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Diseases and Immunology

Like other creatures, amphibians are integrated into their environment, interacting with each other and with the environment. As a result of internal factors such as aging or external influences such as infections, individual body cells, tissues or organs may fail or mutate; regulatory functions may collapse, tissue may die or be attacked by foreign organisms such as protozoa or bacteria that use it as a substrate. As vertebrates, amphibians are in principle exposed to the same pathogenic mechanisms as humans or domestic animals, although some peculiarities exist due to their mode of life and phylogeny.

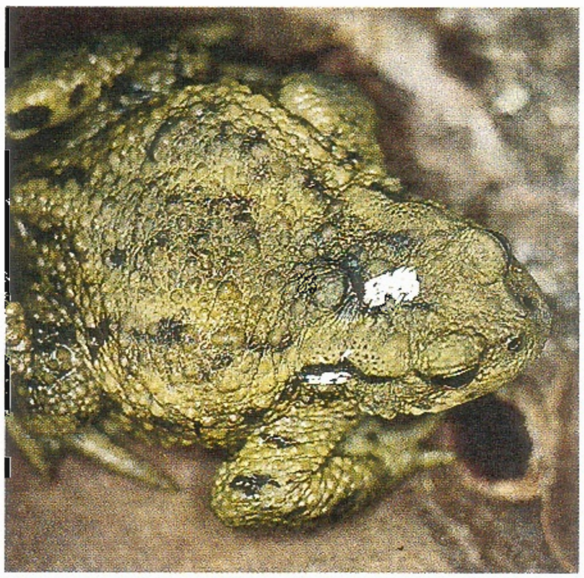
Diseases in Amphibians

Not much is known about systematic diseases, infectious diseases, immunology and disease defense in all amphibians with the exception of the clawed frog (*Xenopus laevis*), which is often used as a laboratory specimen, as few case histories have been recorded in each field. Above all, the relevance of individual factors which are expected to be pathogenic by themselves or to modulate the immune system—for example, increased UV radiation or the resorption of industrial toxins—in disease manifestation is unknown altogether. This is why it is currently impossible to predict the survival of amphibian populations or even the worldwide well-being of this class in the context of the global amphibian decline. However, precise studies in pathology and immunology in special laboratories can reveal the cause of disease or death in an individual.

Before attempting to organize amphibian diseases into logical and systematic categories, we should take a closer look at amphibians' living conditions. Veterinary studies on amphibians in their natural environment yield findings that are considerably different from those obtained from terrarium specimens. In nature creatures that are weakened by disease are the preferred prey of predators. This means that, in comparison to specimens kept in captivity, chronic infections, diseases that result from aging processes and benign tumors are less frequently diagnosed. Furthermore, the range of diagnosed infectious or viral diseases is considerably different.

In captivity the vectors necessary to the life cycle of some germs are almost always absent, but unnaturally high population densities create epidemic conditions that are specific to captivity. In nature, however, limited germ coloniza-

*ilaria-infection in a tree rog (*Hyla geographica*) rom Trinidad. Although the .5-inch (4-cm) long nematodes could be seen shifting nd moving beneath the kin, the host animal did ot seem to be handicapped.*



*Nostrils in a common toad (*Bufo bufo*) ravaged by Lucilia bufonivora larvae.*

Left: Freshly laid Lucilia *eggs on the head of a common toad. The maggots hatch after two to three days and penetrate the nostrils of the toad. Adult toad are most frequently affectec by this infestation.*

tion is normal and sometimes even conducive to the health of the host. This relates to a broad spectrum of usually hardy or non-pathogenic germs that are slowly propagating; among them are many opportunists. Only when considerable stress occurs—for example, during an increase of toxin exposition or constant uneasiness with the connected immunosuppression—do these germs multiply in an uncontrollable way and begin to develop pathogenic characteristics.

The colonization of free-living amphibians by opportunistic germs is clinically barely noticeable and it is thus rarely documented; nevertheless, it is the main factor for a lasting regulation of population density. In captivity, however, we see a preponderance of infections caused by a small number of usually highly pathogenic species of germs that multiply rapidly and have a tendency to autoinfection. Diseases in amphibians can be classified into the following groups:

Cancer (Tumors): Benign tumors are difficult to recognize because there is an even transition to encystation of foreign tissue. Malign tumors in amphibians are almost invariably epithelial in origin. They may be striking because they can reach unbelievable dimensions. The transplantable, renal adenocancer of *Rana pipiens* (Lucké tumor) is a long-known and relatively well-researched cancer whose herpes virus-induced genesis is, however, still in question.

Metabolic Diseases and Regulatory Malfunctions: This category includes ontogenetic abnormalities and malformations that are unusually frequent among amphibians and are caused by abnormal genes or abnormal gene combinations. Abnormalities are well researched because they are easy to induce in a laboratory setting and are well documented in the genetic literature. Amphibian larvae with thyroid hypofunction do not metamorphose, but continue to grow and sometimes even reach sexual maturity.

Deficiency Diseases manifest themselves clinically almost exclusively as a result of poor nutrition in captivity. Skeletal damage has been described for the clawed frog as a result of insufficient calcium intake. The standard values of required vitamin and mineral intake for individual amphibian species have not been determined until now.

Infectious Diseases are caused by transmitable germs that harm the host by robbing it of its energy, but which are not lethal to it, at least at the initial contact. Amphibians are susceptible to viruses, bacteria, fungi, protozoa and metazoa. Although infectious diseases may threaten the life of the host, they are the driving force behind the immunological and—to a smaller degree—the biological evolution.

In recent years certain germs have received a lot of attention for different reasons: virus identification and detection continue to present a diagnostic and technical challenge. Specific viruses (for example, iridovirus-like particles) can now be identified quite easily and efficiently with the help of electron microscopy, so they are now frequently detected in amphibians. The clinically conspicuous and epidemic-causing "red-leg" disease in frogs has excited interest across the world; the germ *Acromonas hydrophila* is a ubiquitous, mobile, gram-negative bacterium regularly found in the intestine of amphibians. The cold-water mycobacteria, which cause tuberculosis in amphibians, are similarly ubiquitous opportunists; they colonize the skin of the host. Protozoa from the phylum Microsporida create completely different

Lankesterella sp. *or* Dactylosoma sp.*-infection of a Rana ridibunda in Thagit oasis in Algeria (Giemsa staining). The protozoa penetrate the erythrocytes, where they modify and displace the host cell nucleus. The probable vector, a leech from the genus Placobdella, is common to the same body of water.*

Amphibians as Vectors

When thinking about a possible link between human health and amphibians, cases in which people were harmed by poison excreted through these animals' skin, by touching or coming into contact with a wound, immediately come to mind. There is, however, another frequently overlooked aspect of amphibian impact on the health of human beings: certain taxa of this group of animals can serve as intermediate hosts for pathogenic germs, and can transport these germs or cause contact between man and infectious foci.

Amphibians as Intermediate Hosts

The most severe and most dangerous illness that humans can contract from a germ transmitted by amphibians is an infection caused by the larvae of the tapeworm genus *Spirometra*. These parasites are located in the muscles of frogs as outwardly undifferentiated worm larvae. They can actively move into human tissue if direct contact occurs, stay there permanently, perhaps even metastasize, move, and increase in size to several cubic inches or centimeters. Especially in South East Asia, where direct contact with frog meat is quite common, since compresses containing amphibian meat are used as an antibiotic remedy and placed on patients' eyes or on festering wounds. Particularly serious cases of these sparganoses [infestations with plerocercoids of the genus Spirometra] may lead to the loss of one's eyes; in minor cases, surgery is required to remove the worm. The development of a mature tapeworm in human beings is impossible.

Amphibians as Carriers of Potentially Pathogenic Germs

Several potentially pathogenic bacteria have such a low host specificity and are so widespread that they can be found in sick amphibians as well as in sick humans. These include *Aeromonas hydrophila* (red leg, sepsis), *Pseuolomonas sp.*, and *Salmonella* (diarrhea, sepsis). Contrary to the situation with reptiles, an actual transmission of these germs from amphibians to humans has not been proven until now. Nevertheless, caution is advised when dealing with excrement or in treating wounds.

Amphibian Cultivation as the Source of Infections

In cases where human beings keep amphibians, it is inevitable that the caretakers come into contact with a special flora of germs, specific to aquaterrariums. The human pathogenic germs of this flora include *Mycobacterium marinum* (but not *M. ranae*!), free-living, potentially pathogenic amoeba *Acanthamoeba spp. Naegleria fowleri*; a germ that produces brain abcesses) and the pathogen that causes a subcutaenous mycosis in Africa, *Basidiobolus ranarum.*

So far, there are no basic scientific studies in existence concerning the health risk to humans in handling amphibians. Also, the risk to the animals during their first contact with humans, when germ exchange and "germ adaptation" occurs, has not been examined, although this phenomenon has a significant impact on physiological studies and on their chances of survival in captivity.

scenarios: In recent years they led to catastrophic mortality among non-adapted hosts (*Bufo bufo*) in southern England. This was caused by intensified commercial fish breeding and the introduction of fishes foreign to the local fauna.

Immunology

Immunology is a basic and vital function of any union of cells. In all higher animals it maintains individual integrity or, put very simply, maintains health. The key function of immunology is to distinguish reliably between the body's own working cells and foreign and/or mutated cells. In amphibians, self-recognition is realized with the help of complex and multi-structured protein molecules at the cell surface (MHC complex). Protein structures that do not carry these recognition signals are captured by means of a complicated mechanism by phagocytic cells that are part of the body (macrophages); divided through digestion, the parts are then kept at the macrophages surface and are utilized by other immune cells as a starting point to control their own activity. This "presentation" sets in motion a cascade of several reactions that ultimately lead to a complete destruction of the foreign structure. Unique characteristics of the amphibian immune system are a strong seasonal activity cycle and a partial functioning at temperatures that would lead to paralysis of the immune functions in other poikilothermic animals.

Another immune function is the production of proteins (antibodies) floating in bodily fluids that attach to foreign proteins according to the "keyhole" principle. Its task is to clot and thus immobilize the foreign structure and to mark the surface for the purpose of targeted attack by phagocytes. These antibodies are divided into several classes according to their specialized tasks. In Anura we find the so-called IgX (dimension: probably 170 kD), during an infection early arising IgM (900 kD) and IgY (170 kD); in the phylogenetic older Urodela only IgM and in some species IgY are present. Antibodies are produced during the active stage of an infection and are still present in small amounts months later. Hence, identifying and documenting antibodies that target a specific germ provides conclusive proof of an infection without having to isolate the germ itself. Such immunological-diagnostic procedures are currently becoming rapidly more significant in amphibian research.