

## VETERINARY MYCOLOGY

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### Summary

Animals are hosts to numerous fungal pathogens most of which can also infect humans. In some cases, these diseases can be transmitted from animals to humans or *vice versa*. Many wild, agricultural, and domestic animals constitute reservoirs of human fungal pathogens. There has been synergy in technological developments between the human and animal systems. Understanding of animal diseases has benefited from basic scientific and therapeutic understanding of human diseases whereas understanding of human diseases has benefited from use of animal models for various experimentations.

Animals harbor some discrete ecological groups of fungi. Most of them are widely distributed environmental facultative parasites. In contrast to these fungi, *Malassezia*

spp., *Piedraia* spp., and *Pneumocystis jiroveci* are obligate pathogens. Some facultative parasites are endemic in limited areas. These fungi include some of the more virulent fungal pathogens and can infect immune-compromised and even immune-competent subjects. Most widely distributed environmental fungi are opportunistic pathogens on debilitated or immune-suppressed subjects. Many fungi produce readily dispersed conidia which can initiate infection. For pulmonary infection, the conidia have to be small-enough to be carried by air currents and deposited deep in the alveoli. A small number of fungi are commensal on the skin surface and some undergo morphogenetic switching in order to initiate infection. Thermally induced dimorphic transition from the saprobic mold to pathogenic yeast phase is important for pathogenicity and virulence in some fungi.

Fungal pathogens of animals range from a unicellular chytrid to numerous unicellular yeast and multicellular filamentous types. They belong to many fungal taxa and exhibit a diversity of morphologies, and asexual and sexual reproduction types. A range of technologies have been developed for diagnosing fungal infections. They generally include symptomatic observations at the veterinary clinic, culturing of the pathogen, cytologic and histological evaluations, and serologic and DNA-based testing.

## 1. Introduction

Fungi serve essential roles in the global ecosystem. Their activities degrade organic matter, recycle nutrients, create soils, and promote plant growth. Without their saprobic activities, humans will drown in a deep layer of leaf litter. They parasitize humans, animals, and plants, and regulate biodiversity. They serve as food for humans and animals, and produce fermentation products for humans. Fungi have versatile biochemistry and produce myriads of chemical compounds. Many of these biochemicals are used by humans as medicines and other industrial chemicals.

Fungal diseases affect the health and consequently the survival of wild and domesticated animals. In this respect there is a close relationship between humans and animals since they suffer from many of the same fungal pathogens. The zoonotic diseases are able to be transmitted from wild, agricultural, and domestic animals to humans either directly or through vectors. The opposite transmission is called reverse zoonosis. Sporotrichosis caused by *Sporothrix schenckii* and ringworm caused by *Microsporum* spp. and *Trichophyton* spp. are well-known examples of zoonoses. Many wild and domesticated animals (including their feces and soil in their burrows) harbor reservoirs of fungi pathogenic on humans. Some examples of such fungi are *Blastomyces dermatitidis*, *Candida* spp., *Coccidioides immitis*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Malassezia* spp., and *Paracoccidioides brasiliensis*. Close relationships exist among different components in some disease syndromes. For example, in South America, outbreaks of coccidioidomycosis have taken place in armadillo hunters and in hunters' dogs. The causal agent *C. immitis* has also been isolated from armadillos and from its burrow soil. Several factors are contributing to evolution of emerging zoonotic diseases. Pre-existing diseases are being dispersed through rapid global transportation of animals and humans. Animal trade is also contributing to dispersal of diseases. Contacts between humans and animals are increasing. Numbers of immune-suppressed subjects predisposed to fungal infection are

also increasing. Additionally, more and more fungal diseases are being recognized through recent advances in diagnostic technologies.

Some novel diseases have been recognized in wild animals in the recent past. These emerging infectious diseases include the frog chytridiomycosis, coral fungus, and crayfish plague caused by *Batrachochytrium dendrobatidis*, *Aspergillus sydowii*, and *Aphanomyces astaci*, respectively. *Fusarium* sp. is also emerging as an important pathogen of captive marine fish, especially elasmobranchs. Out of the above mentioned fungi, *A. astaci* is not regarded as a fungus as it is now placed under Oomycetes. At least one of these pathogens has significantly affected animal biodiversity. *Batrachochytrium dendrobatidis* was first described in 1998 and causes chytridiomycosis in more than 90 species of amphibians worldwide. While the principal drivers of this phenomenon remain unclear, this chytrid has caused decline and extinction of numerous amphibians underscoring the ecological significance of animal mycoses. It is strongly believed that international trade is contributing to introduction of this pathogen in new areas. *Batrachochytrium dendrobatidis* is an aquatic fungus that infects the keratinized stratum corneum of adult amphibians producing sporangia with zoospores in them (Figure 1). The infection causes hyperkeratosis and sloughing of the epidermis.



Figure 1. Section of the ventral skin of upper hind limb of *Atelopus varius* showing sporangia of *Batrachochytrium* sp. with zoospores. Arrowheads point to discharge tubes with exiting zoospores [A Centers for Disease Control and Prevention image in Public Domain copied from Daszak P. *et al.* (1991)].

Fungi causing diseases in animals are broadly classified into two groups. Endemic fungi are restricted geographically and can potentially cause serious infections in immune-competent animals. These pathogens are highly virulent and are called true or primary pathogens. Immune-competent animals exposed to such fungi generally fight-off infection easily and are asymptomatic. However, if exposed to a large enough inoculum, an animal with an intact immune system may develop chronic infection requiring treatment. An immune-compromised animal upon infection may easily develop life-threatening systemic and progressive malady. For causing deep mycoses, conidia of the mold phase of these pathogens usually gain access into the host via the respiratory tract. This group of pathogens consists of *Coccidioides immitis*, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Paracoccidioides brasiliensis*. These fungi are thermally

dimorphic and have a hyphal saprobic form in the environment at temperatures of 25-30° C and a yeast-like (or spherule phase in the case of coccidioidomycosis) parasitic form inside the host at body temperatures. Sporotrichosis resembles the aforesaid mycoses but differs in being worldwide in distribution and in direct lymphocutaneous inoculation of the pathogen conidia leading to infection.

Opportunistic fungi (also called secondary pathogens) are widely distributed in the environment or live as body commensals and cause diseases in hosts with compromised defenses or in animals receiving prolonged immune-suppressive or antibacterial therapy. They are not true pathogens. Other predisposing conditions include long-term use of indwelling devices or intravenous catheters, and extreme age groups. For causing deep mycoses, these pathogens principally gain access into the host via the respiratory tract, alimentary tract, or intravascular devices. Traumatic inoculation can also create a portal of entry. This is a heterogeneous group and consists of numerous fungi such as *Candida*, *Aspergillus*, *Cryptococcus*, many zygomycetes, and *Fusarium* spp.

A more detailed classification emerges when factors such as location of infection relative to skin surface, body parts infected, localized or systemic infection, and causal fungi involved are considered collectively. This classification system includes Superficial Mycoses [examples: pityriasis versicolor and superficial dermatophytoses of hair (ectothrix and endothrix)], Cutaneous Mycoses [examples: dermatophytoses of skin (ringworm, favus, and onychomycosis) and non-dermatophyte cutaneous infections], Subcutaneous Mycoses (examples: sporotrichosis, primary subcutaneous blastomycosis, and zygomycotic rhinitis), and Deep Mycoses [examples: systemic mycosis (blastomycosis, paracoccidioidomycosis, coccidioidomycosis, histoplasmosis, penicilliosis, aspergillosis, cryptococcosis, systemic candidiasis, and zygomycosis)].

## **2. Characteristics and Classification of Fungi**

Fungi are eukaryotic, heterotrophic, unicellular (chytrids or yeast form) or multicellular tubular (hyphal or mold form), rigid cell-walled, and spore-producing organisms. As eukaryotes, fungi contain membrane-bound nuclei and organelles such as mitochondria (with plate-like cristae like in animals), Golgi apparatus, endoplasmic reticulum, ribosomes, microbodies, lysosomes, lipid bodies etc. Fungal cell membrane has the sterol, ergosterol. Biosynthesis of this integral membrane component is inhibited by the azole antifungals. Fungi are insensitive to antibacterial antibiotics. Fungi lack chlorophyll. Hence, they are heterotrophic organisms (dependent on absorption of organic carbon compounds from their habitat for their nutrition) that are saprobes (living on dead organic matter) and/or parasites (utilizing living tissue). They secrete enzymes into the substratum and absorb the digested compounds through their cell walls resulting in extracellular digestion and absorptive nutrition. The cell walls of fungi contain chitin, chitosan, glucan, mannan and some other components. The antifungal compounds, polyoxins and echinocandins, inhibit the biosynthesis of chitin and glucan, respectively. Like animals, in fungi also glycogen is the storage polysaccharide material.

Fungi occur in two basic forms [tubular (filamentous) or yeast forms] responsible for secretion and absorption of materials, and production of asexual and sexual propagating

structures. Fungal filaments are known as hyphae collectively making up the mycelium. The hyphae are branched, colorless or brownish in color, and either mostly aseptate (Zygomycota) or septate (Ascomycota and Basidiomycota). The septa divide the hypha into one-, two-, or multi-nucleate compartments or cells which remain connected through pores in the septa. The aseptate hyphae are coenocytic (one-celled and multi-nucleate). Cytoplasm and cellular organelles can stream through the pores which are simple in Ascomycota and dolipore in many Basidiomycota. These pore morphologies are diagnostic for the two groups. The hyphal growth is apical and is mediated through a Spitzenkörper. Branching patterns and diameters of hyphae are also often diagnostic. The yeast form is unicellular, reproduces by budding, and its growth is mediated by a polarisome. The pattern of budding is often diagnostic.

Spore is a minute propagating, dispersal, and survival unit of fungi without an embryo that serves in the production of new individuals. Spores are produced by asexual (mitospores via mitosis) and sexual (meiospores via meiosis) means. Sexually and asexually reproducing thalli are known as teleomorphs and anamorphs, respectively. The total organism is referred to as a holomorph. Teleomorphs and anamorphs may have their own separate binomial names. Conidium (plu. conidia) is an asexual spore produced on a specialized hypha called conidiophore.

Many systems of classification are in use. They are based on morphological, biochemical, and molecular traits of disease-causing fungi and are being continually refined as more and more phylogenetic criteria are recognized. The binomial names and taxonomic hierarchies provide useful information on disease diagnostics. Also, they provide information on biology and ecology of the disease-causing fungi, such as morphology, modes of dispersal/infection, survival of infectious propagules, potential of evolution of new races/drug resistance, and toxicological/biochemical characteristics. The veterinary and medical personnel often under utilize/realize the significance of taxonomy in diagnosis/management of mycoses. Main taxa of one of the current systems of classification for veterinary and medical pathogenic fungi are given below. Details of further taxa of this system and other systems can be found in references given in the bibliography.

Kingdom: Eumycota Division: Chytridiomycota – Thallus is often unicellular. Gametes and asexual spores (zoospores) are motile and each has a single posteriorly-directed flagellum. Example: Fungus/Disease – *Batrachomyces dendrobatidis*/Chytridiomycosis.

Division: Zygomycota - Thallus is hyphal, coenocytic, generally aseptate, and usually 6.0-30.0 µm wide. Width and branching of the hyphae are irregular. Sporangia produce asexual non-motile sporangiospores. Fusion of gametangia leads to the formation of sexual spores, the zygospores. Examples: Fungi/Disease -- *Absidia* spp., *Mortierella* spp., *Mucor* spp., *Rhizomucor* spp., *Rhizopus* spp., and *Saksenaea vasiformis*/Zygomycosis; *Basidiobolus* spp. and *Conidiobolus* spp./Entomophthoromycosis.

Division: Ascomycota - Thallus is hyphal or yeast-like. The hyphae are usually 3.0-8.0 µm wide, septate, with septa having simple pores. Asexual and sexual fruiting bodies

producing non-motile spores are present. Many kinds of asexual spores are produced. Following karyogamy, sexual spores called ascospores are produced endogenously in asci. Examples: Fungus/Disease – Various teleomorphic and anamorphic stages of *Candida* spp./Candidiasis; *Arthroderma* spp. (teleomorphs of anamorphic *Microsporium* spp. and *Trichophyton* spp.)/Dermatophytosis; *Ajellomyces dermatitidis* (teleomorph of anamorphic *Blastomyces dermatitidis*)/Blastomycosis; *Coccidioides immitis* (Hyphomycetes)/Coccidioidomycosis; *Ajellomyces capsulatus* (teleomorph of anamorphic *Histoplasma capsulatum*)/Histoplasmosis; *Paracoccidioides brasiliensis* (Hyphomycetes)/Paracoccidioidomycosis; *Emericella* spp. and *Eurotium* spp. (teleomorphs of anamorphic *Aspergillus* spp.)/Aspergillosis; *Penicillium marneffeii* (Hyphomycetes)/Penicilliosis; *Ophiostoma* spp. (teleomorph of anamorphic *Sporothrix schenckii*)/Sporotrichosis.

Division: Basidiomycota - Thallus is hyphal or yeast-like. The hyphae are septate and often have clamp connections. The septal pore canal (dolipore) is often swollen. Following karyogamy, sexual spores called basidiospores are produced exogenously on basidia. Examples: *Filobasidiella* spp. (teleomorph of anamorphic *Cryptococcus* spp.)/Cryptococcosis; *Malassezia* spp. (anamorphic)/Dermatitis and Otis Externa.

Anamorphic fungi – Teleomorphic stages of many asexual fungi are not known. Most such fungi are thought to belong to Ascomycota while a small number may belong to Basidiomycota. The former have 2-layered while the latter have multilamellar cell walls. In many such fungi conidia are produced on more or less differentiated hyphal branches (conidiophores) and asexual fruiting bodies are absent. Such fungi are grouped together under Hyphomycetes.

Discussions in this article will focus mostly on morphology/systematics, epidemiology, types of mycoses, and diagnostics of important animal disease-causing fungi. A group of filamentous microbes (Kingdom Chromista, Division Oomycota) causing pythiosis and lagenidiosis were formerly regarded as fungi but are now excluded from the Kingdom Eumycota. Hence, information on these oomycetous pathogens is not included in this article.

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### Biographical Sketch

**Jalpa P. Tewari** was born at Unnao, U.P., India on December 31, 1937 and received his Bachelor of Science in Science, Master of Science in Botany, and Doctor of Philosophy in Botany degrees in 1957, 1959, and 1963, respectively, all from the University of Lucknow, Lucknow, U.P., India. His Ph.D. thesis was entitled "Studies on the Microbiology of Indian Soils with Special Reference to the Soil Fungi"; Supervisor Dr. J.N. Rai.

During his professional career, he has worked in a number of positions. Some of these positions were Lecturer/Assistant Professor of Botany at the University of Lucknow for about 8 years from 1961-1970. He had a concurrent honorary appointment at the University of Lucknow from 1965-1969 as a Research Officer in a United States Public Law 480 project working on diseases of wild, vegetable, and oleiferous crucifers, and their germplasm collection. From 1970-1974, he was a post-doctoral fellow/research associate at the University of Alberta working on fungal and biomedical biological membranes systems. Again at the University of Alberta, he was a Research Associate from 1974-1978 working on the diseases of rapeseed in Alberta. From 1979-1982 he was a Visiting Faculty Service Officer/ Faculty Service office II at the University of Alberta, engaged in administration, teaching, and research on biological membranes. He was an Associate Professor and later Professor from 1982-2003 at the University of Alberta engaged in teaching, research, and extension work in Plant Pathology, and administration. He was Acting Chair, Department of Plant Science, University of Alberta in 1988. He worked as Professor Visitante, Dep. Biología Vegetal, Universidad Politécnica, Madrid, Spain from 1992 and 1993 (both partial); Visiting Professor, Dept. of Botany, University of Delhi, Delhi, India from 1992-1993; Professeur Invité, Laboratoire de Cryptogamie, Muséum National d'Histoire Naturelle, Paris, France in 1993 (partial); and Guest Professor, Labor für Biotechnologie und ökologische Phytomedizin, Universität –GH-Paderborn, Soest, Germany from 1993 and 1997 (both partial). He was appointed as a Research Professor, Department of Microbiology, Immunology, and Biochemistry, Northeastern Ohio Universities College of Medicine (NEOUCOM), Rootstown, Ohio, U.S.A. from 2005-2007 and as a Professor Emeritus, Department of Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, AB, Canada from 2003 – onwards.

He was conferred an Award for Outstanding Research by the Canadian Phytopathological Society in 2002. He has participated in two patents and has about 440 publications in Plant Pathology, Mycology, Microbial Physiology, Microbial Ecology in Soil, Botany of Oil-yielding and Wild Crucifers, Anatomy of Angiosperms and Gymnosperms, Biomedical areas, and Cell Biology of Fungi and Animals. His research has focused on plant disease diagnostics, host-parasite interactions, slow-diseasing resistance, basis of disease resistance, selection for disease resistance, including alien sources of resistance, environmentally-friendly disease management, microbiology of crop stubble, and international development research. Current research interests include medical and animal mycology (isolation and identification of fungi causing mycoses, proteomic biomarkers for diagnosing fungal diseases, virulence factors in fungal pathogens, epidemiology, drug resistance in mycotic agents, and natural plant products as novel antimycotic compounds for management of diseases, and molds and indoor air quality). Dr. Tewari is a Member of Alberta Veterinary Research Institute Council and is a member of the following professional societies: Emeritus Member, Canadian Phytopathological Society, Emeritus Member, American Phytopathological Society, Life Member, Indian Phytopathological Society, Life Member, Society for Plant Biochemistry and Biotechnology, and Emeritus Member, Mycological Society of America.