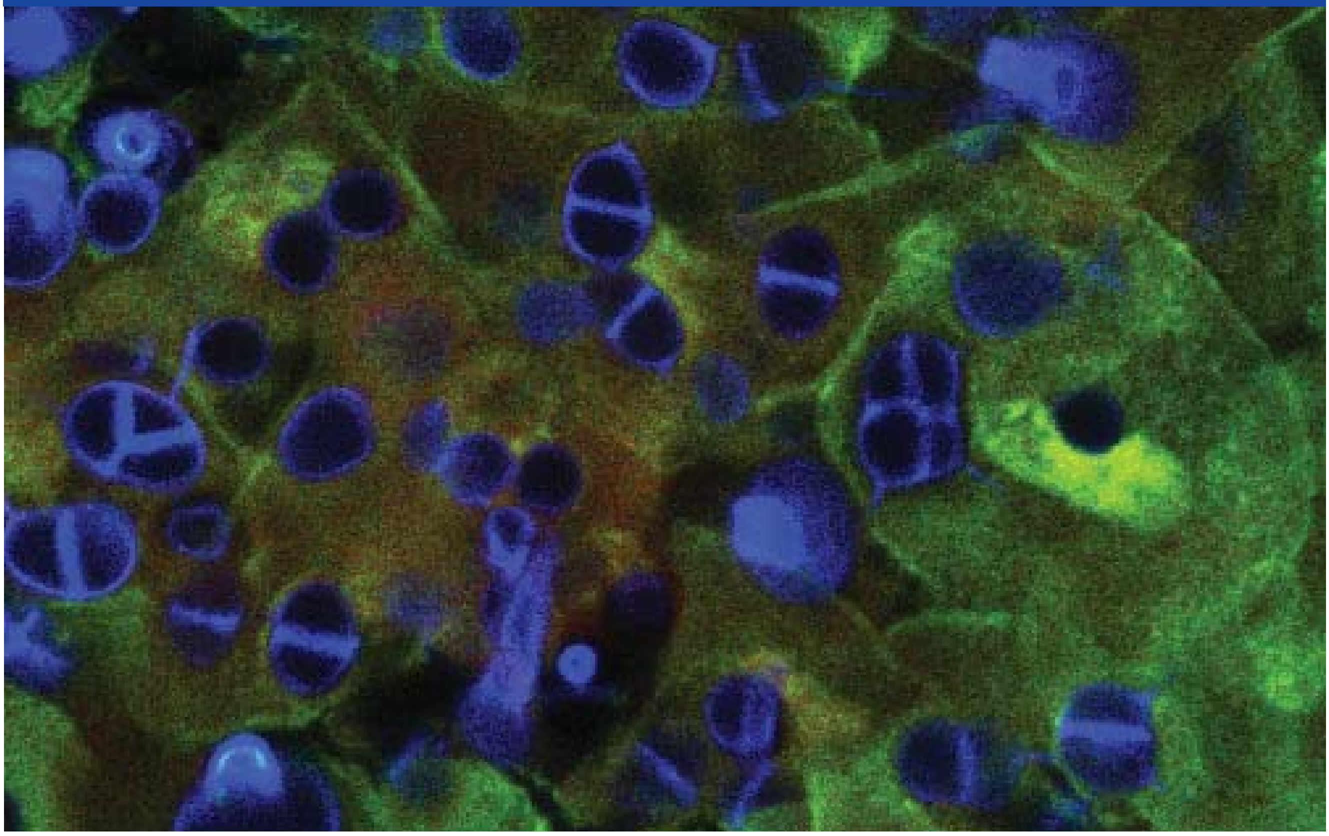
DISEASE RESEARCH

Diagnostics and aquatic emerging fungal diseases

History has shown that pandemics can have major impacts on populations and the environment. The current Covid-19 pandemic is turning the world upside down, and will undoubtedly leave its footprints on all sectors of life to some degree. So writes MR Greeff-Laubscher and C Weldon.



Laser scanning confocal micrograph of amphibian skin (green epidermal tissue) infected with Batrachochytrium dendrobatidis (blue fungal bodies).

The infection pathway and high infection rate of this causative virus affect our social lives, and even our behaviours are impacted and changed. A question we should be asking is if any of these adversities could have been avoided had we been more prepared? Or, even more fundamentally, is it at all possible to be prepared for dealing with the diagnosis of a pandemic?

What are the driving forces behind research in the field of emerging infectious diseases (EIDs) – is it engagement with current known diseases and their related strains, or is it lessons learnt from historic epizootics? In reality, it is incredibly difficult to prepare for EIDs, be it human or animal diseases, especially when the epidemiology and infection rates are not yet understood. This begs the question, how do we develop accurate and reliable diagnostic tools for EIDs if the infectious agent is not yet known? The current Covid-19 pandemic, caused by the infectious virus, SARS-CoV-2, is an example of how this zoonotic coronavirus became pandemic within just a few months. Infectious agents can include a variety of microbial organisms, including bacteria, viruses, fungal and fungal-like organisms.

Over the past two decades, there has been an increase in fungal diseases both in plant and animal (aquatic and semi-aquatic) populations. A record number of fungal and fungal-like diseases have now known to cause mass mortalities and species extinctions. Some examples include ***Batrachochytrium dendrobatidis,*** the causative agent of amphibian chytridiomycosis; ***Aspergillus sydowii***, the causative agent of aspergillosis in marine coral; and ***Aphanomyces invadans*** and ***A. astaci*** causing epizootic ulcerative syndrome in fishes, and crayfish plague respectively.

In the face of unprecedented, rapid declines in global biodiversity, scientists have been investigating how ecosystems with altered biodiversity affect the provision of beneficial ecosystem services. Studies on Lime diseases, for instance, indicated that a greater diversity of hosts lower transmission risks to humans. One can deduct from this finding that a loss of biodiversity would likely result in an increase of infectious disease transmission to humans.

The impact of pandemics can be lowered with quick, reliable and accurate diagnostics. Once infected individuals have been diagnosed, management plans can be put in place to either control or eradicate the infectious agents. While population density and disease susceptibility are some of the major risk factors involved during disease outbreaks, another silent factor is asymptomatic individuals. During the absence of reliable diagnostics, the latter is often underestimated or remains undiagnosed within a population. This is especially true when diagnostic assays are dependent on clinical symptoms (in humans) or visible clinical signs (e.g. in animals).

Although it is referred to as a silent factor, we prefer to refer to it as ***ticking bombs*** because these individuals have the ability to infect other individuals in their environment, apparently unnoticed. If this would happen with a highly infectious disease, an epidemic can occur, resulting in high mortality rates in severely affected populations. To lower the effect of these ***ticking bombs*** on their populations, reliable diagnostic tools are required.

Most diagnostic protocols can be divided into three phases. These are: sampling, sample processing, and sample analysis. The saying "only as strong as the weakest link" is a good way to approach a diagnostic assay. If one of the steps is not reliable, the entire diagnostic assay becomes unreliable.

Validating each step of a diagnostic assay is therefore a prerequisite for achieving reliability. It bridges laboratory work and field application by assessing the application of a specific technique related to the intended use of that technique. The validation step truly reveals if an assay is good enough to perform to the anticipated outcome.

Validation of an assay can be time-consuming and is often neglected during the development of diagnostic (identification from a host) or detection (identification from environment) assays, especially when each step has to be validated. Using an unvalidated diagnostic assay lowers the confidence in the results. Such assays could either lead to the misinterpretation of negative results (false negatives) or an incorrect number of positive individuals (false positives) or sites reported, and therefore lead to inappropriate management of a pandemic.

The causative agent of the next animal pandemic and its impact on aquatic biodiversity is currently unknown to us; however in the midst of a changing climate it has been argued that emerging fungal infections will cause an enhanced abrasion of biodiversity, which spill over to human and ecosystem health. Just like certain bacteria and virus species, fungal and fungal-like organisms can be lethal to naïve species. ***Batrachochytrium dendrobatidis*** is a true panzootic, with a wide host range and the ability to survive outside the host. The global occurrence of this pathogen was facilitated by anthropogenic spread, which resulted in an estimated 90 amphibian species becoming extinct.

The first report of this pathogen on the African continent dates back to the early 1930s. Researchers are now busy using this infectious agent as a model to develop and validate the first two steps of a novel diagnostic assay, which includes field sampling and sample processing, based on environmental DNA technology. The environmental DNA technology will be used in conjunction with a targeted approach at first. This involves detecting DNA of a specific pathogen from water samples which will assist in early detection of future disease outbreaks, specifically in South African waters with its rich biodiversity (e.g. 140 odd amphibian species). In doing so, the first two steps of an assay would only require development and validation of one step, should an outbreak of a fungal or fungal-like species currently unknown to us occur.

In conclusion, the new detection assays that are being developed based on eDNA will hopefully assist not only in understanding the current fungal communities, but also assist in building a robust foundation database of host diversity in aquatic ecosystems. Above all and most importantly, this data will assist in predicting outbreaks and hopefully lower the impact of ***ticking bombs*** among our cherished biodiversity.

Ché Weldon

The Anchieta's ridged frog.