Ranid Herpesvirus 3 Infection in Common Frog *Rana temporaria* Tadpoles

[Announcer] This program is presented by the Centers for Disease Control and Prevention.

[Sarah Gregory] Hello, I'm Sarah Gregory, and today I'm talking with Dr. Francesco Origgi, a veterinary microbiologist and pathologist at the University of Messina and University of Bern. We'll be discussing the detection of ranid herpesvirus 3 in frogs.

Welcome, Dr. Origgi.

[Francesco Origgi] Thank you very much, Sarah. It's a pleasure to be here.

[Sarah Gregory] Well, we're very happy to have you back. What's the situation with frogs globally?

[Francesco Origgi] Well, unfortunately it's not a good situation. I think that let's say, it's becoming more and more evident that frogs and amphibians in general are undergoing a massive global decline, actually, which is really very concerning. And just to give a quick number, of the 42,000 threatened species, actually, that are on this planet, 41 percent are amphibians. And so, they make up almost half of all the threatened species. So this is actually very, very concerning.

[Sarah Gregory] What's contributing to this decline?

[Francesco Origgi] Well, this is actually not a very easy question to answer to because it's probably what is defined as a sort of a multifactorial cause, or actually, group of causes. Surely, the habitat loss has been shown to be really a significant issue. The human impact (direct and indirect impact) is also, let's say, a pretty significant problem. And actually, more recently—and this is really, let's say, something that we are very, very interested in, too—infectious diseases (so, infectious agents) actually have surfaced as significant contributors to this decline.

[Sarah Gregory] What happens to biodiversity when frogs start dying off?

[Francesco Origgi] This is actually a great question. It's kind of interesting to think that, instinctively...actually, we think of everything within the natural world or anywhere, let's say, in general, as some kind of stand-alone element. Instead, the more and the more we investigate, the more we study nature and wildlife (animals) —let's say nature, in general—we realize that we are really all incredibly interconnected. And so, frogs really belong to this incredibly entangled, okay, reality. And so, even just as a single species of frog, actually, or amphibian would be lost, we really cannot predict, okay, the...how can I say, the real extent of this impact, and not only related to, let's say, animals themselves (amphibians themselves), but also related to humans.

There's a paper, actually, that recently came out and showed how a massive extinction—actually, more than a massive extinction—a very significant decline of the frog population in a region could be linked to a spike in malaria in people in the same region. So basically, the loss, the decline, the reduction of the number of frogs could be correlated to essentially the increase of vectors—so basically, of arthropods—which, actually, were essentially not eaten by these amphibians. And so, their increased number then became a problem for the human population itself of that region. And so, this really shows us, let's say, how frogs and essentially any species is really relevant not only in terms of biodiversity (in terms of conservation), but also of human health. And I think this is really critical to understand. You know, more and more we hear about, for example, One Health, considering that actually we're all together.

And so, what is relevant, let's say, for the health of human beings inevitably is connected with the health of animals and the other way around. And also, now we also talk about planetary health, because it's not just a matter of a connection and of interdependence in a way between human beings and animals, but also the environment (the planet). So it's really a very, very complex topic, and frogs are a very important element of this.

[Sarah Gregory] What role do ranaviruses and fungi play in this die-off?

[Francesco Origgi] Yeah, actually ranavirus and a specific group of fungi... actually, there's two species which are mainly, let's say, characterized as a concern of amphibian disease, and these are commonly referred as chytrids, and so...especially *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans*. So ranavirus and these other fungi have been shown to be very significant pathogens and to significantly contribute to actually local extinction and extirpation of an entire population of amphibians. But the most recently discovered of these agents, which is *Batrachochytrium salamandrivorans* (which is normally referred as *B. sal)* is a fungal agent which actually had been detected a few years ago in Europe, and has been shown, okay, to be directly related and responsible of literally the extinction of an entire population of fire salamanders in different European regions. And so, now there's major concerns that this agent might actually cross the ocean somehow, and, let's say, there's lots of concern that if it comes, for example, to the US, the impact might be massive.

And so, these are really very, very important agents. However, we know that these are clearly relevant and important, but I think that we need to keep our eyes open because when we talk, for example, about amphibians, we talk about several thousands of different species. And so, it's probably unlikely that out of these several thousands of species, probably only three agents would be really relevant. And this is actually where we're going in terms about our research and our investigation, trying to figure out what else besides, let's say, ranavirus and chytrids is out there that could be a problem.

[Sarah Gregory] And what is ranid herpesvirus 3? I haven’t heard of this one previously. It’s a novel virus, right?

[Francesco Origgi] It is. And actually, this really relates to what I was saying in my previous answer. So we have been running extensive investigations, trying, let's say, to look and find emerging agents or novel agents which actually could represent an issue for amphibians. And while, actually, let's say, investigating this—while running this research project—we actually found this new virus which we called ranid herpesvirus 3, and we published originally the first paper describing this virus in 2017. And interestingly enough, this probably was not really a new virus, meaning that we actually characterized it, and so we were able to identify which virus it was. But actually, there have been previous reports which describe the infection of frogs with this virus. However, it was really not characterized. So this investigation led to the understanding that we were talking about a herpesvirus, but, let's say, we really didn't know what kind of herpesvirus it was.

And so, with our investigation, we were able to determine which herpesvirus it was, and we could name it in a way, because after that time, only two other herpesviruses had been discovered and characterized in amphibians, which were ranid herpesvirus 1 and 2. And so, this was the third, okay, of the group. And this was really incredible, also because once we published this paper and people all around Europe actually started reading it, they realized that actually they had been seeing lesions, which are classic lesions associated with infection of this

herpesvirus. And so, we now know that this virus is not present only in Switzerland, actually, where we originally found it, but is present in other countries in Europe. And so, it's probably very, very much widespread. And it's kind of interesting, you know, that many years passed by without actually being able to pinpoint the lesions with the actual nature of this virus.

[Sarah Gregory] There's a similar virus apparently found on toads. Which one is that?

[Francesco Origgi] Yes, this is correct. This was actually another very interesting finding, let's say, I think of this investigation that we were carrying out. Pretty much at the same time when we discovered this new virus in frogs, we observed very similar lesions associated with this herpesvirus in frogs were also present in toads. The main difference was the color of the lesions. So infected frogs generally showed gray patches on their skin, and these gray patches are invariably associated with the presence of this virus.

Well, on toads, we started to observe that there were several toads with brown patches, very similar to the gray patches that we saw on frogs. And so, we checked those lesions, thinking that there might have been something similar. And funny enough, we found another herpesvirus which is morphologically and genetically very closely related to ranid herpesvirus 3 but is different. It's a distinct virus (it's a distinct herpesvirus), and we tentatively called this virus bufonid herpesvirus 1, which is, actually, was the fourth herpesvirus which was discovered within amphibians, and all these herpesviruses actually are within the genus...now we know we could actually cluster them within the genus of the *Batrachovirus*, which the taxonomy has been recently changed, but this is actually the older name and so probably most of the people are familiar with this.

[Sarah Gregory] And you mentioned that there's so many different species of frogs. What kind of frogs was ranid herpesvirus 3 found on?

[Francesco Origgi] Yeah. Originally, we found this virus on the common frog (in the classic grass frogs, essentially), and this is called *Rana temporaria* as the scientific name. Instead, additionally to this, we also found the same virus on the so-called agile frog, which is the common name for *Rana dalmatina*. So we know that we found...we can find, actually, this virus at least on two different species of frogs. However, we don't know if these are the only two species that might be susceptible to this virus, or actually others could be susceptible. And this actually is something that we're trying to investigate at the moment—so, the spectrum of this herpesvirus.

[Sarah Gregory] And you mentioned Switzerland, and that's where you did your study, but where particularly there were they found?

[Francesco Origgi] We found these infected frogs in different regions in Switzerland, originally in the northeastern portion. But now, let's say, we realize that there's multiple regions in Switzerland where, actually, we can find this virus. However, as I said, it looks like it is pretty widespread in Europe. We actually have preliminary results suggesting that the virus could be present in France, in Italy, in Germany, in the UK, and other countries, again. So let's say it looks like it's pretty much really widespread, it would make sense there would be distribution of these frogs. When we talk about, for example, the common frog, it is really widespread all around Europe. So if this virus, for example, would have co-evolved with this host, it would really make sense that the virus might be present, essentially, along most of the area (of the territory) where these species of frogs actually are from. So...and we would expect to find this virus in other

regions. And so, it would be really interesting then to understand if there would be differences, okay, genetic differences among the different strains of this herpesvirus.

[Sarah Gregory] And frogs have some stages of life. What stage of life were the frogs in when you found the virus?

[Francesco Origgi] The very first finding...so, when we observed for the first time the presence of this virus, we found this virus on postmetamorphic frogs (basically, individuals). So as we all know, frogs undergo essentially later in their development...so we can divide, actually, their development in two different stages—so we have a premetamorphic and a postmetamorphic stage. The premetamorphic (essentially, the classic tadpole stage) is what we can find, actually, is actually, let's say, the stage that is really necessarily bound to water. And as soon as instead the tadpoles complete their metamorphosis, we have the froglets. And at that point, the frog essentially leaves the pond (leaves the water)—I mean, it still has to be bound, but not strictly to a water environment. And at that point, essentially the frog just grows until it reaches its adult size.

And at the moment, what we have observed is that the lesions which are associated with this virus (or with these gray patches) —which corresponds, actually, to areas of epidermal hyperplasia, which essentially is the thickening of the epidermis—we have observed those, essentially, on postmetamorphic individuals—so basically, let's say, on the frog, when morphologically the frog has really the classic aspect, so, say it's not anymore a tadpole but actually has the classic aspect with the limbs. And so, it's the classic jumping frogs, just to make things very easy.

[Sarah Gregory] Okay. We're talking about die-off here of frogs. Do we know what affects this virus (this particular virus) is having on frogs? Besides the lesions...

[Francesco Origgi] Yeah. This is actually a very important question, and I would like to first of all clarify one aspect, which is actually at the moment, we know that this virus is associated invariably with these lesions, and so we think that there might be a causative link but we haven't been able to demonstrate yet a causative relationship or a causative association, although we strongly believe there is, and actually this is something that we are really working on. With all said, what we have is that this virus (this herpesvirus) is associated with this skin thickening—so, these patches which develop on the skin. The question is, do these lesions have a clinical impact on the frog? Is this something that is going to impact the frog in some way? Well, if you think about it, the skin of the frog is a very important organ. Through the skin, frogs exchange fluid, exchange oxygen, exchange electrolytes. And so, it's really a vital part of the body, and the physiology (the whole physiology) of frogs is really very much dependent of good health of the skin.

So we can assume that these lesions, which in some individuals may be very, very, very severe and pretty diffused, we can assume actually that some impact is going to be there. We don't really know the extent of this impact. However, the question is, you know, is it going to kill the frog, and if it does not kill the frog, maybe, let's say, I don't know, it might predispose the frog to other infectious agents, or essentially it might, for example, reduce the fitness of these frogs. Let's imagine...these are clearly examples, okay? Because the interesting thing is this: we often consider an infectious agent dangerous or problematic only if this agent kills. However, when we talk about wildlife, if we have infectious agents that might not necessarily kill the individuals, but if they would reduce their fitness—and for fitness, I mean their wellbeing in general—let's

imagine that, I don't know, an agent is going to be able to reduce the replication fitness (so basically, the reproductive fitness), so infected animals might, for example, reproduce less successfully. Well, in this case, we're not going to see a die-off, but what we might see after 10, 15, 20 years, we may see a crash in the population which we didn't necessarily see coming. And this might just be because, let's say, we haven't seen a pile of dead animals here and there, but essentially it was a very cryptic problem which anyway at some point became visible.

So the question with ranid herpesvirus 3 is that we really don't know if or actually what kind of impact, let's say, it has on the frog. However, even if we would realize that this virus doesn't kill frogs, this does not necessarily mean that its impact on the frog is absolutely zero or actually, let's say, it has no real clinical relevance of it. So this is really something we are working on and we really would like to try to understand a little bit more about it—so, what is the actual impact of this virus on these frogs?

[Sarah Gregory] So along these same lines, do we understand the actual pathogenesis of this virus?

[Francesco Origgi] Yeah. We know a little bit more, or actually every year we are learning something more, because as I said, we started essentially from scratch. And so, at the beginning, we found this virus and we...little by little, we characterized this virus from the molecular side, using molecular tools to really understand what, for example, its genome was carrying with it and if we could learn something from its genome about its potential virulence (its potential activity and pathogenesis). And then, we looked also on the host side (so basically, on the frog) what was going on. And so, what we learned is that, as I said before, this virus is associated with this proliferation of the skin.

So basically, what happens is that when the virus is in the frog, it looks like that it localizes essentially within the epidermis. And once it's localized in the epidermis, we have this epidermal thickening (this proliferation). Why do we have this? We don't know, but what it looks like is that the virus really replicates in this thicker epidermis. And even more interesting, if you look at the lesions which are really associated with this virus in the epidermis, you can literally draw a line, and so you have...you can see that in the upper portion of the epidermis, there is the virus replicating with all the damage, actually, which is associated with this replication.

In the lower layer of the epidermis, funny enough, there's really no virus that can be detected, and there's no tissue damage. Later on, we observe that, in infected animals, this upper layer starts to undergo degeneration and progressively is sloughed off. And so, at the end, the virus is not detectable any more in the epidermis. However, what we think is that this virus might hide somewhere. Herpesviruses, as a matter of fact, are well-known organisms which are able to hide within the host that they have infected, and they are able to hide in a form which is called 'latency'. Latency is sort of a dormant stage, okay? So basically, during this stage, the virus itself is kind of silent, but is present and is ready to come up, okay, and come out again, essentially, as soon as the environment, as soon as the conditions, and as soon as it's possible.

And so, we think that there might be a seasonality, okay, in the disease associated with this virus, and that infected frogs may undergo a cycle of diseases associated with the season. What we don't know is if during one of the cycles something can happen to the frogs, and so the virus might go somehow out of control and maybe eventually cause severe disease or actually death in the frogs. So this is actually something that we know. But we are missing a very crucial part of the moment—or we were missing—because now, let's say, with what we found and we have

published in the current paper, actually, in *Emerging Infectious Diseases* we are starting to fill in this hole, meaning that we don't know how the frogs actually get infected, and now we are understanding a little bit more. And this is where we're really focusing right now.

[Sarah Gregory] And why did you do this study? What alerted you to anything? What were you looking for?

[Francesco Origgi] And that's exactly to say, the follow up of the previous question. So we were trying...we're trying, okay, to complete, ideally, this pathogenic path of the virus. The big question is and was, "How does the frog get infected by the virus?". So we actually had been running a transmission study using live virus and infecting postmetamorphic frogs, and we could not reproduce the disease. And so, we started to think that maybe the adults or, actually, postmetamorphic stage maybe was not the proper stage for the virus to be able to infect the frogs.

Reading in the literature, back in the 70's, there has been lots of investigation on another frog herpesvirus, which was called ranid herpesvirus 1. This ranid herpesvirus 1 was discovered in leopard frogs in the US, and a number of studies showed that this virus was causing a tumor in kidneys. And this became a very, very investigated virus because back in those days, it was the first herpesvirus which was actually linked to a neoplastic disease. Then also, let's say, as concerning human herpesviruses, actually, this has been shown. But at that time, this was kind of a unique example.

And researchers actually tried to infect frogs with this virus in multiple ways, and none of them basically were able to do it except when working on the larval stage, actually, let's say, on the embryonic stage of the frogs—so, the very, very, early developmental stages—so, essentially right after hatching. So injecting this larval (embryonic) stage actually with the virus, then they were able to reproduce the disease. But if the virus was actually injected in adult frogs, nothing was happening. And so, we started to think that actually maybe we were supposed to not necessarily to look just at the postmetamorphic stages, but actually, let's say to the larval ones—so, the previous ones to the tadpole level. And that was what we were looking for. We were looking...we were trying to see if we could find the virus in the tadpole stages because if we would have been able to find this, then this would have been very important evidence suggesting that the infection would have occurred not at the postmetamorphic stages, actually, but very early in life. And this is what we were looking for.

[Sarah Gregory] You've done some previous investigations on frogs (I think you mentioned that already). You want to tell us about that a little bit?

[Francesco Origgi] Yes. And this is how we got to this point. I've been always very much interested in poikilotherms (so, the so-called 'cold-blooded' vertebrae), so what we talk about in this case: reptiles, amphibians, and fish. And concerning amphibians, we've done several investigations on frogs, always, let's say, linked to infectious agents. And as I said, we have investigated the nature of this virus (basically, of ranid herpesvirus 3 and bufonid herpesvirus 1) at the molecular level. And for example, we have discovered a very interesting thing. It looks like that these viruses contain in their genome very special genes, which are putatively able to encode for proteins that have an immunomodulating effect on the immune system of their host—so, basically of frogs and toads.

So this is really very interesting because it looks like that not only the impact of the virus or of the disease on the frog might not necessarily just depend on the nature of the infectious agent

itself. So basically, it's a virus, so it can infect the host—but, let's say, the virulence of it—and so, the impact that the virus can have on the frogs—may depend also, let's say, on these elements. So it's likely that the virus has learned that if it has specific tools that can somehow prevent the frog to respond with its immune reactivity, let's say, to the infection, well, let's say, the virus seems to have learned that actually it may have kind of an easier life and can carry out its infection, let's say, in an easier way.

And so, we are really trying now also to investigate this aspect of it—so, basically to what extent these genes, to what extent these encoded proteins actually may have an impact on that. And I think that, you know, this really opens up a very fascinating scenario and research field, actually, that I would like to investigate. And even more, if you think about this thickening of the epidermis, it's a proliferative disease. And for example, tumors are proliferative disease. The main difference is that an hyperplasia, as we see in these frogs, is kind of a...sort of a relatively benign, proliferative process. Instead, a tumor instead can be a very malignant process. Well, if we would learn, for example, what are the buttons (the steps) which this infection goes through in order to cause this proliferation, well, for example, we may learn also something about cancer in people and animals. We know that there are actually viruses which can cause cancer. If you think about papilloma virus, it can do that. And if you look at the lesions that papilloma viruses actually cause to animals and to people, well, morphologically speaking it's very similar to, let's say, the lesions that actually we can see in frogs, in fact, with this herpesvirus. So we really think that we're going to learn a lot, okay, from these infections (this herpesvirus) and actually amphibians, not only in terms of conservation, but also in terms of comparative pathology, comparative immunology. So I think that there's really a lot that we can learn from frogs, and this can go really far beyond what we originally imagined.

[Sarah Gregory] Is there anything about how you went about this investigation that you want to say more about?

[Francesco Origgi] Yes. This specific investigation—the detection of this ranid herpesvirus 3 in *Rana temporaria* (so, common frog tadpoles)—as I said, is an investigation that started, or the idea started, let's say, to check if we could find the virus in the tadpoles. And so, this whole thing started thanks to a collaboration with the Norwegian Institute for Nature Research, and specifically with Dr. Annette Taugbøl, who is actually the co-author of this article. And so, discussing what we could have done, because, I mean, our collaboration started originally because of some testing of the frogs in Norway (they observed similar lesions). So you can see, we were talking about, you know, where we do see, let's say, this disease as well. We know that at least, I mean, right now the upper north limit that we have found so far is actually Norway.

And so, when we learned about this, we started discussing about what kind of investigation we could have done, and I was saying that, you know, we really don't know how this virus actually infects these frogs, and most of all, we really don't know at what stages it does. And so, we had the chance to sample different populations of frogs in ponds where we knew that there were infected frogs. So we collected these tadpoles, and we checked for the presence of the virus by PCR—so, basically by molecular methods—and by histology—so, looking specifically at the tissues—to see if there was any kind of change that we could actually have detected, and that would have let us understand a little bit more about this virus and the pathogenesis. So that's how we went along for this investigation.

[Sarah Gregory] And is there anything about what you found that you haven't covered yet?

[Francesco Origgi] Yeah, and what we found is, as I said before, I mentioned before actually is that we found that this virus was present in tadpoles. And this is really something exciting because we thought about it, but we were not sure that we would have found it also because we really didn't know, for example, how many of these tadpoles might have been infected, how extensive, let's say, this infection within this larval stage is. We didn't know if we were looking at the right pond or at the right populations. So there has been also a significant part of luck in our investigation, but I think that, you know, this hopefully sometimes happens. So this is actually really the main result of this investigation. So we can finally say that the virus can infect frogs in their premetamorphic stage, and this is really very important.

[Sarah Gregory] Let's talk about people here for a second. People can get herpesvirus from each other, certainly. Is it possible that children playing and grab a frog or anybody...can people get this from the frogs?

[Francesco Origgi] We don't really have any element at the moment to really think or have any specific concern in this direction. The herpesvirus, actually, which infects frogs and amphibians in general are very different from the herpesviruses that infect people. As a matter of fact, they belong to a different family. So herpesviruses are comprised in three main families. So we have the family called *Herpesviridae*, which actually includes essentially all herpesviruses infecting people. And then we have the second of this family instead is the family which includes herpesvirus which infects fish and amphibians, and we're talking about the family of the *Alloherpesviridae*. So although we are talking about herpesviruses in both cases—so, herpesvirus which infects people and herpesvirus which infects amphibians—we're talking about very different viruses.

And another important aspect to consider is that, as far as we know, frog herpesvirus requires relatively low temperatures to develop and replicate, and these temperatures are very different from the temperature that a human being would have. So 37 Celsius, which is actually the common physiological temperature, actually, that we have in people, is very, very far away from the temperature range where the frog herpesvirus probably would thrive, which we believe are actually, probably across 10 Celsius (a little bit less or a little bit more than 10 Celsius). So the virus, besides being very different, and so. would have tremendous difficulty to adapt to humans. However, even if, let's say, we would imagine this hypothetical infection, probably this would be an aborted infection, meaning that the virus wouldn't be able to cause a productive infection because it wouldn't be able to replicate at this temperature. So from this point of view, I think that kids playing with frogs probably are not going to run any specific risk as concerning ranid herpesvirus 3. As concerning other pathogens, that's another story.

[Sarah Gregory] So we've talked about some really important things, here. Are there one or two most important aspects of this, public health-wise, you want to mention?

[Francesco Origgi] One of the most important things that we found in this investigation, so let's say, the understanding, knowing that the virus can infect tadpoles, opens up an entire venue in terms of investigation, in terms of assessment. Why? Because, you know, what we were talking about before, the impact that this virus may have on frogs. And we have seen that, and we are realizing that maybe on the adult frogs—so, basically on the postmetamorphic frogs—its impact might not be a lethal impact, although we don't know if, as we said before, it might cause some problems in terms of fitness. So it might impact negatively the frog anyway.

Now that we know that the virus can infect tadpoles is a completely different game. Why? Because traditionally and historically, we know that herpesviruses actually are more likely to cause more severe disease or problems in young individuals. For example, there are fish herpesviruses which can cause death in very young individuals whereas not in adult individuals, although they can cause disease in the adults. So if you think about it, in terms of conservation, it's really very important. Why? Because in this case, we can really go and monitor tadpole populations. And now with molecular tests, we can really easily assess the presence of the virus.

And so, now we're going to have tremendous proof to really to go investigating if this virus can cause any problem at the tadpole level—so, if this infection, for example, is associated with death. So let's imagine, I don't know, that—I'm just going to throw numbers here; these are all hypothetical—but let's imagine that, you know, this virus may cause, I don't know, 20 percent death in the infected population in tadpoles. I mean, these are really numbers which actually may have an impact at some point, let's say, on the survival of the population, especially if we add this to other problems (other stressors) that frogs actually may have to endure. So I think that this is really the great aspect and the important aspect of the results we got from this investigation.

But now, we really, let's say, besides having learned something more and something very important about the pathogenesis, now we also know what to look for. So far, we have essentially just concentrated on the adult frogs. Now we're going to concentrate on the larval stages and see really what happens there and see if really this virus may cause issues (may cause problems) to these frogs at this level (at this stage of development). And we really think...we are very excited about it because I think that this could really be instrumental for conservation of these frogs.

[Sarah Gregory] Well, that seems to open up to my next question. What further studies would you like to see done?

[Francesco Origgi] A transmission study would be very important now. So we know that, okay, tadpoles can get infected. Now, we would like to know at what stage, okay...because I mean, the tadpoles go through very, very significant developmental changes before completing the metamorphosis. And so, it might happen that the virus actually is going to be able to infect the tadpoles only at a specific level or at a specific stage of the development or at multiple stages. And so, this is something that really, we would like to understand.

And so, when the tadpole gets infected, what changes do occur in the tadpoles in the sense is this infection somehow followed kind of a sort of an immediate silencing of the virus? So is the virus undergoing latency immediately afterwards and so, to the tadpoles, probably nothing happens until it completes its metamorphosis? Or is it going to cause disease? Is it actually going to cause tissue lesions in the tadpoles? So is the tadpole going to die after the infection? So these are all things that we really need to figure out. And ideally, the condition that may favor...so we have said before about temperature, okay? So we really would like to see a little bit more kind of pinpoint the temperature where this can happen. So we know we are going through this environmental global warming, and we really don't know how this global warming may impact these infectious agents, which are very, very much dependent on the temperature to, you know, as concern their potential of infection of causing diseases and their death. So we really don't know what's going to happen.

So there's lots of things that we really would like to look at. And also, on the host side, as concerning, let's say, postmetamorphic frogs, we really would like to look a little bit more into

the dynamic of this skin thickening—so, how the skin thickening happens. And so, if we really can learn something about tumors that actually, you know, potentially we could use also in a translational way also in human medicine, this would be fantastic. So this is actually the way we would like to go.

[Sarah Gregory] You and I had a wonderful conversation about fungus in snakes in October of '22. Does your study of frog fungus and pathogens relate to this topic?

[Francesco Origgi] Yeah, I remember that. I really enjoyed it. There's no really direct association or actually direct link in terms of the different type of agent, but there is actually a certain number of links (a number of associations), that's for sure. What we have done with snake fungal disease—and so, investigating of *Ophidiomyces* in snakes—goes exactly in the same direction as concerning the work that we're doing with frogs and other amphibians, which is really investigating infectious diseases, understanding what is the role, what is their significance, okay, in what we call the disease ecology of these animals. And so, this really is our main goal, and this is our approach—so, look for agents, try to characterize them, try to put them in context with the host. Are they just there or are they causing some problem? And if this problem is happening, what kind of impact can it have on the population? And so, what can we do in order to kind of mitigate this problem? What kind of management recommendations we actually can come up with, we can provide to the people on the ground—the people, let's say, in the field—in order to try to minimize the impact of this problem.

And yet, there's also a link in terms of possible complementation between these pathogens or these infectious agents. I'm going to make a brief example. We said before that we really don't know, we're not sure how severe the impact of this ranid herpesvirus 3 is on the overall health of frogs. Well, if, for example, we have said that this virus actually has immunomodulating or putative immunomodulating genes. So if the infection with this virus somehow can manipulate the immune response of the infected frogs, well, maybe these infected frogs might become more susceptible to other agents like, for example, chytrids—so, actually fungi. So this is actually something that we're going to try, let's say, to tackle and to really understand a little bit more. So the bottom line, yes. They look like distant topics, but at the end, they are very, very close to each other and they are very much interconnected.

[Sarah Gregory] You're clearly involved in some really fascinating work. Can you tell us about what you do and your work in general?

[Francesco Origgi] Yes. This investigation is an integral part of my interest, my passion, and my work. As I said, I'm a microbiologist and I'm a pathologist, and I'm very, very much interested in wildlife and especially in, as I said before, in poikilotherms—so actually, these cold-blooded animals—in particular, amphibians and reptiles. And I've been working and I'm still working at the University of Bern, and now I have recently...I mean, I got an appointment at the University of Messina in Italy where we are starting to develop the same program. And the idea is really this: it's try to, let's say, to keep going and try to get as many people (as many students) involved in this. And I think that is very important to get young people...I mean, young people are extremely sensitive to this topic—conservation, you know, global health, planetary health.

And so, I really hope to be able to keep going in this direction and try to answer to this question and hopefully, let's say, while answering to this question, hopefully contributing a little bit, okay, to the conservation of this incredible animal that is anyway part of our life in some way. I think the issue of that has been probably... at least when we were kids, to a pond, probably played with

frogs and toads and tadpoles. So they are an extremely important part of the environment (of the ecology), so I think that it's really very, very much important to really, let's say, work on these animals. It's not very easy because very often funding agencies, when you have grants, they have lots of priorities, of course. And unfortunately, up to now, this was not one of the priorities. But it is becoming, and I'm very, very happy about this. I think the funding agencies are becoming very, very much sensitive, and I think it's really...we're getting into a great time, into a great, really, season, okay, to work on this. And I hope that more and more people will get interested in working in this and hopefully contributing, as I said, a little bit to the conservation of these animals.

[Sarah Gregory] Is there any one disease or possible consequence of disease that keeps you awake at night?

[Francesco Origgi] Yeah. I can answer your questions in two ways. What actually keeps me awake is, for sure, the disease that we haven't discovered yet...that, actually, the pathogen that we haven't discovered yet that is just out there. And if we...unfortunately we put this agent in the condition to do damage, then this would be really a big problem. And so, let's say, one of my concerns really is to...how do you say, be able to see the rain before the storm. And so, let's say this is actually what really keeps me awake at night and keeps me really going in the lab. Try really not to give anything for granted; try, let's say to really understand—that we're trying to understand—anything, okay, that is out there. And that maybe we cannot really make sense of it right now, we just can't ignore its actual potential, but the potential might be there. So this is actually really, really something very important.

And more specifically, as I was mentioning before, one of these chytrids (*B. sal*) is really recognized as a very, very aggressive agent, and surely the uncontrolled, let's say spread and diffusion of this agent, will be really a complete disaster (and a global disaster). So hopefully this will not happen. And so, as I said, there's probably lots of agents we are not aware of or that we know but we are completely unaware about their actual potential. That is also, let's say, in my opinion very important to keep in check.

[Sarah Gregory] Well, this has certainly been a very interesting topic and conversation and thank you for taking the time to talk with me today about it, Dr. Origgi.

[Francesco Origgi] Thank you very much, Sarah. It has been really great. Thank you for this opportunity.

[Sarah Gregory] And thanks for joining me out there. You can read the June 2023 article, Ranid Herpesvirus 3 Infection in Common Frog *Rana temporaria* Tadpoles, online at cdc.gov/eid.

I'm Sarah Gregory for *Emerging Infectious Diseases*.

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