Global warming and viral diseases – Tilapia Lake Virus (TiLV) in tilapia, common carp, crucian carp, and rainbow trout - first results –

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Abstract. Recently, a global increase in temperature has been considered with a temperature rise. Therefore, new species and diseases are getting domestic in Europe. Particularly, tilapia is kept in "colder" areas like in European waters in indoor facilities. It was examined if the tilapia lake virus (TiLV) can infect native European fish. Common carp, crucian carp, and rainbow trout were chosen to be with infected tilapia. While tilapia were infected, the other species were always cohabitated. After keeping all species together, samples were collected. Tilapia and crucian carp at 20 °C as well as rainbow trout at 12°C, showed mortality. Besides necrosis, infiltrations and syncytia were observed. TiLV was detected by *in-situ* hybridization in all species. Sera were used for SNT to prove the replication. While tilapia developed neutralizing antibodies after 14 to 21 dpi, carp and rainbow trout started after 12 dpi. The highest neutralizing activities we found in sera from crucian carp. All fish (tilapia, crucian carp, and rainbow trout) that died during the experiment were inspected for the presence of TiLV by RT-qPCR, histology, and ISH. Due to the experience that clinically infected fish showing severe symptoms are not producing antibodies, serology was not carried out.

1 Introduction

Climate change with artificially induced global warming is a worldwide problem [1]. Its effects have consequences for the physical environment, ecosystems, and human societies. Climate change represents a threat to sustainability [2], including a decrease in biological diversity. Mainly, it affects the water temperatures in fresh water and marine environments

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[3]. It is strongly associated with a decrease in species and provokes a northward migration of aquatic animals of the temperate and tropical zones [4]. More and more invasive species will occur in marine and coastal brackish waters and freshwater areas of the mainland [5]. These invaders' microbiomes, including disease agents, viruses, bacteria, and parasites, can also be transferred to the new areas. Not only do the migrating species and their agents adapt to new environmental conditions, but the disease agents may also be able to use native species and their innate disease agents and vice versa, perhaps for hybridization to create so far unknown variations, a process designated as cladogenesis [6]

Tilapia (*Oreochromis sp*.) are mainly produced in countries with warm climates. The leading producers are PR of China, followed by Indonesia, the Philippines, Thailand, and Egypt (FAO, 2020). Tilapia have been exported to most countries of the world for aquaculture purposes; when they escape, they can be responsible for ecological disasters in the wild, especially in areas with constant warm water temperatures [7]. USA, China, and Australia are significantly affected by wild living tilapia [8]. Nile tilapia (*O. niloticus*) and its hybrids are the most commonly kept fish species worldwide [9].

Although tilapia was mostly resistant against diseases induced by viruses, bacteria, and parasites, in 2009, a new disease caused by an orthomyxo-like virus was identified in Israel and retrospectively in Columbia and Ecuador [10]. The agent was designated as the tilapia lake virus (TiLV). Meanwhile, more than 16 countries are affected by TiLV disease (TiLVD) [11]. The disease is considered to be a threat to the entire tilapia industry. Clinical signs after infection were only observed in the summertime in tilapias and giant gourami [12]. Other fish, e.g. common carp (*Cyprinus carpio*), walking catfish (*Clarias macrocephalus*), striped snake-head fish (*Channa striata*), climbing perch (*Anabas testudineus*), silver barb (*Barbodes gonionotus*), or Asian sea bass (*Lates calcarifer*), kept with TiLV infected and diseased tilapia never showed any clinical signs of TiLVD or any mortality [12]. The authors stated that these fish were not susceptible. However, they had not tested them for the presence or absence of the virus. It was unclear if their susceptibility for TiLV infection or a possible TiLVD had been assessed. Additionally, no serological investigations with regards to TiLV had been carried out.

In the recent study, it was investigated for the first time if native European fish species exposed to higher temperatures which seem to be possible in the frame of global warming, can be infected with TiLV if they can display clinical signs or show mortality, and how they can react via adaptive immune response after a possible infection by co-habitation with infected tilapia.

2 Materials and methods

2.1 Fish species

Tilapia fingerlings (n=25, 0.2 – 0.6 g) were obtained from an indoor farm in Germany producing under specific pathogen-free (SPF) conditions. They were kept at 25°C in a 450 L aquarium in a re-circulation system changing 100 L water per day. They were fed six times per day until they had reached a weight of 5 to 10 g. Carp (n=45, three summers old, 400 g to 1.1 kg) and crucian carp (n=45, three summers old, 30 to 80 g) were obtained from a commercial farm in the German federal state Thuringia tested virologically and serologically for the absence of disease agents, e.g., KHV, SVCV, and CEV, several times a year. These fish were quarantined and kept in a re-circulation system with a volume of 1.5 m^3 with 150 L daily water exchange for two years in the wet facility at FLI at 20°C. They were fed three times a day. Clonal female SPF rainbow trout (strain France, n=85, ten months old, 30 to 110 g) kept in a 1.5 m^3 tank with 150 L daily water exchange at 12°C water temperature were

obtained from the quarantine facility of FLI. Before the experiment, all fish were tested for the absence of notifiable disease agents, i.e., carp and crucian carp by qPCR, e.g., for koi herpesvirus and carp edema virus, and rainbow trout by RT-qPCR, e.g., for viral hemorrhagic septicemia virus and infectious hematopoietic virus. Additionally, all fish were tested for the absence of spring viremia of carp virus and infectious pancreas necrosis virus by RT-PCR as well as for the absence of TiLV by RT-PCR and RT-qPCR.

2.2 TiLV isolates, replication, and identification

Two TiLV were isolated from tilapia in an East Asian country. Both were investigated in animal experiments for their virulence in tilapia. While the isolate 2017A [13] was high virulent with 80 to 100% mortality after ip injection to tilapia, the isolate 2017B (this study) was weak virulent, inducing 10 to 25% mortality. The viruses were replicated as described before [14]. Additionally, both isolates were passaged five times onto different cell lines (Table 1). All passages, as well as the negative controls, were tested by RT-qPCR [15].

Table 1. Cell lines used for propagation of TiLV isolates.

no. of CCLV*	species	name	Publication
57	fathead minnow	FHM	[28]
816	common carp	CCB	[29]
826	seabream	SAF-1	[26]
1492	zebrafish	ZF-4	[27]
1542	grass carp	TiB-F	not published, Guangzhou (PRFRI)
1543	common carp	CCM-R	not published, own cell line (FLI)
1550	Nile tilapia	TiB	[14]

* Collection of Cell Lines in Veterinary Medicine (CCLV) at FLI

2.3 Animals experiment scheme

2.3.1 Common carp, crucian carp, and TiLV infected tilapia

The fish were divided into five experimental groups for carp and crucian carp. Each group was kept together in one aquarium. Group 1 consisted of five tilapias (ip infected with 2017A), ten carp, and ten crucian carp kept at 22°C for 35 days. Group 2 had the same number of fish, but tilapia was infected with 2017A by immersion. For groups 3 and 4, a similar number of fish was used, but tilapia of both groups were infected with 2017B. In group 5, all negative control fish were kept in cohabitation at 22°C. Samples for histology and in-situ hybridization (ISH) as well as for virology and serology were collected on 3, 7 and 10 dpi and at the end of the experiment between 38 to 51 dpi of the tilapia. All fish died in the course of the experiments and all survivors were investigated individually by histology, RT-qPCR and for virus re-isolation but also by SNT, if possible.

2.3.2 Rainbow trout and TiLV infected tilapia

Tilapia were adapted to 12 and 17°C water temperature. With the latter group, rainbow trout were adapted at 17°C for one week. Subsequently, tilapia (n=5 per aquarium) was ip infected with 2017A and 2017B. Fish were divided into five groups: Group 1 consisted of five tilapias infected with 2017A cohabitated with 20 rainbow trout at 12°C; group 2 again consisted of five tilapias infected with 2017A cohabitated with 20 rainbow trout but was kept at 17°C. Groups 3 and 4 were kept at the same temperatures and the same numbers of fish were used, but tilapia of both groups were infected with 2017B before cohabitation. Samples were

collected at 3 and 7 dpi of the tilapia and at the end of the experiment. All fish died during the experiments and all survivors were investigated individually by histology, RT-qPCR and for virus re-isolation but also by SNT if possible.

2.4 Histology and ISH

Fish chosen for sample collection were anaesthetized with benzocaine (2 ml in 5 L water) and killed by decapitation. Organs were fixed in 4% buffered formalin for at least 48 h. After paraffin embedding, 4 to 5 nm sections were fixed on Superfrost Plus (Roth, Germany) slides. One slide was stained using a standard method with haematoxylin-eosin, the following for ISH. The DNA probe was prepared using the semi-nested PCR according to [16]. The procedure afterwards was carried out according to [17].

2.5 Detection of TiLV by RT-qPCR

For detection of TiLV by RT-qPCR, the method published by [15] was used. Beside positive controls obtained from the isolates 2017A and 2017B with known titres, IC-2 RNA [18] was included to estimate the absolute but also the relative concentration of the viral RNA copies.

2.6 Detection of neutralizing antibodies against TiLV

Neutralizing antibodies were detected by a new version of SNT. From diseased or dead fish no serum samples were collected. From all survivors or fish not showing any clinical sign of disease, 100 – 800 µl blood was taken from the caudal vein and allowed to clot at 4°C in a microtainer (Beck and Dickinson, Germany) for at least four hours.

Briefly, Polysorb ELISA plates (Nunc, Denmark) with a lid from 96 well cell culture plates (Costar, Germany) were disinfected under UV light for 30 min. Sera from all fish species (2 µl into 200 µl cell culture medium with antibiotics) were serial diluted in the ELISA plate from 1: 100 to 1:400. Subsequently, 100 µl of TiLV diluted into cell culture medium with antibiotics at a titre of 10 $TCID_{50}$/mL were added to each well including a virus control and a virus titration control on each plate. After 24 h reaction time at 4°C, 100 µl of cell line E-11 (no. 1335 CCLV) with antibiotics in transfer medium were added to each well. The plates were sealed with parafilm (Plano, Germany) and then incubated in a CO_2 incubator at 26°C for five to seven days. As controls on each plate additionally a positive serum control from crucian carp, a negative serum control from tilapia and a cell negative control were used. All plates were read using a light microscope at 5, 7 and 10 dpi.

3 Results

3.1 Replication of TiLV isolates 2017A and 2017B onto different cell cultures

Both TiLV isolates were passaged five times onto different cell lines at an incubation temperature of 26°C. While none of the isolates was replicated after two passages onto cell lines 816 (CCB, carp), 1542 (grass carp) and 1543 (CCM-R, carp), the cell lines 57 (fathead minnow, FHM), 1492 (ZF-4, zebrafish) and 1550 (TiB, tilapia) preserved both isolates or replicated them to a similar virus titre visible by RT-qPCR. Only cell line 826 (SAF-1, seabream) replicated the weak virulent isolate 2017B with an increasing titre but stopped propagating isolate 2017A after the third passage (Tab. 2). These results led to the assumption that TiLV can be replicated by other fish than tilapia or their hybrids.

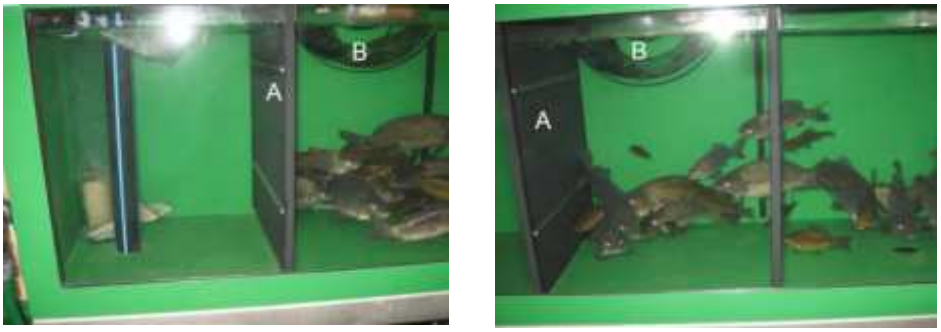
Table 2. Passages of two TiLV isolates (2017A and 2017B) onto different fish cell lines with results for the virus concentration by RT-qPCR (cq values).

cells	57		816		826		1492		1542		1543		1550	
isolate	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1*	23	23	27	28	21	-	25	24	21	21	28	25	21	22
2	24	23	34	33	31	31	22	21	25	25	33	33	23	23
3	20	19	-	-	40	26	25	27	-	-	-	-	22	20
4	20	19	-	-	-	25	21	23	-	-	-	-	21	20
5	21	21	-	-	-	24	20	20	-	-	-	-	23	20

* Passage number

3.2 Animal experiment, histology, ISH and virus re-isolation with samples from tilapia, carp and crucian carp

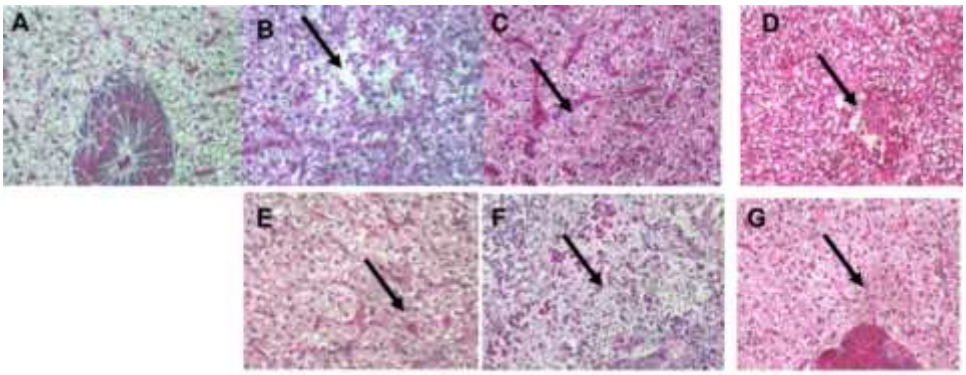
Carp and crucian carp were infected by cohabitation with immersed or ip injected tilapia, respectively (Fig. 1), at 22°C (+/-2 °C). In group 1 (2017A, ip) 70% of tilapia died within 14 dpi. In addition, mortality was only observed in cohabitated crucian carp with 25%. In all groups, carp never showed any clinical signs of disease or mortality. In group 2 (2017A, immersion) 25% of tilapia but also almost 50% of crucian carp died within 20 dpi. In group 3 (2017B, ip) again 25% of both species died. In contrast to all other groups, tilapia in group 4 (2017B, immersion) stayed healthy, but 30 % of the crucian carp died. In group 5 (negative untreated controls) no mortality or any sign of disease was observed over the period of the experiment.



*A: open operable wall, B camera in a plastic dome

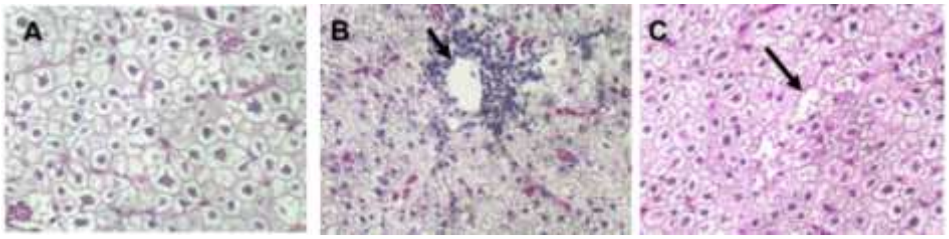
Fig. 1. Co-habitation of tilapia with carp and crucian carp in.

Beside in tilapia, in the section stained by HE, liver necrosis also was observed in carp and crucian carp from 3 dpi to the end of the experiment (Fig. 2). When cohabitated with ip infected tilapia, damages and/or liver necrosis in carp were always weaker than after cohabitation with tilapia infected by immersion. This was different in sections obtained from crucian carp samples. Damages of the liver tissue were stronger with isolate 2017B which was low virulent to tilapia (Fig. 3). Only at the end of the experiment, liver tissue damages induced by 2017A were stronger compared to the fish infected with 2017B. While the group cohabitated with tilapia infected by 2017A showed giant melanomacrophage centres in the liver tissue, crucian carp cohabitated with tilapia infected with 2017B displayed healthy looking liver cells (Fig. 4). By ISH, it was visible that also carp can bear the virus in the liver without any clinical signs of disease (Fig. 5A). In crucian carp it was detected mainly in the kidney (Fig. 5B) but also in the liver.



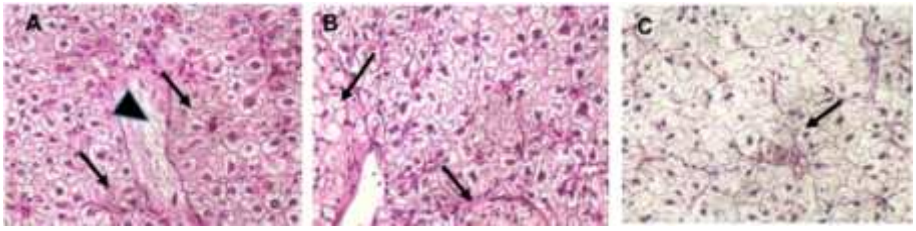
*A: negative control carp, B: 3 dpi (immersion 2017B) C: 10 dpi (immersion 2017B), D: end of the experiment 48 dpi (immersion 2017B), E: 3 dpi (ip 2017B), F: 10 dpi (ip 2017B), G: 46 dpi (ip 2017B)

Fig. 2. HE staining of section from samples obtained from carp at 3, 10 dpi and the end of the experiment after cohabitation with TiLV infected tilapia (2017A and 2017B, ip and immersion) with damages (necrosis) of liver tissues.



*A: negative control crucian carp, B: heavy infiltration and weak necrosis after cohabitation with immersed tilapia 10 dpi (2017A), C: liver tissue necrosis after cohabitation with immersed tilapia 10 dpi (2017B).

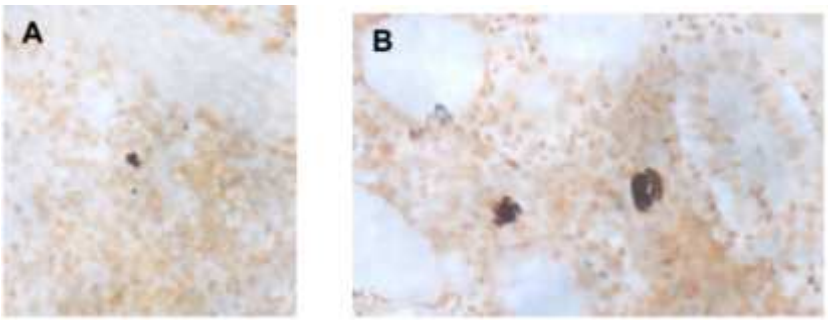
Fig. 3. HE staining of section from samples obtained from crucian carp after cohabitation with TiLV infected tilapia with damages of liver tissues.



*A: 56 dpi with 2017A (and B) immersion infected tilapia with necrosis (arrow) and blood vessel congestion (arrowhead), B: 57 dpi with 2017A (ip infection) with giant melanomacrophage centres, C: 46 dpi with 2017B with cured liver tissue from crucian carp from ip injected tilapia.

Fig. 4. HE staining of section from samples obtained from crucian carp at the end of the experiment with damages of liver tissues.

Virus re-isolation onto E-11 cells and identification by RT-qPCR for both variants showed that, beside from tilapia, it also was possible from carp and crucian carp (Table 3A, Table 3B). Additionally, in all groups ISH was used for TiLV confirmation.



*A: carp liver, B: crucian carp kidney

Fig. 5. Detection of TiLV by ISH in liver and kidney 47 dpi.

Table 3A. Detection and re-isolation of TiLV 2017A from fish samples of the survivors (except tilapia) at the end of the experiment (%).

assay / fish	RT-qPCR		cells no. 1335		ISH
	immersion*	ip*	immersion	ip	
tilapia	100	100	0	0	+
carp	0	0	12.5	0	+
crucian carp	0	25	100	75	+

* Infection of tilapia

Table 3B. Detection and re-isolation of TiLV 2017B from fish samples of the survivors (except tilapia) at the end of the experiment (%)

assay / fish	RT-qPCR		cells no. 1335		ISH
	immersion*	ip*	immersion	ip	
tilapia	100	100	50	50	+
carp	0	25	0	12.5	+
crucian carp	12.5	12.5	12.5	12.5	+

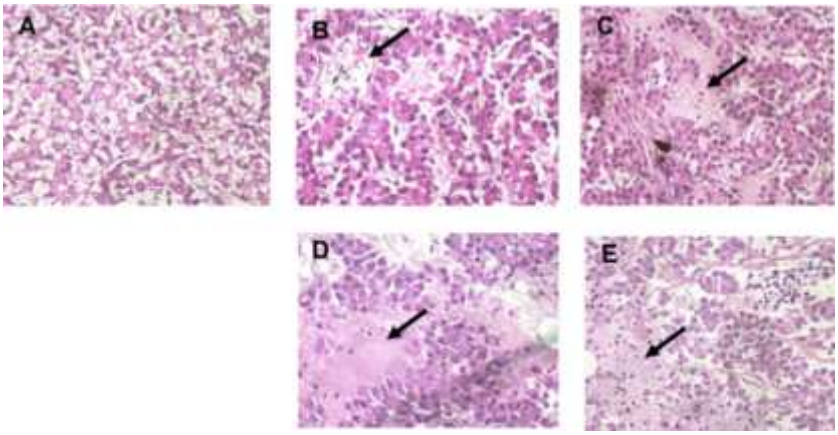
* Infection of tilapia

3.3 Animal experiment, histology, ISH and virus re-isolation with samples from tilapia and rainbow trout

Rainbow trout and tilapia were kept at 12°C and 17°C after a one-week acclimatization period. Tilapia were infected by ip only with both isolates at both temperatures to avoid virus transmission from the fish surface via water. Mortality occurred in 100% of tilapia infected with 2017A at 12°C within 14 dpi, only 10% of rainbow trout died at the same temperature. 50% of tilapia died at 12°C and 17°C after ip injection using 2017B, but no mortality was observed in rainbow trout at both temperatures. Neither clinical signs nor mortality were detected in both fish species infected with 2017A at 17°C.

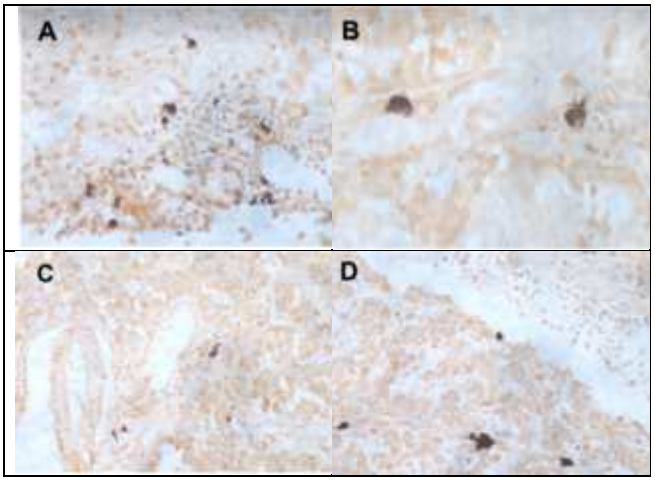
By histology, damage of liver tissues of rainbow trout was visible at 7 dpi and at 38 dpi at the end of the experiments. In the liver tissue of rainbow trout kept at 12 and 17°C, both TiLV isolates induced necrosis (Fig. 6).

At the end of the experiment, both virus variants were detected by ISH in the liver tissues of rainbow trout (Fig. 7) which had not shown any clinical sign of disease. Virus nucleic acid bearing cells were found mainly in necrotic areas (Fig. 7A) when rainbow trout were kept at 17°C in cohabitation with tilapia infected with 2017A. In the liver of rainbow trout kept at 12°C with tilapia infected with 2017A and with 2017B at 12 and 17°C, virus infected cells were visible in normal liver tissues (Fig. 7B, C and D).



*A: negative control, B: 7 dpi of tilapia infected with 2017B at 12°C, C: 7 dpi of tilapia infected with 2017B at 17°C, D: 7 dpi of tilapia infected with 2017A at 12°C, E: 7 dpi of tilapia infected with 2017A at 17°C.

Fig. 6. HE staining of section from samples obtained from rainbow trout after cohabitation with TiLV infected tilapia (ip injection) with necrosis of liver tissues.



*A: 39 dpi of tilapia at 17°C with 2017A, B: 38 dpi of tilapia at 12°C with 2017A, C: 41 dpi of tilapia at 17°C with 2017B, D: 42 dpi of tilapia at 12°C with 2017B.

Fig. 7. Detection of TiLV by ISH in liver tissue of rainbow trout with samples from the end of the experiment.

Additionally, virus re-isolation with samples from rainbow trout at the end of the experiment was possible onto E-11 cells (Table 4A and Table 4B).

Table 4A. Detection and re-isolation of TiLV 2017A from rainbow trout samples of the survivors (except tilapia) at the end of the experiment (%)

assay / fish	RT-qPCR		cells no. 1335		ISH
	12°C	17°C	12°C	17°C	
tilapia	29	35	0	50	+
rainbow trout	0	16	28	41	+

Table 4B. Detection and re-isolation of TiLV 2017B from rainbow trout samples of the survivors (except tilapia) at the end of the experiment (%)

assay / fish	RT-qPCR		cells no. 1335		ISH
	12°C	17°C	12°C	17°C	
tilapia	33	35	100	100	+
rainbow trout	55	0	9	4	+

3.4 Serology by SNT

Frozen serum samples were thawed on ice once and used for SNT immediately. Neutralizing antibodies from survivors were detected in all used fish species. Especially in crucian carp, almost 100% produced neutralizing antibodies against both TiLV isolates independently of the infection rate of the tilapia. The highest titres of all fish used in the experiment were found in group 1 with 2017A after ip infection of tilapia (Table 5A). In group 3 (ip with 2017B), tilapia had not released sufficient virus to the crucian carp. Therefore, only 63% responded with antibodies at relatively low titres.

Table 5A. Results of SNT using sera from tilapia, crucian carp and carp after infection and cohabitation with infected tilapia

isolates / fish	2017A		2017B	
	immersion	ip	immersion	ip
tilapia	50* (200)**	50 (200)	75 (200)	75 (200)
carp	50 (100)	37.5 (50)	75 (162)	50 (50)
crucian carp	100 (125)	100 (450)	100 (150)	63 (75)

* % positive sera, ** rec. average antibody titre against TiLV

During the experiment with rainbow trout and tilapia, the latter had not survived at 12°C after ip infection with both TiLV variants. The antibody titres from tilapia at 17°C were similar. In contrast to tilapia, rainbow trout reacted against both isolates at both temperatures almost constantly with almost 100 % at a similar titre (Table 5B).

Table 5B. Results of SNT using sera from tilapia and rainbow trout after infection and cohabitation with infected tilapia

isolates / fish	2017A		2017B	
	12°C	17°C	12°C	17°C
tilapia	-*	100** (200)***	-	100 (100)
rainbow trout	100 (300)	100 (380)	83 (273)	100 (350)

* no survivors, ** % positive sera, *** rec. average antibody titre against TiLV

4 Discussion

Recently, the world has turned its attention to an artificial phenomenon, global warming. Global warming of air and water additionally involves disadvantages for global aquaculture but also for wild fish in freshwater and marine habitats [19],[20]. Besides their migration into new environments, the migrating species also have taken along new pathogens into the newly colonized area [21].

Based on this observation it must be assumed that viruses from warm water regions may be able to invade cold-water areas, e.g., European fresh and marine waters, due to international trade or accidentally with ballast water from ships. A possible scenario has taken

place with a pathogen that can be devastating for tilapia production, the "Tilapia Lake virus (TiLV)". Tilapia are the second most commonly cultured fish in aquaculture worldwide in fresh and brackish water [22]. Due to the already published mass mortality in tilapia farms in e.g., Ecuador, Israel, Thailand and India, the causative agent is suspected to induce the disease TiLVD in cultured and wild tilapines only [22]. This chapter also provides evidence that more species can be infected by TiLV.

Similar symptoms of disease can be observed in a wide range of fish after infections with *Aphanomyces invadans* (EUS), infectious spleen and kidney necrosis virus (ISKNV)-like viruses, red sea bream iridoviral disease (RSIVD) and viral encephalopathy and retinopathy (VER) [23]. In a few publications it has been reported that other fish than tilapines were cohabitated with infected tilapia to assess the risk of a possible threat. [12] used common carp, walking catfish, striped snakehead, climbing perch, silver barb and Asian sea bass to prove that TiLV does not induce disease or mortality. In-depth analyses were not carried out. A working group in India [24] investigated the susceptibility of rohu (*Labeo rohita*) to TiLV. In their experiment, no TiLV was present after cohabitation of rohu with severely infected tilapia by RT-qPCR and by histology. In contrast, [25] analyzed different ornamental African Cichlids for TiLV by RT-qPCR, ISH and re-isolation onto E-11 cells. The results show that these cichlids are susceptible to the infection but also to the disease.

All these investigations were carried out under local conditions in warm water. The aim of the present study was to investigate the ability of TiLV to infect fish domestic in Europe under normal and abnormal conditions, e.g. warm water for carp and crucian carp or cold and warmer water for rainbow trout. Investigation of the samples was expanded using RT-qPCR, semi-nested PCR, virus re-isolation, ISH using negative fish samples for all assays with the serological response of the infected fish by SNT. If neutralizing antibodies are present, the virus is replicated in the fish. Additionally, fish were always cohabitated with infected tilapia (ip injected or immersed) to mimic a natural infection.

The initial step was the replication of different TiLV isolates onto different fish cell lines including E-11 (1335). Amazingly, the isolates were replicated to high titres onto E-11 (1335), FHM (57), ZF-4 (1492) and TiB (1550) cells but hardly onto common carp cells (CCB (816), CCM-R (1543)) and cells obtained from grass carp (1542). Cells from seabream (SAF-1 (826)) faded out the virulent isolate 2017A after two passages but increased replication with the almost avirulent isolate 2017B up to passage 5. It is likely that TiLV had a tropism to more than one species, at least by cell culture replication. So far, we have no explanation why the avirulent virus was replicated but not the virulent variant after two passages. Further investigations will be necessary on the level of immune response and cell receptors.

While carp and crucian carp were kept in an almost permissive temperature for tilapia (22°C), rainbow trout and tilapia were adapted and exposed to 12°C and 17°C water temperature. The latter temperature is not convenient for salmonids and tilapia, but 12°C is definitely too cold for tilapia. In the experiment, mortality events of course were observed in tilapia but also in crucian carp at 22°C and to a low extent also in rainbow trout at 12°C. No clinical sign or mortality was observed in rainbow trout kept at 17°C independently of the isolates. As expected, tilapia showed clinical signs, e.g. bleeding in the skin, refusal of feed or standing in a silent corner of the aquarium, after ip injection with both isolates at all water temperatures.

Over the entire experiment, clinical signs additionally only were observed in crucian carp. They showed darkening of the skin, massive bleedings in the skin and fins, abnormal swimming behavior, hiding and/or refusal of feed. Rainbow trout showed no external clinical signs but refusal of feed and isolation from covey. These were the fish that died at 12°C water temperature. While TiLV was detectable over the entire duration of the experiments, our main interest was to search for latent or persistent virus at the end of the first experiment.

TiLV was present by RT-qPCR and ISH in all fish species but not in any single fish. The same was found with re-isolation onto E-11 cells.

The detection and re-isolation rates fluctuated between 100% and 0 depending on the isolate and the fish species always in connection with the water temperature. Surprisingly, all species developed neutralizing antibodies against both TiLV isolates. The surviving fish showed a humoral immune response to TiLV between 37.5 % (carp with 2017A, ip infection of tilapia) and 100% mainly in crucian carp and rainbow trout. The highest antibody titres were detected in sera from crucian carp, while almost all rainbow trout were serum antibody positive. The only exception was a rate of 83% of the surviving rainbow trout after ip injection of tilapia with isolate 2017B at 17°C water temperature.

Summarizing all results from the first experiment, it is likely that TiLV may present a risk for the European fish population under inappropriate environmental conditions. Therefore, global warming can turn out to be a threat in terms of new viruses normally adapted to warm water fish. Nevertheless, it also depends on the biodiversity of the wild and farmed fish populations, their genetics and adaption ability. Not every infection will effectively lead to disease and not all fish will be infected with a newly occurring virus. However, there is a hypothetical possibility. Furthermore, experiments are being carried out using common carp and crucian carp at 25°C and rainbow trout at 17°C to re-transmit TiLV to the same species including tilapia. The results of these experiments are currently waiting for analysis with all mentioned assays.

Further experiments will be necessary, especially with fish requiring the same permissive temperature as tilapia between 24 and 29°C water temperature, such as e.g. sturgeon (*Acipenser sp*.) and tench (*Tinca tinca*). They cover a broad range of wild and cultured fish. This scenario may represent a possible future process induced by fish and other aquatic animals, by trade, by pathogens, by immune response influenced by the genetic identity of the fish and in connection with global warming of the waters.

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