mm. Wellehan *et al.* (2004)

**Videla** *et al.* **(2011)** reported 2 camels (Camelus dromedarius), 3- and 4-years-old, respectively, from an eastern Tennessee wildlife farm with persistent weight loss and large vulvar masses. An initial biopsy of the vulvar mass of one of the camels performed by a local

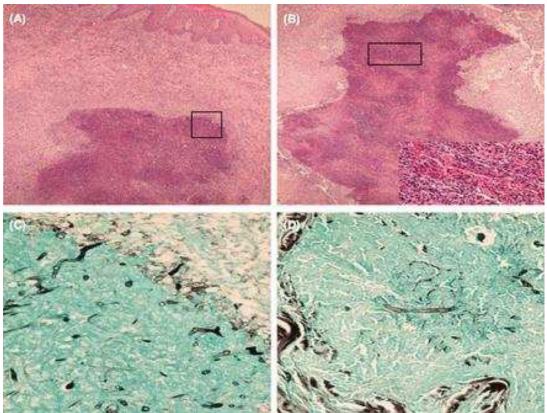
veterinarian showed eosinophilic dermatitis. An allergic or parasitic dermatitis was suspected. The two camels were treated with one dose of sodium iodide (66 mg/kg, in 1.0 L of normosolR, IV) and ivermectin 1% (200 ug/kg PO). Upon presentation at the Veterinary Teaching Hospital, University of Tennessee, additional biopsies of the masses again revealed eosinophilic dermatitis. Microscopic examination of a Gomori methenamine silver (GMS)-stained section prepared from the biopsy of one of the camels revealed the presence of fungal-like hyphae of a mold which was suspected to be Pythium insidiosum. The vulvar masses were surgically debulked in both animals and sodium iodide and Pythium-immunotherapy prescribed. Pythium insidiosum was isolated in culture and hyphae elements were detected in histological sections confirming the diagnosis of pythiosis in both animals. Despite signs of progressive healing of the vulvar surgical areas, postoperative persistent weight lost in one of the camels suggested the possibility of gastro intestinal (GI) tract pythiosis. This camel died 5 months after the first onset of clinical signs and unfortunately a necropsy was not performed. The other camel responded well to the combination of surgery, iodides, and immunotherapy and has currently rejoined the other members of the herd.







Presence of 0.5 to 1.0 cm in diameter soft yellowish kunker-like masses scattered all over the removed vulvar tissues. These masses have been found only in horses with pythiosis. **Videla** *et al.* **(2011)** 



Panels A and B showed H&E stained tissue sections collected from Shadow 1 (A) and Shadow 2 (B). The presence of an eosinophilic granuloma is observed in both sections containing numerous inflammatory cells including eosinophils, mast cells, macrophages and other cells (×4). The presence of large eosinophilic masses (kunker-like structures) showing the presence of unstained hyphal elements (the upper rectangle is displayed on the low right section of Panel B, ×50) could be seen. Panel C is an enlargement of Panel A. The rectangle in Panel A shows the area stained in panel C with Gomori Methenamine Silver (GMS). Sparsely septate 5-10- $\mu$ m-diameter hyphal elements of *P. insidiosum* can be easily observed within the eosinophilic masses (Panel C, ×50). Filamentous hyphae of *P. insidiosum* were also found in the GMS-stained tissue sections of Shadow 2 (Panel D, × 50) **Videla** *et al.* (2011).

#### **Aetiology:**

**Pythium insidiosum** De Cock, L. Mendoza, A.A. Padhye, Ajello & Kaufman, Journal of Clinical Microbiology 25 (2): 345 (1987)

#### Synonym:

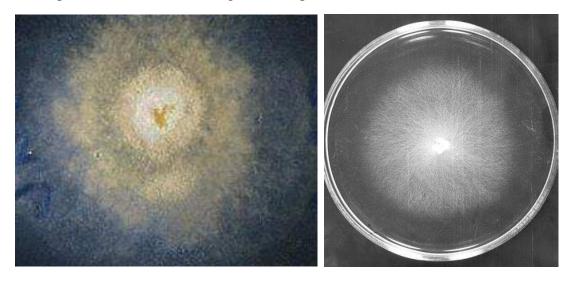
=Hyphomyces destruens C.H. Bridges & C.W. Emmons, Journal of the American Veterinary Medical Association 138 (11): 588 (1961)

Classification Chromista, Oomycota, Oomycetes, Pythiales, Pythiaceae, Pythium

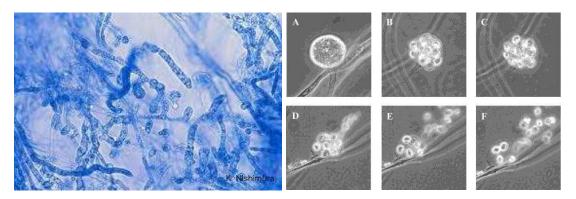
#### **Description**

Cultures (CMA) expanding, white, flat, submerged. Hyphae 4-6 ?m wide, irregularly branched (branches 2.5-4.0 ?m diam), sparsely septate in wider hyphae, locally disarticulating. Club-shaped appressoria present. Zoosporangia undifferentiated, filamentous, with two lateral flagella. Sexual organs (Oogonia) intercalary, subspherical, 23-30 ?m wide.

Antheridia produced from adjacent hyphae, clavate, terminally up to 10 ?m wide.Optimal development at 35°C, maximum growth temperature 45°C.



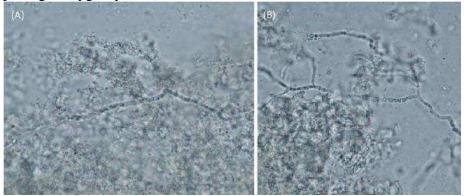
Culture of P. insidiosum. Mycology online Colony of P. insidiosum (4 days old) on SGA



dailyparasite.blogspot.com . (A to C) Zoospore production inside the zoosporangium at time intervals of 3 min. (D to F) Rapid release of zoospores.

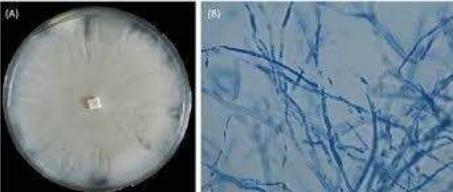
#### **Diagnosis**

1. **Wet Mounts**: The collected tissue from the infected areas is sent to the laboratory in sterile distilled water at room temperature. Pieces of the tissue are placed with 10% KOH. The finding of sparsely septate hyphae may be indicative of *P. insidiosum*, or other fungal pathogen (zygomycetes).



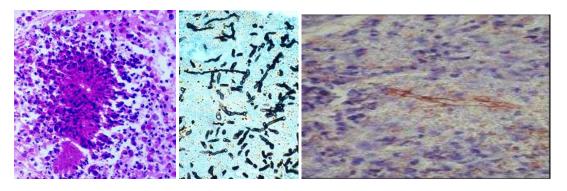
A 10% KOH wet mount preparation of a kunker. Note the long sparsely septate hyaline hyphae of Pythium insidiosum . The presence of numerous vesicles within the hyphae is usually observed, www.researchgate.net

2. **Culture:** To isolate this organism is important to remember that *P. insidiosum* is severely inhibited by low temperatures. Thus, transportation of the biopsy tissue in ice will decrease the chance to isolate this pathogen in culture. Samples, therefore, should be sent to the laboratory in sterile water at room temperature. Small pieces of the biopsy tissue should be placed onto Sabouraud dextrose agar plates and incubated at 37C, the ideal temperature for *P. insidiosum* primary isolation. *Pythium insidiosum* grows rapidly at 37C, but incubation at room temperature delays its growth rate. Small colonies should be observed around the tissue sample after 24 to 48 hours at 37C. At 5 days the plate will be filled with submerged filamentous fungi-like growth. **Microscopically**, hyphae without sporulation are observed. To identify *P. insidiosum* the formation of zoospores must be induced in water cultures with, grass leaves, with specific ionic strength. Sporangia containing zoospores (asexual stage) will be observed at edges of the grass. The production of oogonium (sexual stage) is rare.



A five days old culture of Pythium insidiosum at 37 8 C .www.researchgate.net670 × 284Search by image

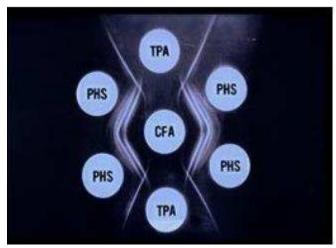
**4. Histopathology**: Tissue sections of the biopsy samples in H&E stains show a typical eosinophilic inflammatory reaction. The hyphae of *P. insidiosum*, however, are difficult to observe with this stain. Silver stain and Periodic Acid-Schiff (PAS) are suggested for the proper identification of the hyphal elements of P. insidiosum in tissue



Histological section of a tissue stained with H&E from a horse with pythiosis. The hyphae of *P. Insidiosum* is observed as transversal rings in the center of the eosinophilic reaction lookfordiagnosis.com,Left :Silver stain of a histological section from a horse with cutaneous pythiosis. www.studyblue.com.

- 5. **Serology:** Several serologic tests have been developed to diagnose pythiosis in humans and animals
- a. *Complement fixation* for the diagnosis of equine pythiosis was developed in Australia. It is a sensitive test but lacked specificity. This test is no longer in use in the laboratories dealing with *P. insidiosum*.
- b. *Immunodiffusion (ID)* has been the most widely employed serological test to diagnose pythiosis in

humans and animals. The test is very specific but it has demonstrated a low level of sensitivity. The finding that the sera from some humans and dogs with proven pythiosis gave negative results indicated that the diagnosis using ID has to be confirmed with a more sensitive test.



- c. *Enzyme-Linked Immunosorbent Assay (ELISA)* was developed to be overcome the drawbacks of the ID test. The ELISA for *P. insidiosum* is specific and sensitive. This assay detected all proven cases of pythiosis in humans and dogs which were negative by the ID. It also has been proven to be helpful in detecting cat, cattle, and equines infected with *Pythium insidiosum*.
- d. *Immunoperoxidase assay* was developed to specifically detect the hyphae of *P. insidiosum* in the biopsy tissue from humans and animals. The technique uses peroxidase labeled polyclonal antibodies against *P. insidiosum* to bind to the hyphae in infected tissue. The tissue sections are examined microscopically to visually identify the stained hyphae.
- e. *Fluorescent antibody technique* was developed to diagnosis pythiosis from fixed tissue samples and to identify *P. insidiosum* from culture. The technique specifically detected *P. insidiosum* hyphae and gave negative results when tested against Entomophthorales and Mucorales zygomycetes.

#### **References:**

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- 5. Pérez R, Luis-León JJ, Vivas JL, Mendoza L. Epizootic cutaneous pythiosis in beef calves. *Vet Microbiol* 2005;109:121-128.
- 6. Santurio JM, Monteiro AB, Leal A. Cutaneous pythiosis insidiosi in calves from the Pantanal region of Brazil. *Mycopathol* 1998;141:123-125.
- 7. Google Scholar
- 8. <u>Videla R, van Amstel S, O'neill SH, Frank LA, Newman SJ, Vilela R, Mendoza L</u>. Vulvar pythiosis in two captive camels (Camelus dromedarius). <u>Med Mycol.</u> 2012 Feb;50(2):219-24.
- 9. Wellehan JFX,, Farina LL, Keoughan CG. Pythiosis in a dromedary camel (*Camelus dromedarious*). *J Zoo Wildlife Med* 2004;35:564-568.
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# 11. Blastomycosis

- Since 2012 blastomycosis caused by Blastomyces dermatitidis has been confirmed in a number of sporadic cases in camelids showing vague neurological signs in midwestern USA. Disease is endemic amongst small animal practitioners in that region but now four cases have been confirmed in camelids (**Richards**, 2015).
- Blastomyces dermatitidis is a fungal pathogen which exists as a mould at cooler temperatures (25-30°C) and a yeast at warmer temperatures (greater than 37°C).
- Endemic regions include certain areas of the USA, parts of Africa and Central and South America.
- The usual route of human or animal infection is inhalation of conidia, although cutaneous or oral inoculation has been reported.
- Humans, cats, dogs and horses are the more commonly affected species.
- Clinically the infection can manifest as localized pulmonary or cutaneous disease or can spread to establish disseminated infection.
- Lung, skin, bone, and the genitourinary tract are the most frequent sites involved although almost any tissue can be affected.

#### **Aetiology:**

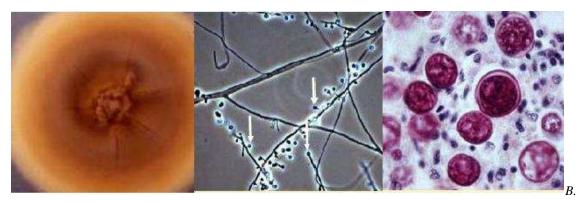
## Blastomyces dermatitidis GILCHRIST et STOCKES 1898

## **Synonyms:**

- Oidium dermatitidis RICKETTS 1901-
- Cryptococcus gilchrisii VUILLEMIN 1902-
- Zymonema gilchrisii BEUREMANN et GOUGEGOT 1901
- Glenospora gammeli POLACCI et NANNIZZI 1927-
- Blastomycoides tulanensis CASTELLANI 1928- 109

#### • Monosporium tulanensis AGOSTIN 1932

*B. dermatitidis* grows at room temperature as mould, developing glabrous, tan, non-conidiating colonies, or colonies with fluffy white mycelium. The colonies mature in 2 weeks and may attain dark brown colour on age. Microscopically, the mycelium consists of hyaline and septate hyphae which bear delicate conidiophores that carry on their tips round, oval or pear-shaped conidia. The fungus readily converts to the yeast phase when plated on blood agar and incubated at 37 C. The yeast colonies are wrinkled and folded, glabrous and tan or creamy in colour. Microscopically, the yeast cells are characterized by broad-based buds.



dermatitidis colony at 25oC oval or pear-shaped conidia broad-based buds in tissues

#### **Diagnosis**

**At necropsy**: firm nodules (up to 2.0 cm in diameter) may be observed in the lungs and in other affected tissues

**Direct microscopic examination:** smear of the exudate from the lesion reveals round, basophilic, thick-walled, refractile structures with broad based budding. Round, basophilic, thick-walled, refractile structures with broad based budding (arrow) are cytologically consistent with *Blastomyces dermatitidis*. **Méndez-Angulo et al. 2011** 

**Histological examination** of the affected tissues (i.e., skin, subcutaneous tissues, pericardium, lung, tracheal fluid and pleural tissue) with PAS or GMS staining reveals the picture of chronic inflammation in all grossly affected sites; yeasts may be clearly visualized.

**Isolation and identification**: Suspected material is plated on Sabouraud agar and blood agar and incubated at 25 and 37 C, respectively. Growth is quite slow and may need 2 months or more. *B. dermatitidis* at 25C *B. dermatitidis* at 37C

#### Serological tests

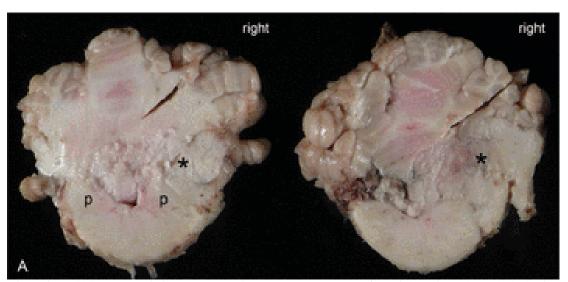
- Immunodiffusion test (precipitin). This requires 2 to 3 weeks to become positive. This test is positive in about 80% of the patients with blastomycosis. When it is positive, there is close to 100% specificity.
- Complement fixation (CF) test. This test requires 2 to 3 months after the onset of disease to develop detectable antibody. It cross reacts with other fungal infections

(coccidioidomycosis and histoplasmosis). The advantage is that it is a quantitative test.

Treatment: the administration of amphotericin B and ketoconazole can be used

# **Reports:**

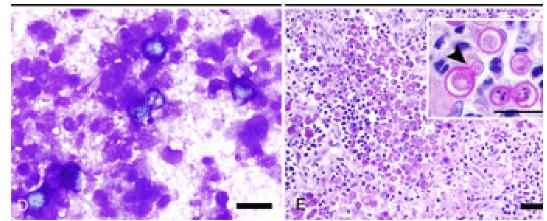
Imai et al. (2014) reported a 7-year-old, female Huacaya alpaca presented with a 3-week history of left-sided head tilt and mild ataxia. Despite aggressive supportive therapy with empirical antibiotic, anti-inflammatory, thiamine, and anthelminthic therapy, neurologic signs progressed to a right-sided head tilt, positional nystagmus, and episodes of severe ataxia and obtundation. On necropsy, discrete expansile to poorly delineated infiltrative masses with tan, creamy to grumous exudate were observed in the right petrous temporal bone, right middle and inner ear, right cerebellum and medulla as well as in lung, kidney, liver, spleen, lymph nodes, and the gastrointestinal tract. Impression cytology and histology revealed a myriad of intralesional yeast, 10-25 µm in diameter, with double-contoured walls, granular protoplasm, and broad-based budding. Culture characteristics, including conidial morphology and temperature-dependent yeast phase transformation, were consistent with Blastomyces sp. Positive Blastomyces antigenuria was identified on a postmortem sample of urine. Microsatellite typing and sequencing of the internal transcribed spacer region 2 identified the infecting strain as a genetic group 2 isolate of Blastomyces dermatitidis. The present report of camelid blastomycosis adds intracranial blastomycosis to the differential list for camelid neurologic disease in endemic regions.



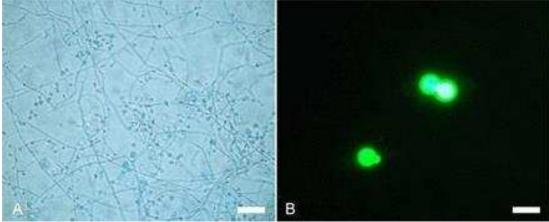
Disseminated blastomycosis in a Huacaya alpaca (*Vicugna pacos*). **A**, serial sections through the brain demonstrate a pyogranulomatous meningoencephalitis (asterisk) that expands the right cerebello- pontine angle, extends into the peduncles (p), infiltrates into the adjacent cerebellum and medulla, and fills the fourth ventricle. **Imai** *et al.* (2014)



pulmonary pyogranulomas coalesce to form a large cavitated mass in the left caudal lung lobe. C, multifocal to coalescing pyogranulomas shower the renal cortex. **Imai** *et al.* (2014)



**D**, tissue impression cytology demonstrates the typical yeast morphology. Wright–Giemsa stain. Bar =  $10 \mu m$ . **E**, myriad yeast organisms, intermixed with neutrophils and macrophages, fill the alveolar spaces in affected sections of lung. Periodic acid–Schiff (PAS) stain. Bar =  $20 \mu m$ . Inset: the yeast organisms are characterized by refractile, double-contoured walls, and broad-based budding (arrowhead), morphologically consistent with *Blastomyces* sp. PAS stain. Bar =  $10 \mu m$  **Imai** *et al.* (2014)



Blastomyces dermatitidis isolated from a Huacaya alpaca (*Vicugna pacos*). A, mycelial form cultured on potato flake agar at 28°C, demonstrating delicate 2–3  $\mu$ m in diameter hyphae. The typical "lollipop" conidia are formed by right-angled conidiophores and single terminal 2–10  $\mu$ m in diameter, oval to pyriform microconidia. Lactophenol cotton blue stain. Bar = 10  $\mu$ m. B, yeast form cultured in 7H9 broth at 35°C, exhibiting broadbased budding. Calcofluor potassium hydroxide stain. Bar = 10  $\mu$ m Imai et al. (2014)

# 12. **Coccidioidomycosis**.

Coccidioidomycosis, also called Valley Fever, is a disease primarily found in the lungs and caused by a fungus Coccidioides sp., and its fungal spores are typically dormant until the monsoon in the southwest, when it becomes active and sometimes airborne. According to veterinarians in the east valley of Maricopa County, the incidents of Valley Fever have become more common due to the disturbance of soil from the active construction, farming and vehicles disturbing the dirt. The fungal spores are disturbed within the soil, become airborne, and are inhaled by animals and people alike.

- Once within the lungs, the spore changes physically due to the moist environment of the lungs and becomes a spherule. A spherule is a larger, multicellular structure, which eventually grows and bursts. It then releases endospores that then develop into more sperules.
- At this point, one will start noticing a change in the llama. This generally occurs within three weeks of exposure.
- The spores are not only found in the soil; the hay used to feed lamas may also contain Valley Fever spores. Again, the spores are inhaled and because lamas are semi-obligate nasal breathers, the dangers of exposure are just as great from the hay as the soil.

#### Prevention

- It is advisable to avoid feeding hay grown in arid or semi-arid soils this not only includes the Phoenix and Tucson area, but the San Fernando valley of California, southern Nevada, southern Utah, southern New Mexico, and Mexico.
- Distributing feed in raised feeders rather than the ground and removing the lamas from the southwestern portions of AZ prior to monsoon season will decrease exposure and risk. Prevention is key to avoid the loss of your animals. Symptoms of primary pulmonary Valley Fever are:

## **Symptoms**

- Coughing
- Fever
- Weight loss
- Lack of appetite
- Lack of energy

Signs of disseminated Valley Fever in llamas and alpacas are much like dogs and can include:

- Lameness or swelling of limbs
- Back or neck pain
- Seizures and other manifestations of brain swelling
- Soft abscess-like swelling under the skin
- Swollen lymph nodes under the chin, in front of the shoulder blades, or behind the stifles
- Non-healing skin ulcerations or draining tracts that ooze fluid

- Eye inflammation with pain or cloudiness
- Unexpected heart failure
- Swollen testicles

Many times a lama will skip signs of having the primary infection in the lungs and will only develop the symptoms of disseminated disease.

# **Aetiology:**

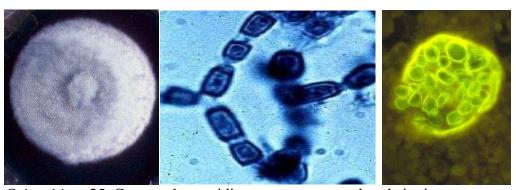
## Coccidioides immitis RIXFORD et GILCHRIST 1896

Synonyms: Posadasia esferiformis CANTON 1898

- Blastomycoides immitis CASTELLANI 1928
  - Pseudococcidioides mazzai DA FONSECA 1928
  - Geotrichum immite AGOSTINI 1932
  - Coccidioides esferiformis MOORE 1932
  - Glenospora metaeuropea CASTELLANI 1933
  - Glenospora louisianoideum CASTELLANI 1933
  - Trichosporon proteolyticum NEGRONI et DE VILLAFANE 1938

C. immitis is a thermically dimorphic fungus that grows in nature, soil, or in the laboratory at room temperature as a mould and in tissues or in the laboratory at 37C as a yeast. The mould phase grows at first as moist, glabrous and grayish colonies that rapidly develop abundant, floccose, aerial mycelium. The mycelium is initially white, but usually becomes tan to brown with age. Microscopically, the fungus develops thin and septate hyphae that produce side branches that are much more thicker and have numerous septations. Thick-walled arthroconidia are produced in these side-branches. The arthroconidia alternate with thinwalled empty cells. The arthroconida are barrel-shaped, 2.5-4 by 3-6 um and are released by fragmentation of the mycelium





C. immitis at 25oC arthroconidia a spherule in tissues

# Coccidioides posadasii M.C. Fisher, G.L. Koenig, T.J. White & J.W. Taylor, Mycologia 94 (1): 78 (2002)

Coccidioides posadasii is morphologically identical with Coccidioides immitis, but genetically and epidemiologically distinct. C. posadasii was identified as a separate species other than C. immitis in 2002 after a phylogenetic analysis. The two species can be distinguished by DNA polymorphisms and different rates of growth in the presence of high-

salt concentrations: C. posadasii grows more slowly



C. posadasii at 25°C

arthroconidia

spherule in tissues

# Diagnosis of coccidioidomycosis:

## **Direct microscopic examination:**

Spherules can be detected in sputum, pus, exudate and biopsy material, either in fresh or stained preparation by methods of Papanicolaou or Gomori's methenamine silver staining. These stains can demonstrate spherules and surrounding inflammation.

#### **Isolation and identification:**

Material to be examined is plated on Sabouraud's dextrose agar and incubated at room temperature and on brain-heart dextrose agar and incubated at high carbon dioxide concentration at 37 C. Growth of the mould phase is rather rapid and the colony matures within 2 weeks. Confirmation requires the conversion into yeast phase by subculturing the mould on brain-heart or blood agar and incubating at 37 C.

## Serology

- **Complement-Fixation** is excellent for coccidioidomycosis because it is quantitative. However, these antibodies cross-react with some other fungi (Blastomyces and Histoplasma).
- EIA and/or immunodiffusion are used to measure IgM and IgG

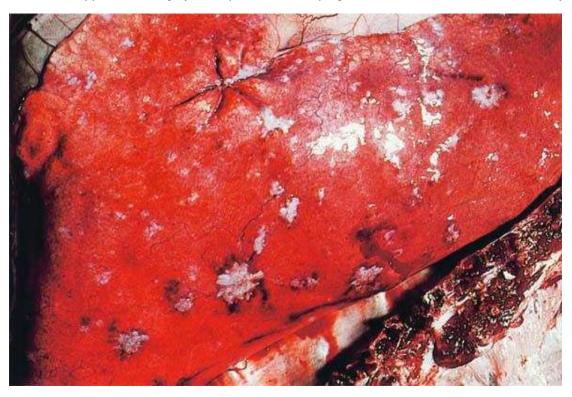
## Histopathology

Spherules of various sizes (10 to 100 µm) with multiple endospores (2 to 5 µm) are characteristic of coccidioidomycosis and can be seen with routine H&E staining. The walls of some of the spherules may appear to be ruptured, and the endospores spill into surrounding tissues. The inflammatory reaction to endospores is predominantly neutrophilic, while reaction to spherules is granulomatous

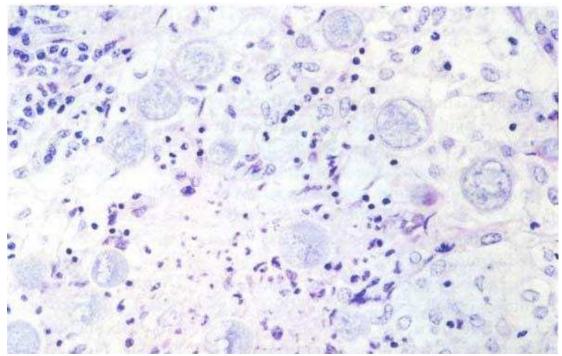
## **Reports:**

Muir and Pappagianis (1982) reported an 8-year-old nongravid female llama with a 1-month history of progressive posterior paresis was referred because of suspected degenerative myelopathy secondary to copper deficiency or plant poisoning. Neurologic examination revealed loss of conscious. The case was diagnosed as Coccidioidomycosis.

**Fowler** *et al.* **(1992)** diagnosed coccidioidomycosis in 19 llamas (California, 9; Arizona, 10). All but 1 had a disseminated form, with pyogranulomas principally in the lungs, thoracic lymph nodes, liver, and kidneys. However, lesions were found in tissue specimens from most organ systems. Antemortem diagnosis was based on radiographic evaluation of the lungs, serologic testing, or presence of spherules of Coccidioides immitis in pyogranuloma aspirates, skin scrapings, or biopsy specimens. Animals residing in endemic areas of southwestern United States are at risk, and llamas appear to be highly susceptible to developing disseminated forms of coccidioidomycosis.

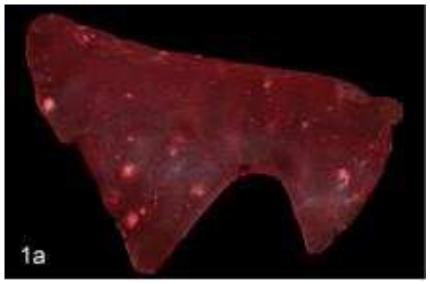


Lung granulomas caused by C. immitis Fowler et al. (1992)



Thick walled spherule filled with endospores Fowler et al. (1992)

**Diab** *et al.* (2013) described a case of placental-fetal infection and abortion in an alpaca with disseminated *C. posadasii* infection. PCR amplification and DNA sequencing were used to confirm the etiology, *C. posadasii*, in fetal tissues.



1 (a) Fetal lung depicting multifocal, variably sized, round, elevated, tan pyogranulomas **Diab** *et al.* (2013)



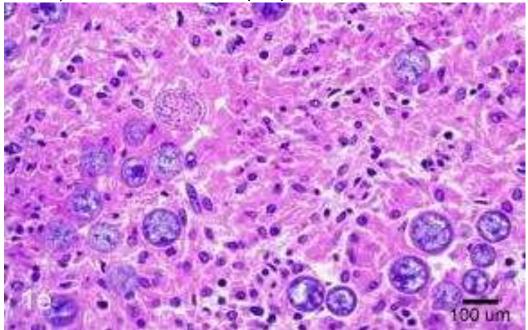
1(b) Fetal spleen showing multifocal, variably sized, round, elevated, tan pyogranulomas. **Diab** *et al.* (2013)



1 (c) Placenta showing a focal area of hyperemia and hemorrhage covered by a fibrinous exudate. **Diab** *et al.* (2013)



1(d) Microphotograph of the fetal lung showing a large, centrally mineralized pyogranuloma. Hematoxylin and eosin stain. **Diab** *et al.* (2013)



1(e) Higher magnification of  $\underline{\text{Fig. 1}}$ d depicting numerous fungal spores compatible with *Coccidioides* spp. Hematoxylin and eosin stain. **Diab** *et al.* (2013)



Dam's lung showing widespread, multifocal, granulomatous pneumonia caused by *Coccidioides* spp. **Diab** *et al.* (2013)

# 13. Histoplasmosis

*H. capsulatum* is most prevalent in the Ohio and Mississippi river valleys. The disease caused by *H. capsulatum* is called histoplasmosis, also known as "cave disease," "Darling's disease," "Ohio Valley disease," "Reticuloendotheliosis," "spelunker's lung" and "caver's disease." Symptoms primarily affect the lungs, but other organs can be affected if the fungus spreads in the body.

Histoplasma capsulatum var. capsulatum infections is a dimorphic fungus with a worldwide distribution, although it is highly prevalent along the Mississippi and Ohio River valleys of North America. It usually occurs in soil, especially if contaminated with bird and bat dropping. Histoplasma capsulatum var. capsulatum can infect a broad range of hosts, such as humans, dogs, cats, cattle, and many other animal species, including horses. Although skintest surveys (i.e., delayed hyper-sensitivity skin test for histoplasmin) have shown that 50–73% of the equine population in endemic areas harbours the infection, only a few case reports are available (Hall, 1979; Goetz and Coffman, 1984; Rezabek et al., 1993; Johnston et al., 1995; Richter et al., 2003; Nunes et al., 2006).

- Histoplasma capsulatum is a dimorphic fungus which exists in its mycelial form in soil.
- Pulmonary infection of mammals occurs when the microconidia are inhaled and carried to the alveoli.
- The higher ambient temperature of the host's body induces the inhaled fungus to assume a yeast form.
- The organisms are phagocytized by the monocyte-macrophage system and, in most cases of infection, recovery occurs uneventfully over a few weeks' time.

- Clinical disease due to infection with H. capsulatum has been reported in dogs, cats, horses, cattle, and a variety of nondomestic animals.
- In both humans and animals, histoplasmosis can occur in a chronic progressive form and a disseminated form.

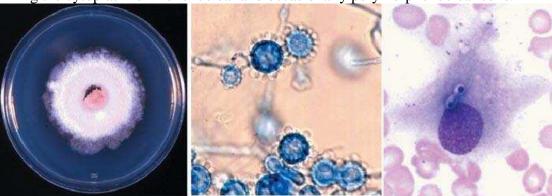
# **Aetiology**

# Histoplasma capsulatum Darling 1906

**Synonyms:** *Cryptococcus capsulatus* Castellani et Chalmers, 1910-*Posadasia capsulate* Moore 1934- *Histoplasma pyriforma Dodge 1935* 

Perfect stage: Emmonsiella capsulate KWON-CHUNG 1972

On Sabouraud dextrose agar, at temperature below 35C, the fungus is slow growing, usually requiring 2-6 weeks. The growth initially appears moist and waxy, then aerial mycelium develops, which is gray to white in colour and turns to buff or dark with age. Microscopically, the hyphae are small, hyaline and septate. They bear both micro- and macroconidia. The microconidia are small, round, sessile or stalked, 2-6 microns in diameter. The macroconidia, which are diagnostic, are round to pear-shaped, 8-14 microns in diameter, tuberculate and born on narrow conidiophores. In tissues, the fungus exists in the form of small, round or oval yeast-like cells, 1-4 microns in diameter. They are intracellular, often filling the cytoplasm of mononuclear and occasionally polymorphonuclear cells.



H. capsulatum at 25°C

tuberculate macroconida

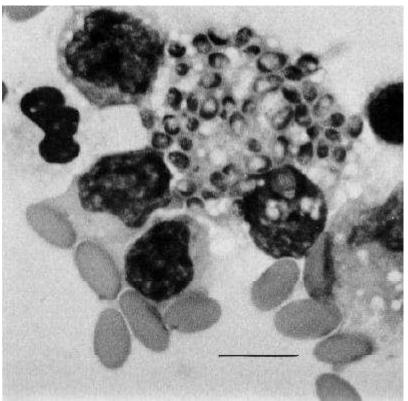
intracellular yeast cells

# **Reports:**

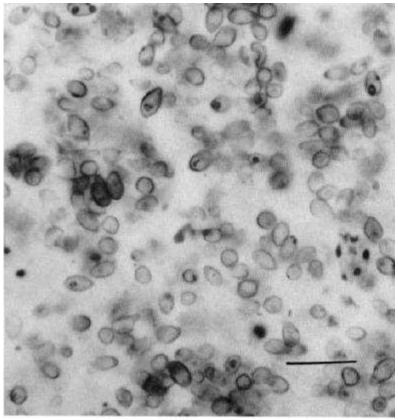
**Handel Khere** (1994) reported clinical signs including anorexia, weight loss, lymphadenopathy, dyspnoea and occasional coughing in 2 male, 13-year-old dromedaries. Both animals died, despite symptomatic treatment. PM examination revealed multiple calcified nodules in the lungs, the only organs or tissues submitted for diagnosis.

Woolums et al. (1995) reported a case of pulmonary histoplasmosis in a llama. A female llama, approximately 5 years old, was presented to the Purdue University Veterinary Teaching Hospital for depression and anorexia. The llama was one of a group imported from Bolivia 7 months prior to presentation. The llamas were held in quarantine for 2 months upon arrival in ]the United States; all other llamas in the group were apparently normal. Physical examination revealed that the llama was moderately depressed and emaciated. Lung

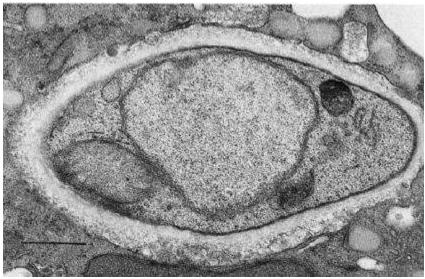
sounds could not be auscultated over any field, and forestomach motility was absent. As part of the medical workup a transtracheal aspirate (TTA) and a bronchoalveolar lavage (BAL) were performed. Both fluids were evaluated cytologically; a portion of the BAL fluid was also centrifuged and the pellet was fixed in glutaraldehyde and submitted for transmission electron microscopy. Cytologic evaluation of fluids from both procedures revealed moderate numbers of mature neutrophils and fewer macrophages. Macrophages were in various stages of activation, and the cytoplasm of occasional macrophages contained moderate numbers of 2–4-um, mildly pleomorphic, round to oval, pale basophilic structures. In many cases these structures had a fine delineating membrane and in other cases there was a thick unstained peripheral zone resembling a prominent cell wall. Specific identification was not possible; a differential including toxoplasmosis, neosporiasis, leishmaniasis, trypanosomiasis, and histoplasmosis was considered. The presence of prominent cell walls made Toxoplasma, Neospora, Leishmania, and the amastigote stage of Trypanosoma cruzi less likely. Additionally, more plump ovoid forms were recognized than would generally be seen in toxoplasmosis. There were also no detectable kinetoplasts which are characteristic of Leishmania. However, the degree of cellular pleomorphism and the very thick cell wall present on many of these organisms was not typical of Histoplasma. In light of the animal's recent importation from Bolivia, a broad differential was maintained.



Numerous 24- $\mu$ m, oval, yeast-like structures with a prominent unstained cell wall are present within a large activated macrophage from a bronchoalveolar lavage fluid cytocentrifuge preparation. Wright's stain; bar = 10  $\mu$ m. **Woolums** *et al.* (1995)



Fungal organisms within a pulmonary granuloma. GMS stain; bar =  $10 \mu m$ . Woolums et al. (1995)



Intracytoplasmic organism characterized by a distinct capsule, nucleus, and mitochondria. An apical complex or kinetoplast is not present. Uranyl acetate and lead citrate; bar = 1  $\mu$ m. Woolums *et al.* (1995)

# 14. Sporotrichosis

Sporotrichosis is a worldwide disease caused by *Sporothrix schenckii*, a yeast-like fungus present on vegetation, particularly on the thorns of roses and on plants that carry sharp spicules, or needlelike projections. A draining sore develops at the site of a puncture wound. This usually occurs on the leg but sometimes on the upper body. Nodules appear beneath the skin along the course of lymphatics. The nodules ulcerate, discharge pus, crust over, and heal slowly.

#### **Aetiology:**

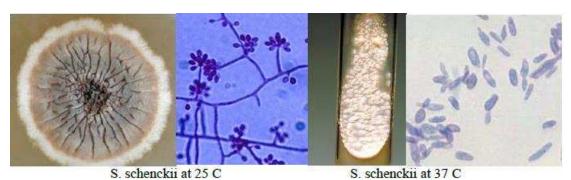
# Sporothrix schenckii HEKTOEN et PERKINS 1900

#### Synonyms:

Sporotrichum schenckii MATRUCHOT1910 Sprotrichum beurmannii MATRUCHOT et RAMOND 1905 Sporotrichum asteroids SPLENDORE 1909

Perfect stage: Ceratocystis stenocera

*S. schenckii* is a thermically dimorphic fungus. On Sabouraud dextrose agar at 25 C colonies develop in 3-5 days, at first blackish and shiny but become fuzzy with age as aerial hyphae are produced. Initially, the colony is moist, glabrous and yeast-like, but becomes tough, wrinkled and folded in time. Microscopically, thin, branching, septate hyphae and small, 3-5 microns, conidia are seen. The conidia are delicately attached to the distal tapering ends of slender conidiophores. The conidia are arranged in flower-like clusters. At 37 °C, on media containing high concentration of sugars the organism grows in the yeast phase. Conversion to the yeast phase requires 3-5 days. The yeast colony is pasty and grayish Microscopically, the yeast cells are variable in shape, but often fusiform, 1-3 by 3-10 microns, with multiple buds. The elongated yeast cells, resembling cigars with buds, are characteristic.



#### **Diagnosis**

## **Direct microscopic examination:**

Microscopic examination of pus, exudates, biopsy material or aspirates may reveal the presence of the characteristic cigar-shaped yeast cells.

#### **Isolation and identification:**

*S. schenckii* grows well on Sabouraud dextrose agar within 4-5 days at 25 C. To induce mycelium-to-yeast transformation, the fungus is inoculated on blood agar and incubated at 37<sub>°</sub> C.

## Serological diagnosis:

Complement fixation and immunodiffusion tests may be used.

#### Histopathology

S. schenckii in tissues appears as round, oval, or cigar-shaped yeasts of 2 to 6 µm or larger in diameter that may show narrow-based or tube-like budding. S. schenckii yeasts are not easy to identify with H&E stains, and thus GMS and PAS stains should be used to highlight their contour. In cases of sporotrichosis, star-like, eosinophilic material (Splendore-Höeppli phenomenon) surrounding yeasts can be observed in 40 to 92% of cases. These structures have been called asteroid bodies, and Sporothrix has been demonstrated in the centers of these structures using immunohistochemistry. The infection is usually present in a background of granulomatous inflammation with neutrophils and microabscesses and various degrees of fibrosis. The epidermis in cutaneous lesions shows pseudoepitheliomatous hyperplasia and microabscesses. Yeasts are most abundant in the microabscesses. In epidermal microabscesses, the yeasts may be mixed with hyphae. S. schenckii has also been identified using fine-needle aspiration of lesions.

**Tuteja** *et al.* (2013) reported lymphocutaneous sporotrichosis in camels in India. The animals showed small, firm dermal to subcutaneous nodules at the site of inoculation. As infection ascended along the lymphatic vessels, cording and new nodules developed. Lesions ulcerated and discharged a serohemorrhagic exudate. The cutaneous form remained localized to the site of inoculation, although lesions may be multicentric. Moist, multicentric fast spreading lesions were seen on the belly



S. schenckii; moist, multicentric lesions on the belly, S. schenckii; fast spreading moist lesions on the belly.

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