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Field Studies, characterization and classification of opportunistic fungal pathogens in an infected Bovine, Bos taurus indicus

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Summary:

Bovine, *Bos taurus indicus* are defenseless to different infection by an assortment of pathogens. They succumb to illness due its low immunity and unhealthy environment. Bovine diseases contribute to an important set of problems within livestock production systems. This leads to problems such as low in productivity of food and milk, indeterminate sustenance security and wellbeing. Various pathogens are known and have been studied. More studies have been done on infection that commonly caused in bovine such as mastitis, anthrax and few others. Fungal diseases also have been studied but their correlation with respect to infection has not been understood.

Bovine are infected by number of pathogens and they succumb to diseases. For this study, we have considered the most common infection seen in bovine such as mastitis and pyrexia. The present study aims to investigate the infected blood sample of bovine suffering from mastitis and pyrexia for screening of fungi and then further characterization by the rate of growth, general topography, texture, surface pigmentation, reverse pigmentation and microscopic examination for mycelial studies and slide culture.

By plating technique the infected cow blood sample was screened for fungi, using potato dextrose agar and incubated at 25 ^{0} C. Once the visible colonies were formed on the plate, they were characterized by colony morphology.

To further identify the fungi, mycelial studies were done. The fungi culture slide were prepared by tease mount technique using lacto phenol cotton blue stain [LPCB]. The slides were then analyzed to characterize the fungi. The identification was done as per the guidelines recommended by Larone (1976) and Al-Doory (1980).

The mastitis infected bovine blood sample which was screened for fungi were characterized as *Aspergillus niger, Gliocladium, Trichoderma, Fusarium oxysporum, Trichophyton tonsurans* and *Candida albicans.* Similarly the pyrexia infected bovine blood sample which was screened for fungi was characterized as *Aspergillus nidulans, Chrysosporium, Aspergillus sydowii* and *Cladosporium.*

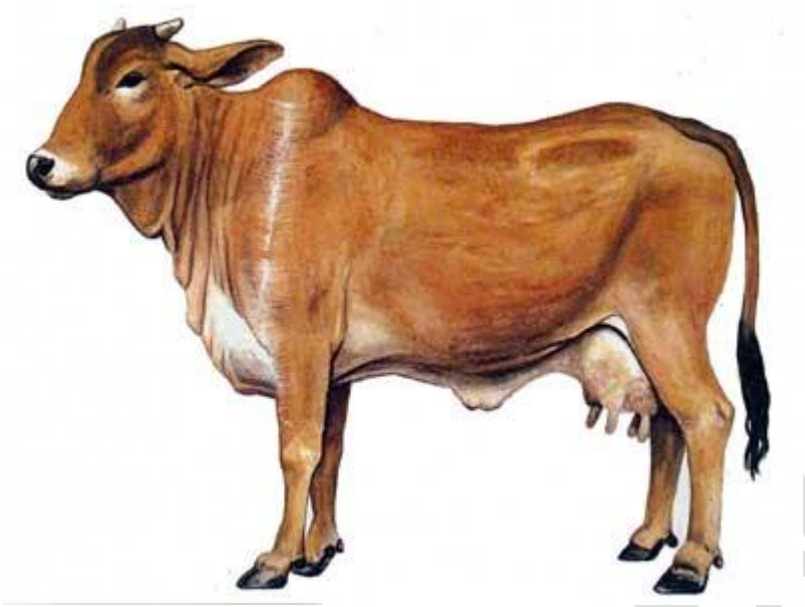
Aspergillus niger, Aspergillus nidulans and Aspergillus sydowii are known to cause Aspergillosis in bovine leading to mycotic abortion. *Candida albicans* causes Candidasis in bovine and also one of the causes of mastitis. *Fusarium oxysporum* is the causative agent of mycotic eye infections and *Trichophyton tonsurans* causes skin diseases. Chrysosporium causes foot rot in bovine . These are the opportunistic pathogens that are present in the host bovine when they are severely infected by disease Mastitis and pyrexia. Gliocladium, Trichoderma, and Cladosporium are commensal to bovine.

This study has revealed that when bovine are infected with diseases such as mastitis and pyrexia, henceforth succumb to opportunistic pathogens that were commensal. Due to weakened immune system during an infection, fungal pathogens can take advantage and cause opportunistic pathogenesis in bovine. This is the first of its kind to study the presence of

opportunistic fungal pathogen during an infection in bovine. Further studies have to be carried out in order to study the mechanism and role involved in pathogenesis.

Introduction:

***Bos taurus indicus**:*



Kingdom: Animalia

Phylum: Chordata

Class: Mammalia

Order: Artiodactyla

Family: Bovidae

Subfamily: Bovinae

Genus: Bos

Species: *B. taurus*

Subspecies: *B. t. indicus*

Trinomial name

Bos taurus indicus

Fig 1: *Bos taurus indicus*

Bos taurus indicus, the scientific name of the Indian cattle commonly called as Cow are the most common type of large domesticated ungulates. They are a prominent modern member of the subfamily Bovinae, and are the most widespread species of the genus Bos. Now, these have been reclassified as one species, *Bos taurus*, with three subspecies: *Bos taurus primigenius*, *Bos taurus indicus*, and *Bos taurus taurus*.[1][2]

The domestication of cattle began as early as 10,000 to 5,000 years ago. From ancient times up to the present, they are bred to provide meat and dairy. Cattle are also employed as draft animals to plow the fields or transport heavy objects. Cattle hide is used for the production of leather, and dung for fuel and agricultural fertilizer. Cattle also have significant religious meaning.

Almost two-thirds of the world's cattle can be found in India, Brazil, China and The United States. According to an estimate from 2012 to 2016, the global cattle population amounted to about 979.64 million animals in 2015. In 2009, cattle became one of the first livestock animals to have a fully mapped genome.[3].

Anatomy of a bovine:

They are large quadrupedal ungulate mammals with cloven hooves. Most breeds have horns, and are ruminants, meaning their digestive system is highly specialized to allow the use of poorly digestible plants as food. Bovine have one stomach with four compartments, the rumen, reticulum, omasum, and abomasum, with the rumen being the largest compartment. The abomasum is like the human stomach; this is why it is known as the "true stomach".

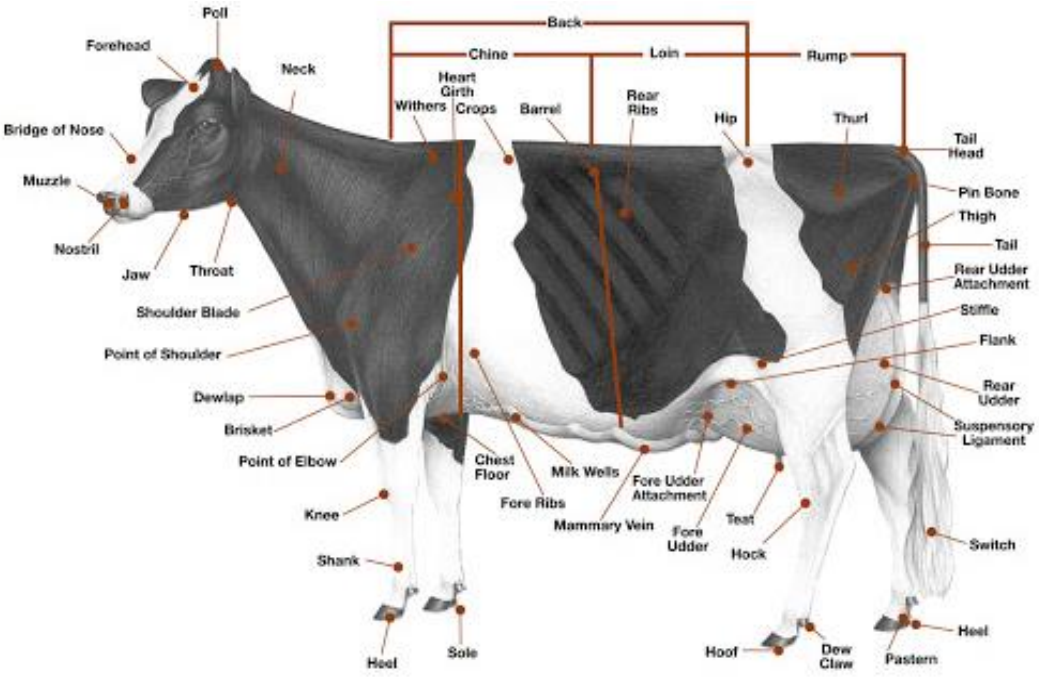


Fig 2: Labeled Diagram of a Bovine

Bovine are known for regurgitating and re-chewing their food, known as cud chewing, like most ruminants. While the animal is feeding, the food is swallowed without being chewed and goes into the rumen for storage until the animal can find a quiet place to continue the digestion process. The food is regurgitated, a mouthful at a time, back up to the mouth, where the food, now called the cud, is chewed by the molars, grinding down the course vegetation to small particles. The cud is then swallowed again and further digested by specialized microorganisms in the rumen.

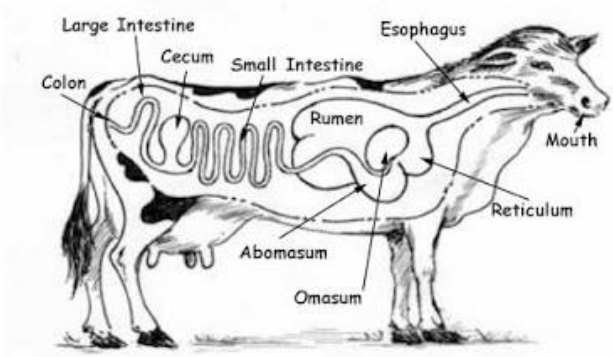


Fig 3: Digestive system of a Bovine

A cow's udder contains two pairs of mammary glands, (commonly referred to as teats) creating four "quarters".[7] The front ones are referred to as fore quarters and the rear ones rear quarters.[8]

The gestation period for a cow is about nine months long. A newborn calf's size can vary among breeds, but a typical calf weighs between 25 to 45 kg (55 to 99 lb). The weight of adult cattle always depends on the breed. It is difficult to generalize or average out the weight of all cattle because different kinds have different averages of weights. However, according to some sources, the average weight of all cattle is 753 kg (1,660 lb). Finishing steers in the feedlot average about 640 kg (1,410 lb); cows about 725 kg (1,600 lb), and bulls about 1,090 kg (2,400 lb).

Infection in Bovine:

Like humans, bovine are susceptible to various infection caused by a variety of pathogens. Due to its low immunity or poor hygiene conditions they succumb to diseases causing them ill. The increasing frequency of heat stress, drought and flooding events could translate into the increased spread of existing vector-borne diseases and macro-parasites, along with the emergence of new diseases and transmission models.

In developing countries they are characterized by rapid change, driven by factors such as population growth, increases in the demand for livestock products as incomes rise, and urbanization. Climate change is adding to the considerable development challenges posed by these drivers of change. Appropriate sustainable livestock management practices are required to ensure healthy cattle in the face of changing and increasingly variable climates.

Bovine diseases contribute to an important set of problems within livestock production systems. These include animal welfare, productivity losses, uncertain food security, loss of income and negative impacts on human health. Livestock disease management can reduce disease through improved animal husbandry practices. These include: controlled breeding, controlling entry to farm lots, and quarantining sick animals and through developing and improving antibiotics, vaccines and diagnostic tools, evaluation of ethno-therapeutic options, and vector control techniques.

Few of the common bovine diseases are as follows:

Anthrax.

Anthrax is a very contagious disease and is communicable to all warm blooded animals and man. Due to the presence of a germ called the Bacillus of Anthrax and is one of the oldest diseases attributed to germs. These Bacilli thrive in warm climates, although found in cold countries. The infection is carried to various parts of the world by box-cars, ships, hides, hoofs, horns, wool and hair taken from sick or dead animals affected with Anthrax. This, perhaps, is the most common method of spreading the disease.

Mastitis

Mastitis in dairy cattle is the persistent, inflammatory reaction of the udder tissue. Mastitis, a potentially fatal mammary gland infection, is the most common disease in dairy cattle

Bovine Respiratory Disease Complex (BRDC)

Bovine Respiratory Disease Complex (BRDC), or “Shipping Fever”, is a general term for the pneumonia commonly seen in shipped or stressed calves. Several disease agents or other interacting factors may cause the syndrome. Stress, such as weaning, dehorning, shipping and weather changes can make the animal susceptible to disease-causing viruses and bacteria

Cowpox.

Investigations lead us to believe that it is due to protozoa. So far, the true micro-organism has not been discovered. This disease is very contagious and is transmitted by direct communication but not through the air.

Clostridial Disease, or "Blackleg"

"Blackleg" is a common name for a class of bacterial infections called clostridial. Clostridial usually occurs in calves or young cattle less than 2 years old and is caused by gangrene that forms in the muscles. Clostridial normally results from young calves not getting the proper amount of colostrum. Clostridial can appear in older cattle and is usually the result of vaccine needle contamination.

BRSV (Bovine Respiratory Syncytial Virus)

This is a sometimes fatal, stress-related infection that can cause mild to severe respiratory disease and reduce the animal’s resistance to other diseases. Signs include coughing, high fever, and runny eyes and nose.

BVD (Bovine Viral Diarrhea)

This is one of the most costly diseases of cattle. Signs include scours, nasal discharge, coughing, and fever. Type 2 BVD is a severe form of this virus that can cause hemorrhaging in young calves, as well as adults.

Haemophilus Somnus

H. Somnus is a bacterial infection implicated in a variety of respiratory, neurological and reproductive disorders. *H. Somnus* can be the primary cause of respiratory disease, or it can be an underlying infection that is masked by other disease-causing agents. Signs of *H. Somnus* include fever, coughing, nasal discharge and labored breathing. Death without symptoms can occur.

IBR (Infectious Bovine Rhinotracheitis)

Also known as 'Red nose,' this highly contagious virus causes respiratory disease. Signs include inflamed nasal passages, fever, rapid breathing, deep cough, and loss of appetite.

PI3 (Parainfluenza Type 3)

This is a common, mild respiratory disease that suppresses the animal's immune system, allowing other diseases and infections to develop. The virus is shed in nasal and eye secretions, and infects non-vaccinated animals through the mouth and nasal passages.

Pasteurella Haemolytica* and *Pasteurella Multocida

These highly infectious bacteria are the major cause of pneumonia, and the most commonly found pathogens in cattle dying of respiratory disease. *P. Haemolytica* and *P. Multocida* multiply quickly in the presence of stress, poor weather, or primary viral infections. Signs include depression, lethargy, loss of appetite, and high fever. Death can occur suddenly with few signs of disease, or the animal can survive only to become a "'poor doer" due to the lung damage caused by this disease.

Pruritus/Pyrexia/Haemorrhagic Syndrome (PPH)

PPH is a rare disease of dairy cows that has been seen in several countries including the US, the UK and the Netherlands. The cause of PPH is unknown. Many causes have been suggested including fungal toxins, coumarins and a urea derivative, di-ureido-isobutane. It is possible that all of these factors can cause PPH.

Fungal Infection in Bovine:

Systemic mycoses are infections with fungal organisms that exist in the environment, enter the host from a single portal of entry, and disseminate within the host usually to multiple organ systems. The soil reservoir is the primary source of most infections, which can be acquired by inhalation, ingestion, or traumatic introduction of fungal elements.

Various manifestations of mycosis in cattle have been recorded by several authors. Pathogenic fungi establish infection in apparently normal hosts, and cause diseases such as histoplasmosis, coccidioidomycosis, blastomycosis, and cryptococcosis are regarded as primary systemic mycoses. Opportunistic fungi usually require a host that is debilitated or immunosuppressed to establish infection. Prolonged administration of antimicrobials or immunosuppressive agents appears to increase the likelihood of infection by the opportunistic fungi that cause diseases such as aspergillosis and candidiasis, which may be focal or systemic.

Clinical findings and gross lesions are often suggestive of systemic mycoses, but definitive diagnosis requires microscopic identification, culture of the organism, or PCR. Identification of the fungus and the tissue reaction via microscopic examination of exudates and biopsy material is adequate for diagnosis of histoplasmosis, cryptococcosis, blastomycosis, coccidioidomycosis, and rhinosporidiosis. Other diseases, such as candidiasis, aspergillosis, zygomycosis, phaeohyphomycosis, hyalohyphomycosis, and oomycosis (pythiosis and lagenidiosis), usually require more than microscopic evaluation for a definitive diagnosis. Some of these fungi are also common contaminants of cultures; thus, tissue invasion and reaction must be demonstrated for the culture isolation to be considered significant. Serology may be useful for diagnosis (and prognosis) of some mycotic diseases such as coccidioidomycosis, pythiosis, and lagenidiosis. Antigen titers have proved useful for cryptococcosis, histoplasmosis, and blastomycosis.

Few of the fungal diseases that are seen commonly among the cattle are as follows:

1. ***Microsporum spp*** - Ringworm disease

Ringworm is most common in calves, characterised by nonpruritic periocular lesions and alopecia. Generalized skin disease may develop. Cows and heifers are reported to develop lesions on the chest and limbs most often, and bulls in the dewlap and intermaxillary skin

2. ***Aspergillus* spp** - Mycotic placentitis

Aspergillus spp are a group of soil mould which are an aerosol cause of mycotic abortion, respiratory diseases and aflatoxicosis in cattle worldwide. In affected cattle, infections with *Aspergillus* may be asymptomatic. In respiratory aspergillosis, respiratory symptoms such as coughing, dyspnea and hemoptysis may be apparent. In some cattle, this can be rapidly fatal as dissemination of spores occurs through the pulmonary circulation.

3. ***Cryptococcus* spp** - Cryptococcosis

It is a relatively rare fungal infection in cattle which causes sporadic outbreaks of mastitis, pulmonary infections and encephalitis. Cryptococcosis is caused by a Gram-positive yeast that has worldwide distribution.

4. ***Candida spp*** - Candidiasis

Candidiasis is a localized mucocutaneous disease caused by species of the yeast-like fungus most commonly *C albicans*. It is a normal inhabitant of the nasopharynx, GI tract, and external genitalia of many species of animals and is opportunistic in causing disease. Factors associated with candidal infections are disruption of mucosal integrity; indwelling, intravenous, or urinary catheters; administration of antibiotics; and immunosuppressive drugs or diseases.

5. ***Chrysosporium spp*** - Foot rot disease

Chrysosporium spp are a ubiquitous saprophytic fungus which has been associated with foot rot in cattle worldwide. These keratinophilic fungi are airborne around dairy barns and can contaminate milk. They also normally reside on bovine skin, particularly the interdigital region of the hoof and can be associated with interdigital dermatitis although they are often secondary invading organisms.

Objective:

1. To screen for fungal pathogen in an infected bovine
2. To identify and characterize the fungi by colony morphology and Tease mount technique and explore the susceptibility of infected bovine.

Materials and Methods:

Apparatus:

Glass Petri dish – 10nos, Pipette 10ml – 5nos, Pipette 1 ml – 5nos, bent glass rod -1nos, Inoculation loop, tissue paper, gloves, newspaper, thread, cotton plug, conical flask 250 ml- 3 no's, measuring cylinder, test tubes 20 no's, spirit lamp

Chemicals:

Alcohol, distilled water

Stains:

Lacto phenol cotton blue stain, Methylene Blue.

Instrument:

Autoclave, Laminar Air flow

Sampling:

The blood sample of the infected bovine was aseptically collected in a sterilized container wearing gloves. The sample is then immediately refrigerated for optimum growth of the culture and to avoid cross contamination. The samples were then labeled them accordingly noting down, date, time, location, symptoms involved for the infection

Preparation of media:

PDA agar was prepared by weighing the required composition of the chemicals of the particular media accordingly. The required composition of the particular media was dissolved separately in a conical flask using distilled water. Then buffered the media to appropriate pH and then agar was added and dissolved it completely. The media was sterilized by autoclaving and were poured into plates aseptically in an inoculation chamber.

Plating technique:

1. **Serial dilution of the sample.**

The working area was surface sterilized with alcohol in a laminar air flow. The test tubes were placed in a stand having 9ml sterile distilled water which has been autoclaved previously. 1ml of the sample was taken and added it to the first test tube and labeled it as 10^{-1}. From this using a sterile pipette 1ml was transferred to another test tube where the dilution factor10^{-2}**.** Similarly the sample was serially diluted until the dilution factor is 10^{-5}using a sterile pipette for each dilution.

2. **Plating into agar.**

The sterilized medium which has been autoclaved is taken and cooled to 40^0C. For **pour plating** method 1 ml of the inoculum from appropriate dilution was poured into the sterile petriplate. To that around 20 ml of the medium is poured and swirled well for uniform distribution. The plates were labeled appropriately and kept on a flat surface for solidification.

For **spread plate method**, the medium is solidified by pouring them into sterile petriplates. To the solidified medium 0.1ml of the inoculum was added from appropriate dilution. The inoculum was spread evenly with the sterilized bent glass rod. The plates were incubated at 25^0C for more than a week to isolate fungal culture.

Microscopic observation of fungi:

The fungal cultures that were isolated were observed microscopically to identify its characteristics. Tease mount technique of fungal culture was used. Two drops of lacto phenol cotton blue stain was placed on a clean glass slide. With a help of a sterile forceps, a small amount of hyphal mass was placed from the culture plate to the slide. Then hyphal mass were teased apart using sterile needle. A cover slip was gently placed onto the specimen with the help of a needle. Then the slides were observed under a microscope.

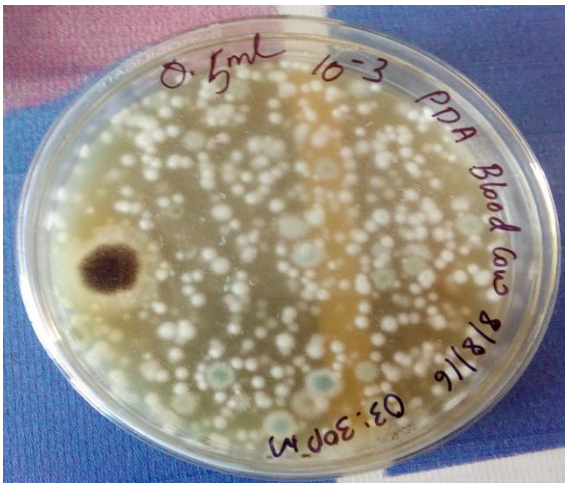
Results:

Analysis for growth on plates:

The blood sample from bovine being infected by mastitis and pyrexia were screened for presence of fungi and its susceptibility to fungal infection. The blood samples were serially diluted and 0.5 ml of the sample was pour plated and 0.5ml of blood sample was also spread plated and kept for incubation at room temperature 25^0 C. The plates were observed on day to day basis for any fungal growth. After a week of incubation the plates had fungal growth. Further the colonies were analyzed for its characteristics and through direct microscopy method the fungal colonies were identified.

Study of Fungal colony morphological characteristics:

Sample 1: Mastitis infected Bovine Blood sample



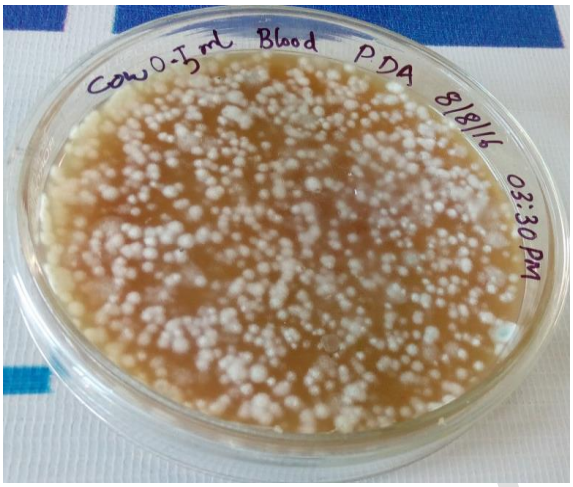


Fig 1 **Fig 2**

Fig1: Plate 1 of Mastitis infected bovine blood sample (0.5ml of 10^{-3} serially diluted sample) on PDA after 4 days of incubation at 25^0C

Fig 2: Plate 2 of Mastitis infected bovine blood sample (0.5ml of blood sample) on PDA after 4 days of incubation at 25^0C.

Colony Morphological Characteristics:

Colony No.	A	B	C	D	E
No of colonies	1	3	10	2	180
Pigment	Black	Green	Light Green	Light Blue	White
Texture	Mucoid	Mucoid	Mucoid	Mucoid	Rough
Optical Characteristics	Opaque	Opaque	Opaque	Opaque	Opaque
Whole colony	Circular	Circular	Circular	Circular	Circular
Edge	Filamentous	Filamentous	Entire	Entire	Entire
Surface	Powdery	Powdery	Powdery	Powdery	Powdery
Elevation	Raised	Raised	Flat	Flat	Raised

Table 1: Colony morphological characteristics of fungal culture grown on PDA of Plate 1 inoculated with mastitis infected blood sample (0.5ml of serially diluted sample).

Colony No.	A	B	C
No of colonies	4	1	640
Texture	Mucoid	Mucoid	Mucoid
Pigmentation	Blue	Green	White
Optical characteristics	Opaque	Opaque	Opaque
Whole	Circular	Entire	Circular
Edge	Filamentous	Filamentous	Entire
Surface	Powdery	Powdery	Smooth
Elevation	Flat	Raised	Convex

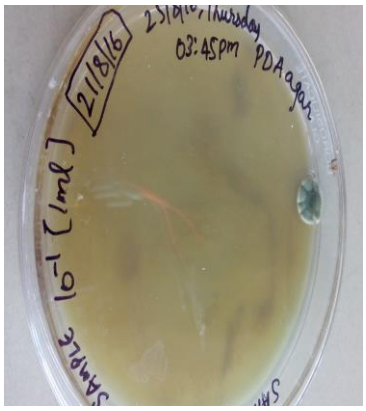
Table 2: Colony morphological characteristics of fungal culture grown on PDA of Plate 2 inoculated with mastitis infected blood sample (0.5ml of blood sample).

Sample 2: Pyrexia infected Bovine Blood Sample

Fig 3 **Fig 4**

Fig 3: Plate 1 of Pyrexia infected Bovine Blood sample (0.5ml of bovine blood sample) on PDA after a week of incubation at 25^0C

Fig 4: Plate 1 of Pyrexia infected Bovine Blood sample (0.5ml of bovine blood sample) on PDA after 2 weeks of incubation at 25^0C



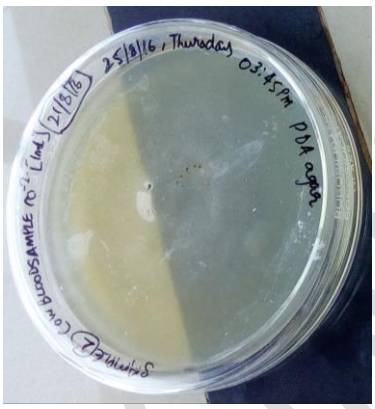


Fig 5 **Fig 6**

Fig 5: Plate 2 of Pyrexia infected Bovine Blood sample (0.5ml of 10^{-1}serially diluted bovine blood sample) on PDA after 2 weeks of incubation at 25^0C.

Fig 6: Plate 3 of Pyrexia infected Bovine Blood sample (0.5ml of 10^{-2}serially diluted bovine blood sample) on PDA after 2 weeks of incubation at 25^0C.

Colony Morphological characteristics:

Colony No.	A	B	C
No. of colonies	1	2	170
Texture	Rough	Mucoid	Smooth
Opt	Opaque	Opaque	Opaque
Pigmentation	Greyish green	White	Light green
Whole	Circular	Circular	Rhizoid
Edge	Entire	Entire	Undulate
Surface	Powdery	Smooth	Powdery
Elevation	Raised	Raised	Flat

Table 3: Colony morphological characteristics of fungal culture grown on PDA of Plate 1 inoculated with pyrexia infected blood sample (0.5ml of bovine blood sample).

Colony No	A
No of colonies	1
Texture	Smooth
Pigmentation	Opaque
Opt	Green
Whole	Circular
Edge	Entire
Surface	Powdery
Elevation	Convex
Fungal Identification	Cladosporium

Table 4: Colony morphological characteristics of fungal culture grown on PDA of Plate 2 inoculated with pyrexia infected blood sample (0.5ml of 10^{-1} serially diluted bovine blood sample).

On Plate 3 inoculated with pyrexia infected blood sample (0.5ml of 10^{-2} serially diluted bovine blood sample), there was no fungal growth seen after few weeks of incubation.

Direct Microscopy:

This method is used to identify and characterize the fungal colonies that were grown on PDA plates after weeks of incubation. After studying the colony characteristics of each fungal colony the fungal slides are prepared by tease mount technique using LPCB stain. The prepared slides are then observed under compound microscope at 10 X and then 45 X.

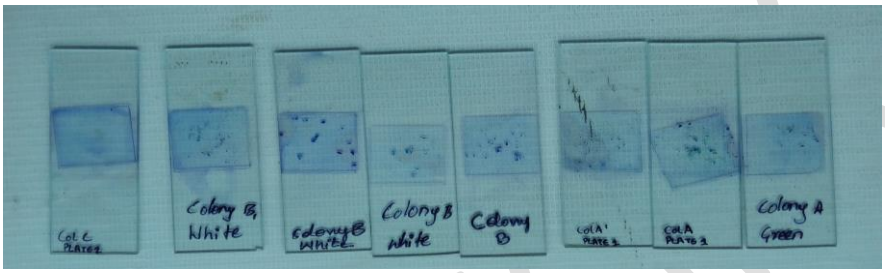


Fig 7

Fig 7: Prepared slides of fungal culture that were grown on PDA by tease mount technique using LPCB stain to identify and characterize under microscope.

SAMPLE 1 – Mastitis infected Bovine blood sample.

Plate 1: Mastitis infected bovine blood sample (0.5mlof 10^{-3}serially diluted bovine blood sample).

Colony No.	A	B	C	D	E
Fungal Identification	*Aspergillus niger*	*Gliocladium*	*Trichoderma*	*Fusarium oxysporum*	*Trichophyton tonsurans*

Table 5: Fungal identification of colonies that are grown on PDA of Plate 1 inoculated with mastitis infected bovine blood sample (0.5ml of 10^{-3}serially diluted bovine blood sample).

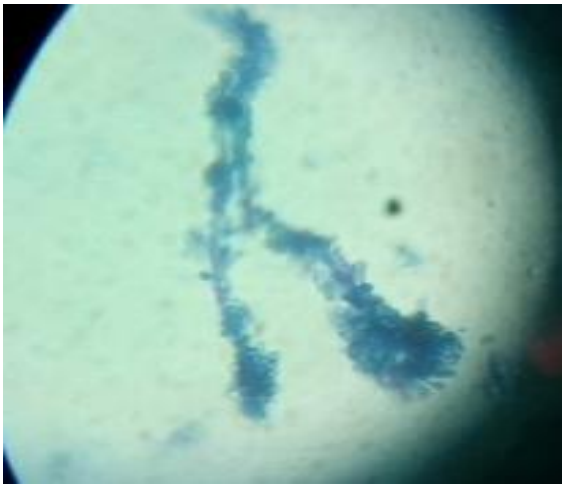


Fig 8

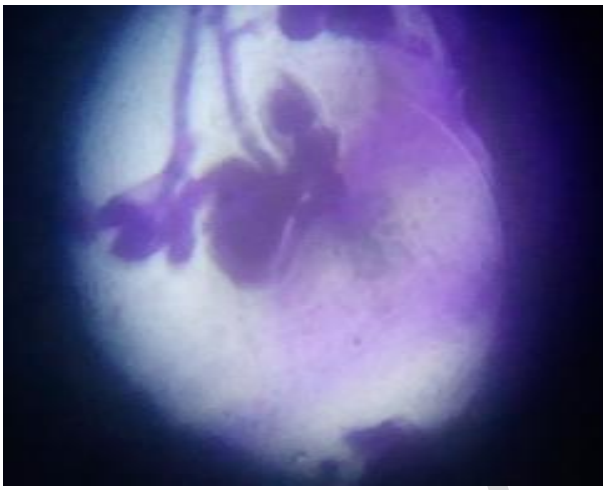


Fig 9

Fig 8: Microscopic view of Colony A of sample 1 on plate 1, mastitis infected bovine blood sample under 45X - *Aspergillus niger*

Fig 9: Microscopic view of Colony B of sample 1 on plate 1, mastitis infected bovine blood sample under 45X - *Gliocladium.*

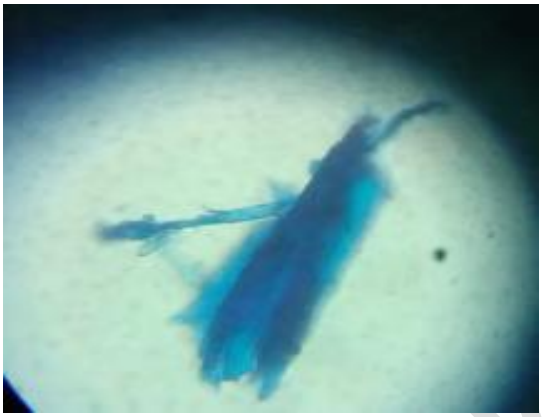


Fig 10

Fig 11

Fig 10: Microscopic view of Colony C of sample 1 on plate 1, mastitis infected bovine blood sample under 45X - *Trichoderma*

Fig 11: Microscopic view of Colony D of sample 1 on plate 1, mastitis infected bovine blood sample under 45X - *Fusarium oxysporum*.

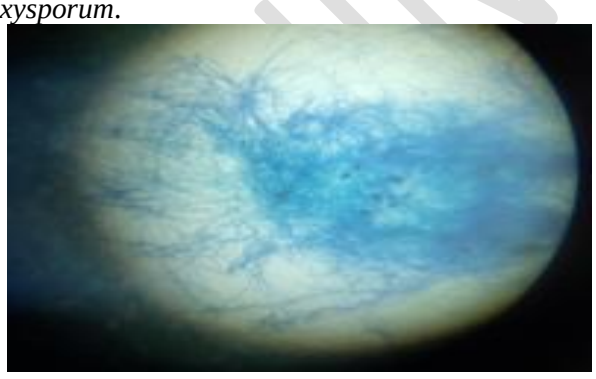


Fig 12

Fig 12: Microscopic view of Colony E of sample 1 on plate 1, mastitis infected bovine blood sample under 45X - *Trichophyton tonsurans*

Plate 2: Mastitis infected bovine blood sample (0.5mlof bovine blood sample).

Colony No.	**A**	**B**	**C**
Fungal Identification	*Fusarium oxysporum*	*Gliocladium*	*Candida albicans*

Table 6: Fungal identification of colonies that are grown on PDA of Plate 2 inoculated with mastitis infected bovine blood sample (0.5ml of bovine blood sample).

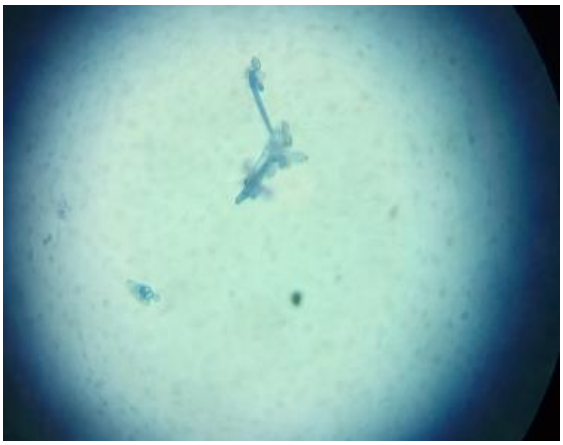


Fig 13

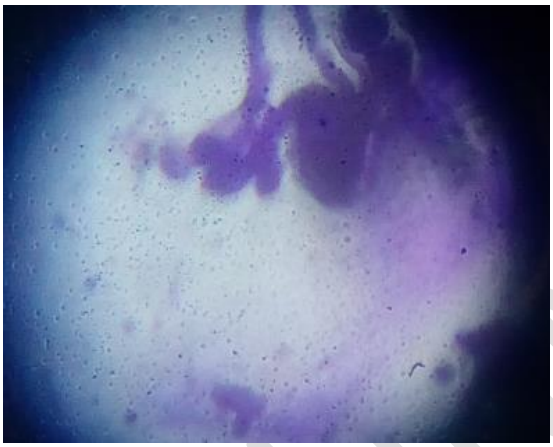


Fig 14

Fig 13: Microscopic view of Colony A of sample 1 on plate 2, mastitis infected bovine blood sample under 45X - *Fusarium oxysporum*.

Fig 14: Microscopic view of Colony B of sample 1 on plate 2, mastitis infected bovine blood sample under 45X - *Gliocladium.*

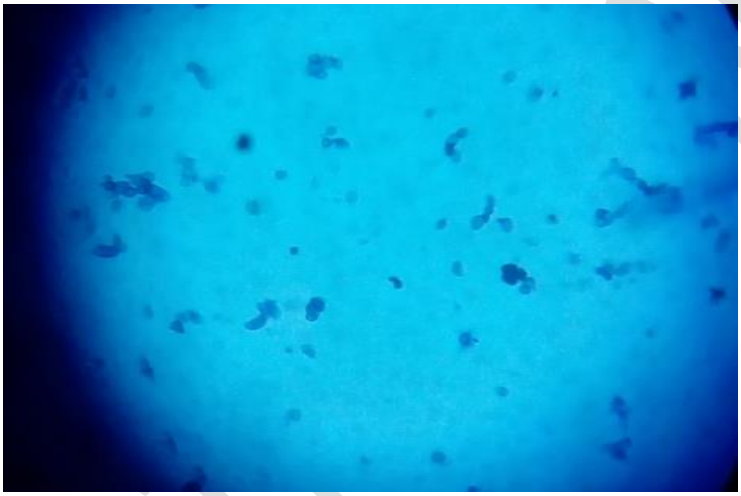


Fig 15

Fig 15: Microscopic view of Colony C of sample 1 on plate 2, mastitis infected bovine blood sample under 45X – *Candida albicans.*

Sample 2: Pyrexia infected Bovine blood sample:

Plate 1 - Pyrexia infected bovine blood sample (0.5mlof bovine blood sample).

Colony No.	**A**	**B**	**C**
Fungal Indentification	*Aspergillus nidulans*	*Chrysosporium*	*Aspergillus sydowii*

Table 7: Fungal identification of colonies that are grown on PDA of Plate 1 inoculated with pyrexia infected bovine blood sample (0.5ml of bovine blood sample).

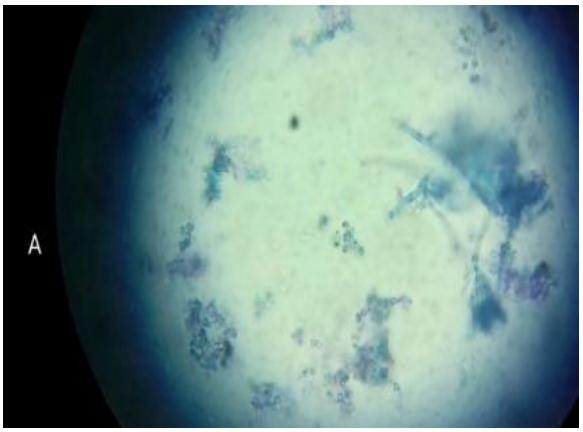


Fig 15A

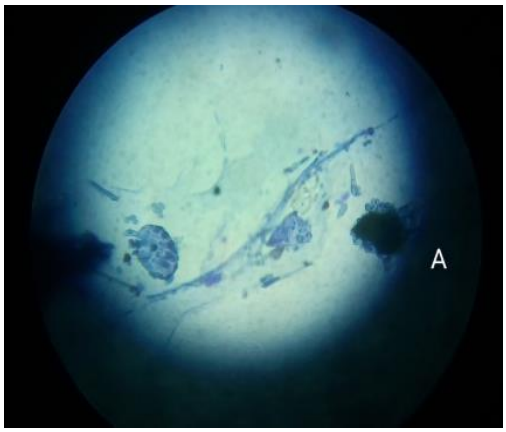


Fig 15B

Fig 15A & 15B: Microscopic view of Colony A of sample 2 on plate 1, Pyrexia infected bovine blood sample under 45X – *Aspergillus nidulans.*

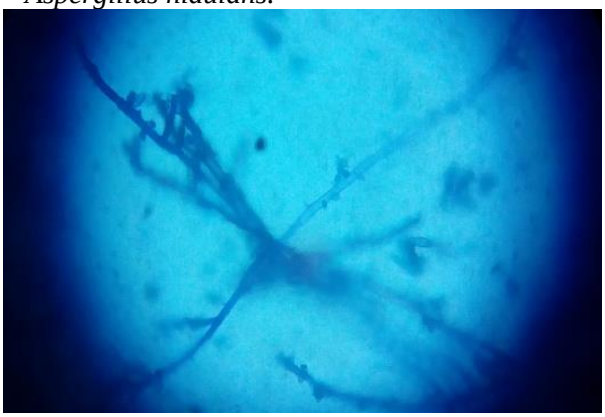


Fig 16

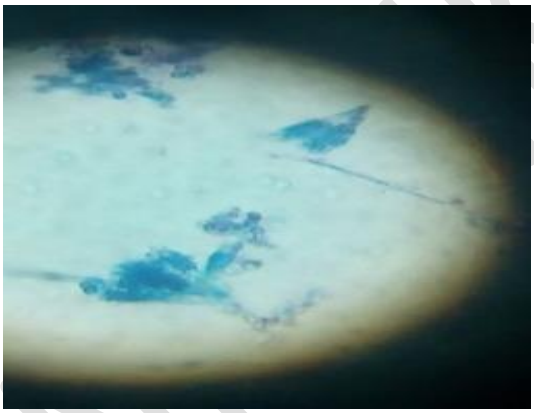


Fig 17

Fig 16: Microscopic view of Colony B of sample 2 on plate 1, Pyrexia infected bovine blood sample under 45X – *Chyrsosporium.*

Fig 17: Microscopic view of Colony C of sample 2 on plate 1, Pyrexia infected bovine blood sample under 45X – *Aspergillus sydowii*

Plate 2 - Pyrexia infected bovine blood sample (0.5mlof 10^{-1} serially diluted bovine blood sample).

Colony No	**A**
Fungal Identification	Cladosporium

Table 8: Fungal identification of colonies that are grown on PDA of Plate 2 inoculated with pyrexia infected bovine blood sample (0.5ml of 10^{-1} serially diluted bovine blood sample).

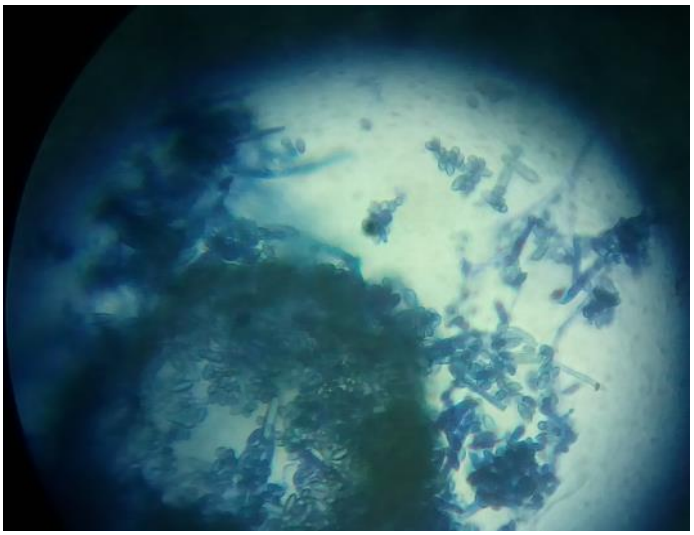


Fig 18

Fig 18: Microscopic view of Colony A of sample 2 on plate 2, Pyrexia infected bovine blood sample under 45X – *Cladosporium*

Discussion:

Mycoses including aspergillosis, candidiasis and zygomycosis in animals are usually sporadic infections and cause non-specific syndromes because of variation in the organs in which they localize. Peracute, septicemic infections are very rare in adults, except in the occasional case in a parturient female, but are not infrequent in newborn calves and foals.

Aspergillus and Candida spp., and the *Zygomyces Absidia corymbifera*, *Rhizopus oryzae*, *Rhizomucor* (Mucor) pusillus and Mortierella wolfii are the common infections which cause systemic mycotic disease. Rare infections occur with Scopulariopsis spp. The infections most commonly recorded in bovine mycotic abortion are Mucor, Aspergillus spp., Petriellidium (Allescheria) boydii, Candida parapsilosis and Mortierella wolfii.

Yeasts of the genus Malassezia inhabit the skin of a variety of mammals and birds and are considered as opportunistic pathogens in animals and man.3 In a survey of a sample of domestic animals in Spain, Malassezia spp. were isolated from 60% of horses, 28% of sheep, 44% of goats, and 58% of cows. The species isolated included: M. sympodialis, M. globosa and M. restricta from sheep. M. pachydermatis, M. fujur, M. sympodialis, M. obtusa, M. globosa, and M. restricta from goats and M. furfur, Epizootic lymphan gitis (pseudoglanders, equine blastomycosis, equine histoplasmosis) 1478 Sporotrichosis 1 479 Equine phycomycosis (swamp cancer, pithyosis, hyphomycosis destruens, Florida horse leech, bu rsattee) 1 480 Madu romycosis 1 481 Murcomycosis 1 481 M. slooffiae, M. obtusa, M. globsa and M. restricta from horses.

Many different species of fungi have been isolated from the conjunctival sac horses, including Aspergillus spp., and other molds such as Cladosporium, Mucor, Fusarium, Alternaria, and Candida Spp.4

This is distinctive in fungal diseases; they are not contagious, with the exception of maternal infections which cause the disease in their calves. Each infection arises from the fungal habitat as a saprophyte in organic matter, commonly moldy hay or straw or moist feeds such as beet pulp or brewers' grains which are allowed to go moldy. Risk factors thought to increase the prevalence of oral-gastric mycosis in young pigs and calves are continued and heavy oral dosing with antibiotics and feeding on poorly formulated or administered artificial diets.

Candida glabrata may have been associated with fungal gastritis and ulcerative colitis in a foal treated with rifampin and spiramycin for rhodococcal bronchopneumonia. An outbreak of neurological disease in dairy cattle associated with consumption of beer residues contaminated with Aspergillus cZavatus has been described in Brazil. Beer residues from malting and brewing factories are widely used for dairy cattle feeding in southern Brazil. The affected farm had been feeding beer residues successfully for 15 years.

Fecal samples and intestinal contents of preweaned dairy calves contain yeasts, among which Candida glabrata is most common and may be associated with some cases of diarrhea. Feeding calves their dam's milk reduced the shedding of the yeasts compared to commercial milk replacer. The incidence of mycotic abortions is much greater (up to 30% of all abortions in the herd) in the winter months in housed cows than in any other group, probably because they are exposed to an environment which is likely to be heavily contaminated with spores from moldy hay and ensilage. A correlation also occurs between the abortion rate and a high rainfall in the haymaking season prior to conception. The increasing incidence of mycotic placentitis leading to abortion may be related to the general use of antibiotictreated semen in artificial insemination programs but the incidence is no higher in artificially bred cows than in those mated naturally. It is more likely that a transient systemic infection is followed by localization in the pregnant uterus causing placentitis and abortion. In humans there is an increasing prevalence of systemic mycoses because of the increasing frequency of immunodeficiency states, but prolonged and intensive antibiotic therapy is a commoner precursor in farm animals, especially in aspergillosis.

Microbiological investigations of the guttural pouch of horses affected with mycotic infections have frequently demonstrated the presence of several different Aspergillus Spp 9 Candida krusei has been isolated from a case of bronchopneumonia in one-year old heifer which was incurable and had to euthanized.1o Fungal keratitis (keratomycosis) in horses has been regarded as rare but now account for about 30% of horses diagnosed with keratitis.4 In the northern , hemisphere the disease is more common . in the summer and autumn, possibly because climatic and environmental factors favor the proliferation of fungal spores.

Human zygomycosis has been on the increase, and this trend is expected to continue due to increases in the number of immunocompromised hosts The literature on the animal models of Zygomycosis- I Absidia, Rhizopus, Rhizomucor, and Cunninghamclla has been reviewed.ll Zygomycetes are filamentous fungi which are natural inhabitants of soil and fairly common in the environment. They comprise Mucorales and Entomophthorales. Muconl1ycosis due to Absidia corymbifera has been described occurring in ponies and 4 of 15 animals died within a few days of onset.13

Although inhalation of dust containing fungal elements is an obvious portal of entry for the fungi, with a primary focus developing in a lung, it is generally believed that the more common portal is in the alimentary tract, where the mycosis establishes in a pre-existing abomasal or gastric ulcer or is established in the normal lining of the forestomachs, abomasums and intestines. Hematogenous spread from these foci occurs to all organs, especially splanchnic lymph nodes, liver, lungs and the placenta in pregnant females. Only the placenta is invaded and subsequent fertility is not impaired. Infection of the placenta and uterus can be established by intravenous injection during pregnancy but not by of the injected animals develop placentitis and granulomas in the liver and lungs, and this proportion can be increased by increasing the dose of spores injected. In keratomycosis in horses, traumatic injury to the cornea by plant material, the prolonged use of topical antibiotics may cause a shift in the normal conjunctival flora from Gram-positive to Gramnegative organisms, and the use of topical corticosteroids can all contribute to fungal growth 4 In temperate climates keratomycosis is normally a chronic disease associated with ocular trauma. Small corneal ulcers may heal, trapping the organisms deep within the stroma, and result in the development of a stromal abscess. Large stromal ulcers may fail to heal and be slowly progressive, and the corneal stroma may melt. Various categories of stromal ulcers occur including: superficial fungal keratitis (punctuate lesions of the epithelium and subepithelium stroma); keratomycosis with a surrounding furrow; keratomycosis with a 'cake frosting' appearance; and stromal abscess.

Neurological disease in cattle associated with the consumption of beer residues contaminated with Aspergillus clavatus is due to the neuromycotoxicosis effect of the mycotoxins of the fungi

APPENDIX A:

Pictorial representation

Screening of Infected Bovine Blood Sample for Fungi

1. Sample Collection

Fig 1: Sample Collection of Bovine

2. Serial dilution and Inoculation of the infected Bovine blood sample

Fig 2: Preparation of Media

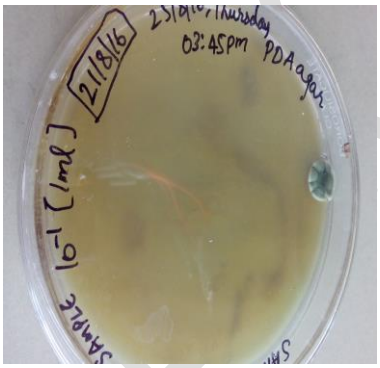
Fig 3: Inoculation of infected Bovine Blood sample

Fig 4: Serial Dilution of infected Bovine Blood Sample

3. Pour plate and Spread plate method of the serially diluted blood sample that has kept for incubation.

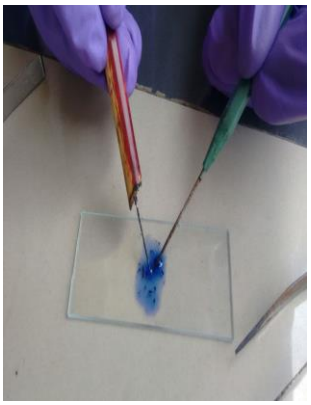

4. PDA plates after weeks of incubation at 25^0 C.



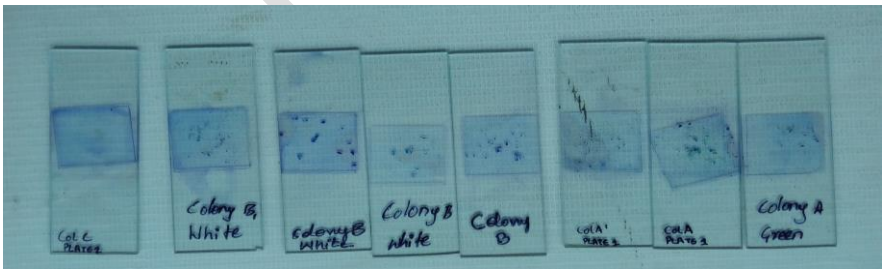


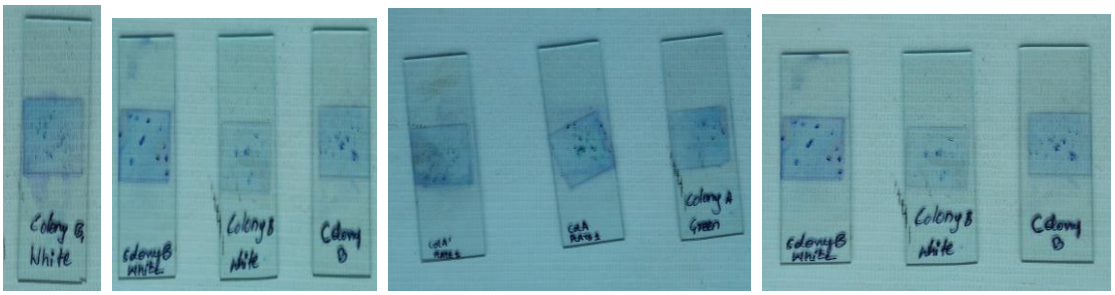
5. Tease Mount technique of staining fungal culture for identification





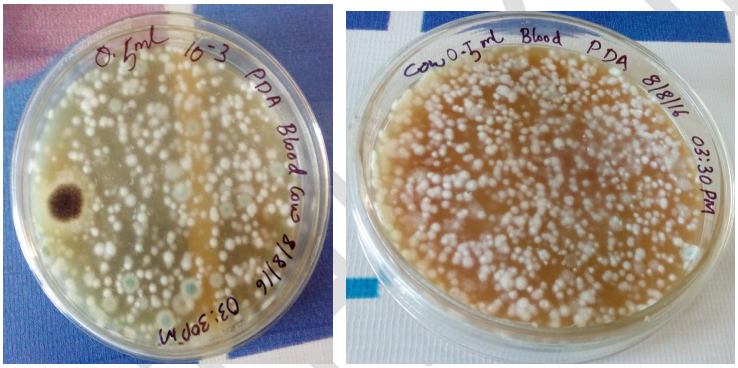
6. Prepared tease mount slides of fungal colony for identification under microscope





Identification of fungal culture under microscope:

Sample 1: Mastitis infected cow blood sample culture on PDA



Sample 2: Pyrexia infected Cow Blood sample culture on PDA

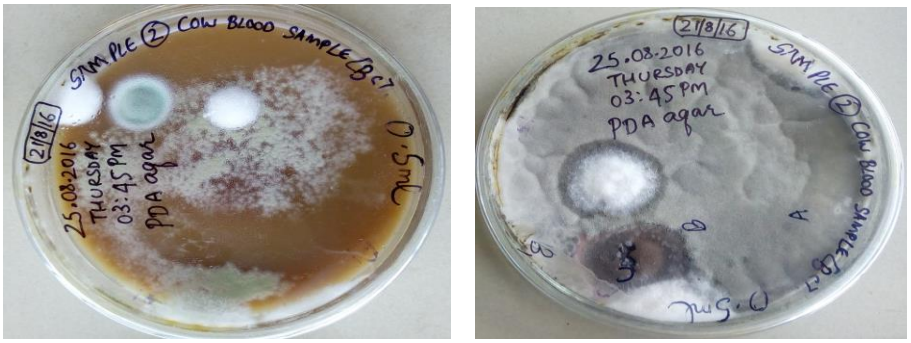
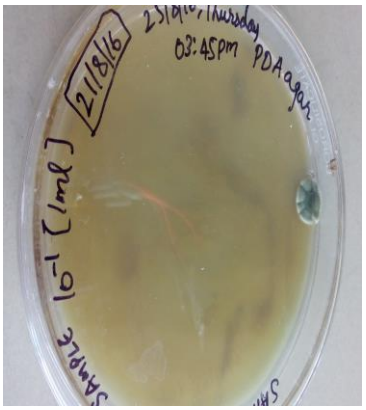


Plate 1 of Cow Blood sample on PDA after 1 week and 2 weeks of incubation



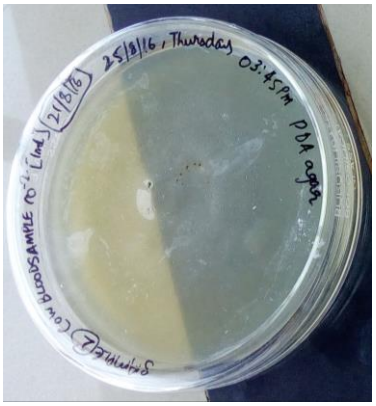


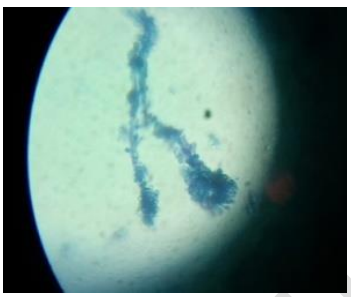
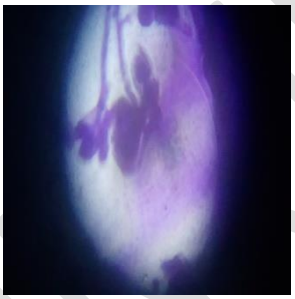
Plate 2 and 3 of Cow Blood sample after 2 weeks of incubation.

Microscopic Observation of fungal colonies under 45x

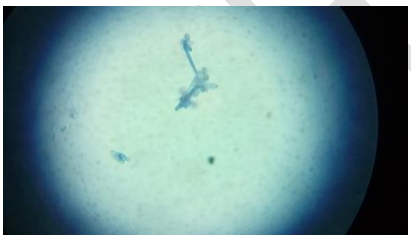
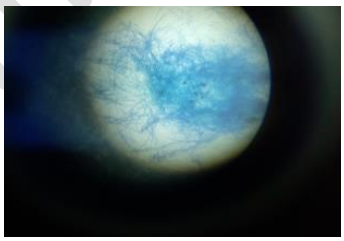
SAMPLE 1 – Mastitis infected blood sample of Bovine

Plate 1 - 10-3 (Cow Blood Sample 0.5ml)

Colony No.	**A**	**B**	**C**	**D**	**E**
Fungal Identification	***Aspergillus niger***	***Gliocladium***	***Trichoderma***	*Fusarium oxysporum*	***Trichophyton tonsurans***

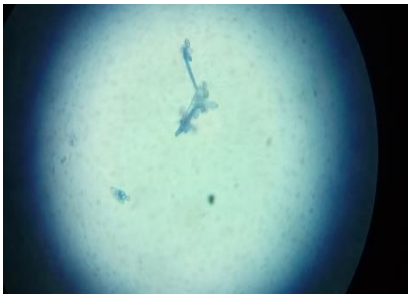
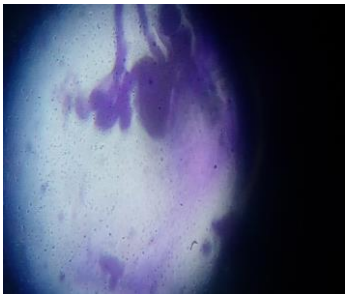
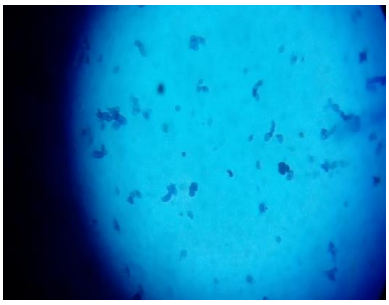

Colony A, B, C

Colony D & E

Plate 2 (Cow Blood Sample 0.5ml)

Colony No.	**A**	**B**	**C**
Fungal Identification	**Fusarium oxysporum**	**Gliocladium**	Candida albicans

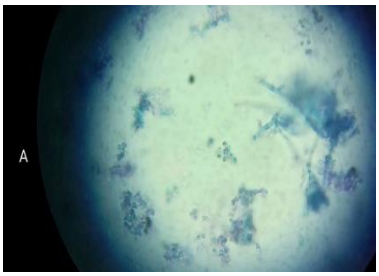




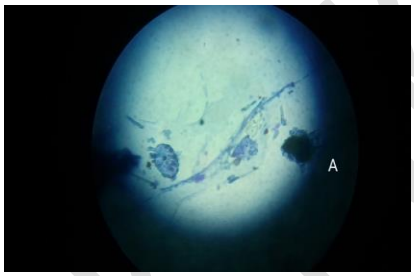
Colony A, B & C

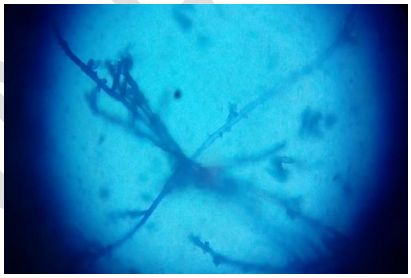
Sample 2: Pyrexia infected blood sample of Bovine:

Plate 1 - 0.5 ml of Cow Blood Sample

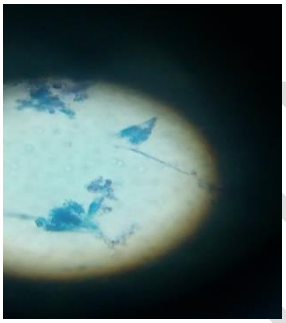
Colony No.	**A**	**B**	**C**
Fungal Indentification	*Aspergillus nidulans*	*Chrysosporium*	*Aspergillus sydowii*







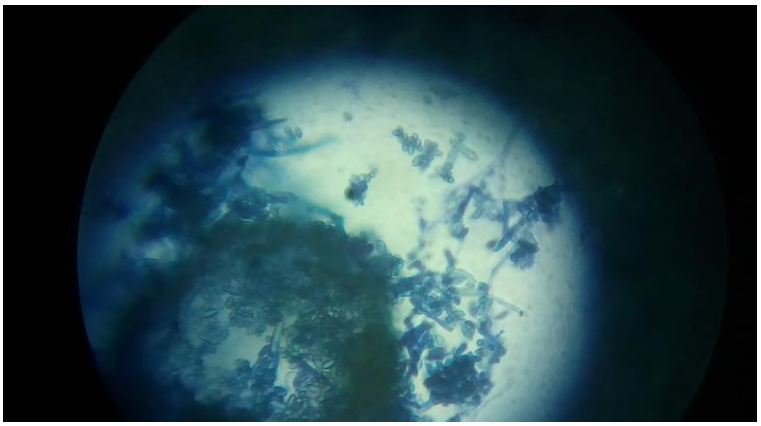
Colony A & B



Colony C

Plate 2 - 10^{-1} cow blood sample (0.5ml)

Colony No	**A**
Fungal Identification	Cladosporium



Colony A

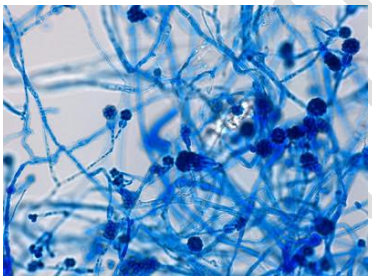
APPENDIX B:

1. *Aspergillus niger*

Domain: Eukaryota
Kingdom: Fungi
Phylum: Ascomycota
Subphylum: Pezizomycotina
Class: Eurotiomycetes
Order: Eurotiales
Family: Trichocomaceae
Genus: Aspergillus
Species: A. niger

Aspergillus niger and several *Aspergillus* spp causes **Aspergillosis**. Aspergillus infection and it is found worldwide and in almost all domestic animals. It is primarily a respiratory infection that may become generalized; however, tissue predilection varies among species. The most common forms are pulmonary infections in poultry and other birds; mycotic abortion in cattle.

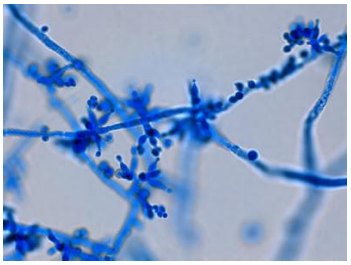
2. Gliocladium



Kingdom: Fungi
Division: Ascomycota
Subdivision: Pezizomycotina
Class: Sordariomycetes
Order: Hypocreales
Family: Hypocreaceae
Genus: Gliocladium

Species of Gliocladium are considered to have pathogenic potential although they are not commonly thought of as a disease causing agent in humans and animals. Gliocladium species occur worldwide in soil and decaying organic matter. Some species of Gliocladium are parasitic on other fungi. Gliocladium is found world-wide. Gliocladium is classified as a RG-1 organism; it is assessed to have low to no individual or community risk. Also, this microorganism is unlikely to cause human or animal disease.

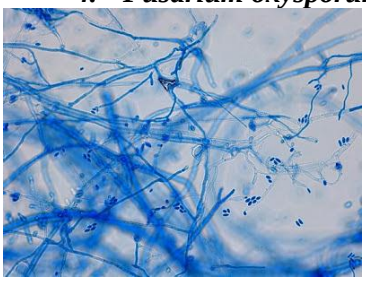
3. *Trichoderma*



Kingdom: Fungi
Division: Ascomycota
Subdivision: Pezizomycotina
Class: Sordariomycetes
Order: Hypocreales
Family: Hypocreaceae
Genus: Trichoderma

Trichoderma is a genus of fungi that is present in all soils, where they are the most prevalent culturable fungi. Many species in this genus can be characterized as opportunistic avirulent plant symbionts

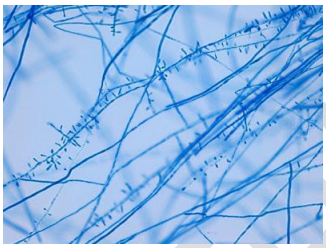
4. *Fusarium oxysporum*



Kingdom: Fungi
Division: Ascomycota
Class: Sordariomycetes
Order: Hypocreales
Family: Nectriaceae
Genus: Fusarium
Species: F. oxysporum

Fusarium species are frequent agents of mycotic eye infections, particularly the cornea (keratomycosis, endopthalmitis). They have also been implicated in onychomycosis (nail infections), catheter infections, peritonitis, sinusitis and septic arthritis. As with many other fungi immunocompromised and neutropenic patients may be at greater risk. Fusarium may contaminate stored grain where some species can produce potent mycotoxins. Food prepared from these contaminated grains may cause illness on ingestion. Fusarium species may also be found as laboratory contaminants but must not be dismissed outright without further investigation.

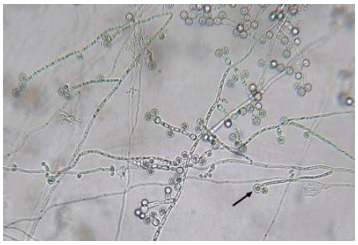
5. *Trichophyton tonsurans*



Kingdom: Fungi
Division: Ascomycota
Class: Eurotiomycetes
Order: Onygenales
Family: Arthrodermataceae
Genus: Trichophyton
Species: T. tonsurans

Trichophyton tonsurans is a cosmopolitan (found worldwide) dermatophyte (a fungus of skin, nails or hair). It is the etiologic agent most frequently implicated in 'ringworm' infections of the scalp (Tinea capitis) in America. Causes an endothrix infection of the hair (penetrates hair shaft to grow within). *T. tonsurans* is anthropophilic (prefers humans to animals) however sources vary on its infectivity. One prominent source suggests *T. tonsurans* is Zoophilic, but is frequently transmitted to man. Others have speculated that equine strains may have mutated to become anthropophilic. Infections are more commonly found in heavily populated urban regions.

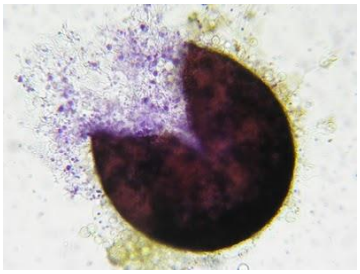
6. *Candida albicans*



Kingdom: Fungi
Division: Ascomycota
Class: Saccharomycetes
Order: Saccharomycetales
Family: Saccharomycetaceae
Genus: Candida
Species: C. albicans

Candidiasis is a localized mucocutaneous disease caused by species of the yeast-like fungus Candida, most commonly C albicans. It is distributed worldwide in a variety of animals. C albicans is a normal inhabitant of the nasopharynx, GI tract, and external genitalia of many species of animals and is opportunistic in causing disease. Factors associated with candidal infections are disruption of mucosal integrity; indwelling, intravenous, or urinary catheters; administration of antibiotics; and immunosuppressive drugs or diseases. The organism most frequently infects birds (see Candidiasis), in which it involves the oral mucosa, esophagus, and crop. Superficial infections limited to the mucous membranes of the intestinal tract have been described in pigs and foals. Systemic candidiasis has also been described in cattle, calves, sheep, and foals secondary to prolonged antibiotic or corticosteroid therapy. However, Candida spp have been considered a cause of arthritis in horses and mastitis and abortion in cattle

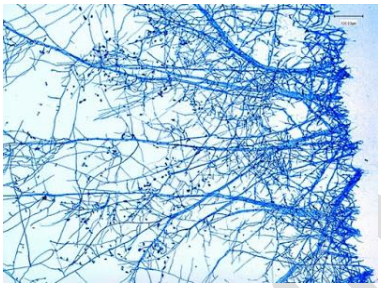
7. Aspergillus nidulans



Domain: Eukarya
Kingdom: Fungi
Phylum: Ascomycota
Class: Eurotiomycetes
Order: Eurotiales
Family: Trichocomaceae
Genus: Aspergillus
Species: A. nidulans

Aspergillus nidulans is one of many species of filamentous fungi in the phylum Ascomycota. It is one of the few species in its genus able to form sexual spores through meiosis, allowing crossing of strains in the laboratory. A. nidulans is a homothallic fungus, meaning it is able to self-fertilize and form fruiting bodies in the absence of a mating partner. Aspergillosis is also caused by several *Aspergillus* spp, especially *A fumigatus* and *A terreus*. *A niger*, *A nidulans*, *A viridinutans*, *A flavus*, and *A felis* are being recognized

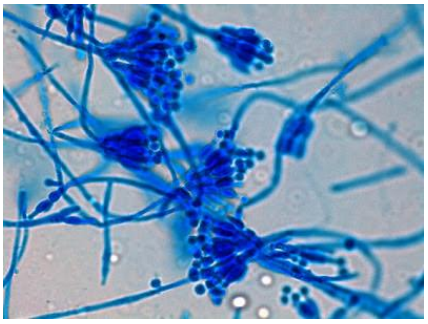
8. Chyrsosporium



Kingdom: Fungi
Phylum: Ascomycota
Class: Eurotiomycetes
Order: Onygenales
Family: Onygenaceae
Genus: Chrysosporium

Chrysosporium is a type of hyaline hyphomycetes fungi in the family Onygenaceae. Chrysosporium colonies are moderately fast-growing, flat, white to tan to beige in color; they often have a powdery or granular surface texture. Hyaline, one-celled (ameroconidia) are produced directly on vegetative hyphae by non-specialized conidiogenous cells. Species of Chrysosporium are occasionally isolated from skin and nail scrapings, especially from feet, but, because they are common soil saprotrophs, they are usually considered as contaminants. Chrysosporium has been identified as an emerging infectious disease. There are about 22 species of Chrysosporium, several are keratinophilic with some also being thermotolerant, and cultures may closely resemble some dermatophytes, especially *Trichophyton mentagrophytes*, and some strains may also resemble cultures of Histoplasma and Blastomyces.

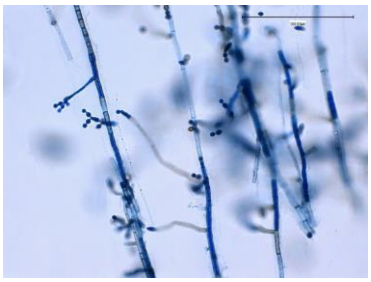
9. Aspergillus sydowii



Kingdom: Fungi
Division: Ascomycota
Class: Eurotiomycetes
Order: Eurotiales
Family: Trichocomaceae
Genus: Aspergillus
Species: A. sydowii

Aspergillus sydowii is a pathogenic fungus that causes several diseases. It has been implicated in the death of sea fan corals (Gorgonia spp.) in the Caribbean Sea. Aspergillus sydowii has been implicated in the pathogenesis of several human diseases, including aspergillosis, onychomycosis, and keratomycosis.

10. Cladosporium



Kingdom: Fungi
Division: Ascomycota
Class: Dothideomycetes
Order: Capnodiales
Family: Davidiellaceae
Genus: Cladosporium

Cladosporium species are rarely pathogenic to humans, but have been reported to cause infections of the skin and toenails as well as sinuses and lungs. The airborne spores of Cladosporium species are significant allergens, and in large amounts they can severely affect asthmatics and people with respiratory diseases. Cladosporium species produce no major mycotoxins of concern, but do produce volatile organic compounds (VOCs) associated with odours.

Annexure:

Media:

1. Potato Dextrose Agar [PDA]

   Composition:

Potato extract-	1000ml
Dextrose -	20 g
Agar -	20 g
pH -	6.9

2. Lactophenol Cotton Blue

   Lactophenol Cotton Blue is used as a staining solution for fungi.

Phenol crystals	20.0gm
Cotton blue	0.050gm
Lactic acid	20.0ml
Glycerol	20.0ml
Distilled water	20.0ml

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