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ORIGINAL ARTICLE

It is everywhere—A survey on the presence of carp edema virus in carp populations in Germany

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Abstract

Carp edema virus (CEV) is the causative agent of koi sleepy disease (KSD), a serious gill disease affecting common carp, *Cyprinus carpio*, and its ornamental variety, koi. After recent detections of the virus in various countries around the world, KSD has emerged as a new global disease in carp. However, the prevalence of the infection in carp populations in a given geographical region has not been studied thoroughly. The present communication reports an investigation into the presence of CEV in carp and koi populations in Germany. For this purpose, gill samples collected from carp and koi populations suffering from gill diseases or collected for a routine examination of their health status were tested for the presence of CEV by PCR. In total, 651 fish samples from 401 carp or koi cases were examined in 2015 and 2016, additional 118 samples from previous studies were included in the examination. CEV was detected in archive samples from carp dating back to 2007, and in koi samples dating back to 2009. From 2015 to 2016, CEV was detected in 69% of cases from carp populations examined from the main carp-producing areas in Germany, and in 41% of the examined cases from koi populations from all over Germany. Clinical KSD occurred mainly from April to June in carp populations at water temperatures ranging from 8 to 12°C and in koi populations at water temperatures ranging from 18 to 22°C. Most fish from clinically affected carp or koi

populations harboured high virus loads of above 10,000 copies of CEV-specific DNA per 250 ng DNA, while gills from fish of other fish species from the ponds, including goldfish, grass carp and European perch were found CEV negative or harboured a low virus load. A phylogenetic analysis revealed the presence of multiple CEV variants from genogroup I in carp and genogroup II in koi populations in Germany. Genetically identical genogroup I isolates were detected in carp from different geographical locations in Germany and in other European carp populations. Some German genogroup II variants were identical to variants previously recorded from koi in Asian and other European countries. The data presented here show that CEV is highly prevalent in German common carp and koi populations and implies the spreading of this virus by intense trading of common carp and koi without necessary risk mitigating measures. As infections with this virus may induce serious disease, CEV diagnostic should be included in health surveillance and disease monitoring programmes.

KEYWORDS
Carp edema virus, common carp, koi, koi sleepy disease, survey

1 | INTRODUCTION

Common carp, *Cyprinus carpio*, is an important fish for inland aquaculture and the ichthyofauna in Europe and Asia (EU, 2012). Its coloured variety, the koi, is also very popular as an ornamental fish. Germany is one of the largest producers of common carp in continental Europe and is also an important market for ornamental fish. Despite its popularity, common carp suffers from several health problems caused by infectious agents, which seriously affect carp production and trade. Among these diseases, koi herpesvirus disease (KHVD) caused by infection with cyprinid herpesvirus 3 (CyHV-3) has been considered as the greatest threat for carp and koi for about 20 years (OIE, 2019). The risk of introducing this infection into carp stock was considered so serious that this pathogen was classified as a notifiable disease by the World Organisation of Animal Health (OIE) (OIE, 2019). The focus on CyHV-3, however, might have led to the neglection of other viral pathogens for years, with carp edema virus (CEV) being an example thereof.

CEV is a DNA virus belonging to the family *Poxviridae*. It mainly infects gills of common carp and its ornamental variety, koi (Adamek, Oschilewski, et al., 2017; Ono et al., 1986), causing hypertrophy of cells from the branchial respiratory epithelium, fusion of secondary gill lamellae, gill swelling and gill necrosis (Adamek, Oschilewski, et al., 2017; Miyazaki et al., 2005). These pathological changes are considered to induce respiratory distress and lethargic behaviour followed by death due to anoxia (Miyazaki et al., 2005). In addition to lethargy, affected fish are often seen lying on the bottom of the tank on one side of their body (Adamek, Oschilewski, et al., 2017). The disease is therefore called "koi sleepy disease" (KSD). Interestingly, the clinical signs and mortality can be prevented and reduced by the addition of salt to keeping waters (Miyazaki et al., 2005). Outbreaks of the disease with significant mortality (up to 80%) have been observed in koi populations at a temperature range of 15–25°C, while the disease has been described in common carp at a lower temperature range of between 6 and 9°C (Way et al., 2017).

KSD caused by infection with CEV was initially detected in koi populations in Japan in the 1970s (Murakami et al., 1976). Since then, the virus has been found in ornamental koi and carp in North America (Hedrick et al., 1997; Lovy et al., 2018), in many European countries including the UK (Way & Stone, 2013), the Netherlands and France (Haenen et al., 2016; Haenen et al., 2014), Austria (Lewisch et al., 2015), Germany (Jung-Schroers et al., 2015), Poland (Matras et al., 2017), Hungary (Adamek et al., 2018), the Czech Republic and Slovakia (Matějíčková et al., 2020), Serbia (Radosavljevic et al., 2018), Italy (Marsella et al., 2021) and Croatia (Zrnčić et al., 2020) as well as in Asia besides Japan and China (Luo et al., 2020; Zhang et al., 2017), India (Swaminathan et al., 2016) and Thailand (Pikulkaew et al., 2020). Although the virus appears to be present in European koi and carp populations, its prevalence and virulence are not well studied. CEV is not replicated in any of the routinely used cell cultures used for the detection of carp virulent viruses, and PCR-based methods for the detection of virus-specific DNA of all genetic variants were developed only recently (Adamek, Matras, et al., 2017; Matras et al., 2017). Hence, in previous years, several disease outbreaks in carp populations associated with gill pathology remained unexplained and putatively could have been caused by CEV. Therefore, in the present investigation, carp and koi from all over Germany suffering from diseases with gill pathology, which was not related to a known viral pathogen, in particular to an infection with CyHV-3, bacterial or parasitic pathogens, were tested for the presence of CEV. In addition, specimens from carp and koi populations submitted for a routine health examination without any signs of a disease were included to the survey. Further information on the cases, including observed signs of disease, mortality or water temperature were requested in a survey form.

TABLE 1 Fish species examined and detection of carp edema virus genetic material in individuals of these species in a survey in Germany (2015/2016)

Fish species		Status of infection	
Scientific name	Common name	CEV positive/ Examined	Virus load
Cyprinidae			
Carassius auratus	Goldfish	0/11	–
Ctenopharyngodon idella	Grass carp	1/11	4
Cyprinus carpio	Common carp, farmed	62/131	$1–6.5 \times 10^6$
Cyprinus carpio	Ornamental koi	179/480	$1–4.0 \times 10^6$
Leuciscus idus	Ide	0/1	–
Myxocyprinus asiaticus	Chinese sucker	0/2	–
Rhodeus amarus	European bitterling	0/1	–
Scardinius erythrophtalmus	Rudd	0/1	–
Tinca tinca	Tench	0/3	–
Percidae			
Gymnocephalus cernua	Ruffe	1/1	16
Perca fluviatilis	European perch	2/5	3–10
Sander lucioperca	Pike-perch	1/1	1.2×10^3
Esocidae			
Esox lucius	Northern pike	2/3	1–65
Total	248/651		

2 | MATERIAL AND METHODS

2.1 | Sample collection

Samples for this study were collected from fish submitted by practicing veterinarians and regional fish health services from all over Germany, or were collected from fish submitted for disease diagnostic to the consulting service at the Fish Disease Research Unit (FDRU), Veterinary University Hannover, Germany. The sampling was performed according to best veterinary practice and to rules valid for sample collection for diagnostic purposes in Germany. The animals were not subject to a regulated experimental procedure. All samples were sent to FDRU and examined for the presence of CEV as described below. The submitted sample material included gill samples fixed with isopropanol, as well as, complete moribund alive or deceased frozen fish from diseased stocks with an unknown genesis of a gill disease. Samples were also collected from fish submitted to determine the health status as part of a routine examination. In total, 273 fish samples were collected in 2015 and 378 in 2016. In addition, 91 specimens were analysed, which had been collected from 2009 to 2014 from koi with clinical symptoms suggestive of an infection with CyHV-3, but found negative for CyHV-3 DNA sequences. A further 27 specimens of farmed common carp were included from field studies on the presence of CyHV-3 performed in 2007 to 2014. During the survey in 2015 and 2016, samples were mainly collected from common carp and from its ornamental variety (koi), but also from additional fish species kept together with carp or koi in the same ponds or tanks (Table 1). From each fish, gill tissue was collected and kept at −80°C until further analysis.

In order to collect further data on the disease pattern caused by CEV, a survey form (Supplementary file 1) was prepared to be submitted together with tissue samples for analysis. This survey form requested data on the origin of diseased fish specimens, presentation of the disease, mortality, water parameters, further pathogens present, as well as information on the possible further spread of the infection.

In CEV-positive samples, the virus load was determined by means of quantitative PCR. A phylogenetic analysis based on the nucleotide sequence of a fragment of the P4a gene was performed to analyse the diversity of variants present in Germany. The data were analysed to provide an insight into the spatial distribution of a CEV infection in carp and koi populations in Germany, the seasonal pattern of CEV-associated disease outbreaks and the virus phylogeny with association with potential differences in the virulence of different genetic variants of CEV.

2.2 | Molecular biological analysis of samples

From each gill sample, approx. 25 mg tissue was collected. After mechanical lysis of the tissue by using a Tissue Lyser II (Qiagen), DNA was subsequently extracted using the QIAamp DNA Mini Kit (Qiagen) in accordance with the manufacturer's instructions. After extraction, the DNA quantity was determined with a Nanodrop ND-1000 (Peqlab) spectrophotometer, adjusted to 50 ng μL^{-1} by the addition of PCR grade water (Thermo Fisher Scientific) and stored at −80°C. For detection of CEV-specific DNA sequences, three different PCR protocols were used: (i) The end-point PCR assay developed by the Centre of Environment, Fisheries and Aquaculture Sciences (CEFAS) and

published by Matras et al. (2017), (ii) the TiHo quantitative (probe) PCR assay published by Adamek et al. (Adamek et al., 2016) and, (iii) the TiHo SYBRGreen quantitative PCR assay published by Adamek et al. (Adamek, Oschilewski, et al., 2017). Primer sequences are presented in supplemental Table 1. The assays were performed as previously described (Adamek, Matras, et al., 2017). A positive result from one of the PCR assays qualified a sample as positive. All samples were examined by all three PCR protocols. In order to obtain a PCR product for determination of the nucleotide sequence, samples were analysed using the end-point PCR from CEFAS. For determination of the virus load, the samples were initially analysed using with the TiHo quantitative (probe) PCR. A comparative analysis of PCR protocols revealed however, that this PCR failed in detecting CEV from genogroup I (Adamek, Matras, et al., 2017). Therefore, all samples were analysed by means of the subsequently developed TiHo SYBRGreen quantitative PCR, which detects CEV variants from both genogroups (Adamek, Matras, et al., 2017). For virus load, results from the TiHo SYBRGreen quantitative PCR assay were used and are presented as genome copies normalized for 250 ng of extracted DNA. The samples were not routinely tested for the presence of CyHV-3.

The PCR products obtained by using the end-point PCR from the CEFAS were sequenced by means of Sanger sequencing (LGC Genomics, Berlin) and compared with sequences of the CEV P4a gene fragment deposited in the GenBank in order to confirm their CEV identity. Overlapping sequences obtained from the samples were trimmed to 357 bp and were analysed with tools available at www.phylogeny.fr (Dereeper et al., 2008). Sequences were aligned with MUSCLE, curated with Gblock and phylogenetic analyses based on maximum likelihood were performed with PhyML. A phylogenetic tree was rendered with TreeDyn (available at www.phylogeny.fr).

2.3 | Statistics

All data were tested for normality and equal variances using the Kolmogorov Smirnov test. Data from the survey form were entered into a Microsoft Excel worksheet and compared by means of Fisher's Exact Test, or Chi Square Test by means of the computer program R version 3.4.1 (R Studio Boston). Odds ratios were displayed using ggplot within R. The geographical distribution of samples was depicted using the software ArcMap 10.3 (ESRI, Kranzberg, Germany) using GIS data obtained from the Federal Agency for Cartography and Geodesy (BKG).

3 | RESULTS

The survey in 2015 and 2016 included samples from 401 cases with a total of 651 fish individuals from 12 different fish species. The majority of samples originated from carp, with 131 samples collected in 74 cases from farmed common carp and 480 samples collected in 327 cases from koi. Another 40 samples were collected from fish individuals from a further 11 species from carp ponds which had been raised in co-culture with carp or which were traded together with koi as cold-water ornamental fish. In the majority of cases, one sample from one individual, or one pooled sample from several individuals from one population was submitted. In 52 cases, samples from two individuals, in 14 cases samples from three individuals, and in 16 cases samples from four up to 40 individuals from the same population were submitted. In two cases, samples from the same population were submitted on two separate occasions. CEV-specific DNA sequences were detected in samples from farmed carp in the proportion 51 from 74 cases (69% CEV positive cases) with 62 from 131 examined individual fish (47% CEV-positive results). In koi CEV was detected in a proportion of 130 from 327 cases (41% CEV positive cases) with 176 from 480 examined fish individuals (37% CEV-positive results). CEV-positive common carp or koi carried the infection at an intensity of 1.0 to 6.5 × 10^6 virus copies per 250 ng DNA isolated from gill tissue. CEV genomic DNA was also detected in gill tissue of some fish specimens from different species when kept together with PCR-positive koi or carp. In gill samples from these fish, CEV-specific DNA was detected at an intensity of 1.0 to 1.2 × 10^3 DNA copies per 250 ng DNA (Table 1).

TABLE 2 Presence of carp edema virus-specific DNA in samples from carp or koi individuals from archive material

Year	Farmed carp positive/total	Ornamental koi positive/total	Total positive/total
2007	7/7	n.t.	7/7
2008	7/7	1/1	8/8
2009	n.t.	7/16	7/16
2010	9/11	6/23	15/34
2011	n.t.	5/14	5/14
2012	n.t.	n.t.	n.t.
2013	n.t.	1/2	1/2
2014	2/2	19/35	21/37
Total	25/27	39/91	64/118

n.t.: no specimens tested.

Archive samples from koi with clinical signs suggestive of an infection with CyHV-3 and from farmed carp collected in CyHV-3 monitoring programmes dated back to 2007. In these samples, CEV DNA could be detected in gill samples from farmed carp from 2007 to 2014 and in gill samples from koi from 2009 to 2014 (Table 2).

3.1 | Spatial distribution of carp edema virus positive findings

During the survey in 2015 and 2016, gill samples were submitted from various regions of Germany. However, samples originated in particular from locations in Northern Germany in the vicinity of FDRU, and from the carp-producing areas in Bavaria and Saxony. Information on the geographical origin of the samples was available for 407 cases (401 cases in 2015 and 2016, and samples from additional 6 cases from archive material in 2007/2008). An overview of the geographical distribution of samples from the cases submitted for analysis and the presence of CEV DNA in these cases is given in Figure 1(a). Samples from

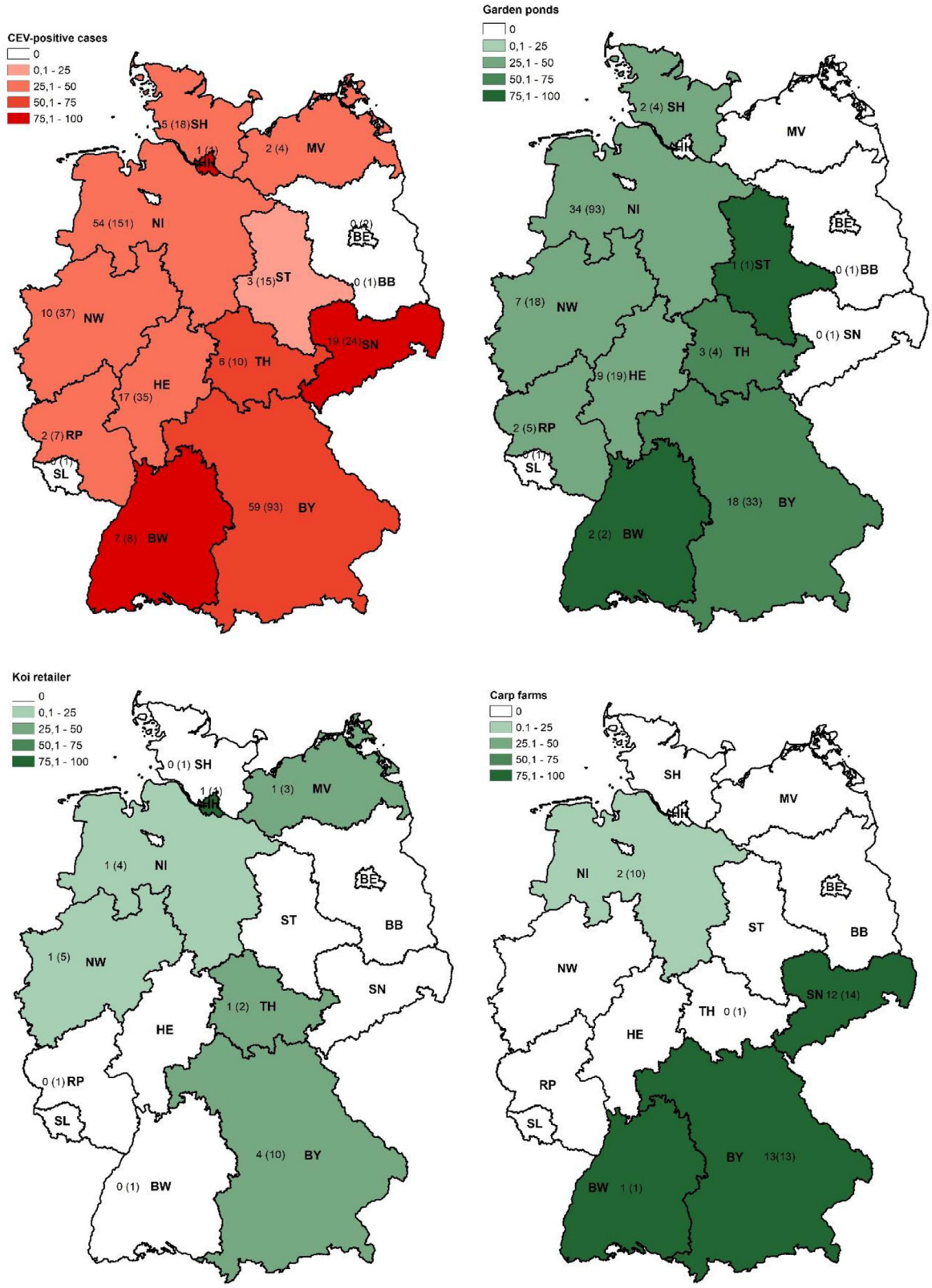


FIGURE 1 Geographical distribution of samples examined for the presence of carp edema virus (CEV) genetic material in ornamental koi and farmed carp populations in Germany in 2015 and 2016. (a) Presence of CEV in all submitted cases from different regions of Germany. Presence of CEV in koi cases submitted (b) from private garden ponds, (c) from koi retailers, and (d) in carp cases from carp farms. Given is the number of CEV positive cases in relation to the total number examined (in brackets). BB: Brandenburg, BE: Berlin, BW: Baden Wurttemberg, BY: Bavaria, HH: Hamburg, HE: Hesse, MV: Mecklenburg-Western Pomerania, NI: Lower Saxony, NW: North Rhine-Westphalia, RP; Rhineland-Palatine, SH: Schleswig-Holstein, SL: Saarland, SN: Saxony, ST: Saxony-Anhalt, TH: Thuringia

koi were mainly submitted from private garden ponds and from ornamental fish trading enterprises. In total, 182 cases, of which 78 were CEV positive (43% CEV positive), were submitted from private garden ponds located in various regions of Germany (Figure 1(b)). In total, 28 cases, of which nine were CEV-positive (32% CEV-positive), were submitted from stocks of ornamental fish retailers distributed over Germany (Figure 1(c)) and 38 cases with 15 CEV-positive originated from ornamental fish wholesalers (39% CEV-positive). Samples from farmed carp were mainly submitted from ponds on carp farms, in particular when clinical signs of a disease were observed. Therefore, most samples originated from carp-producing areas in Bavaria and Saxony (Figure 1(d)). However, an additional number of carp samples originated from different sources, including natural waters, angling ponds, or were submitted without providing any information on the husbandry system. Therefore, these latter samples are not included in Figure 1(d).

3.2 | Seasonal distribution of carp edema virus-positive samples

Fish samples found positive for CEV-specific DNA sequences were detected throughout the year, however, the majority of these cases occurred in April, May and June. An overview of the seasonal distribution of CEV detection in cases submitted during the survey in 2015 and 2016 is given in Figure 2(a). Information concerning the water temperature in the husbandry system at the time of sampling was given for a limited number of cases only. Based on the information available, clinical disease associated with a confirmed presence of CEV genetic material was observed at a water temperature as low as 5°C and as high as 23°C. In farmed carp, the majority of cases occurred at a water temperature of between 8 and 12°C, while in koi, the majority of cases were reported at a water temperature of between 18 and 22°C (Figure 2(b)).

3.3 | Clinical signs characteristic of an infection with carp edema virus

The current survey included samples from 212 cases in which carp or koi submitted for the diagnosis of a CEV infection displayed clinical signs of a disease. In 123 of these cases (58.0%; 95% confidence interval 45.8–60.0%), the presence of CEV-specific DNA sequences was confirmed. Irrespective of whether the presence of CEV-specific DNA was confirmed from the specimens of a case or not, various clinical signs were reported from the cases of farmed carp or ornamental koi submitted for pathogen detection. Most of these clinical signs were not recorded from all cases. An overview of clinical signs observed in cases from carp and koi with or without a confirmed CEV infection is presented in Table 3. Based on the information provided in the filled-in survey form, apathy, anorexia, gill swellings, gill necrosis and enophthalmia were characteristic clinical signs of a CEV infection in farmed carp and koi in the current survey. In order to identify clinical signs characteristic of the disease associated with a CEV infection in carp and koi cases separately, an odds ratio and a confidence interval for the

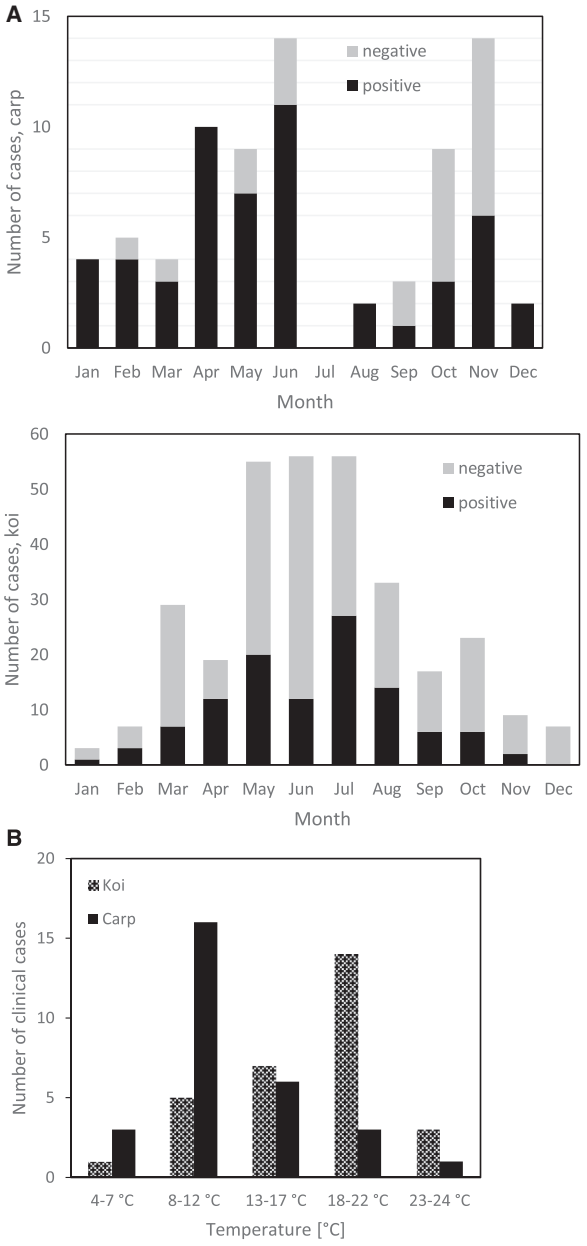


FIGURE 2 (a) Seasonal detection of carp edema virus (CEV) in cases of farmed carp (left panel) and ornamental koi (right panel) during a survey conducted in Germany from 2015 to 2016. Grey bars: uninfected, black bars: infected cases. Average water temperature in small lakes in Germany: Jan–Mar: 2°C, April: 5°C, May: 8°C, June: 14°C, July: 18°C, Aug: 19°C, Sep: 17°C, Oct: 13°C, Nov: 9°C, Dec: 5°C. (www.wassertemperatur.org). (b): Presence of clinical disease with a confirmed CEV genetic material in stocks of farmed carp and ornamental koi in Germany at different water temperatures. $N = 28$ cases for carp and 30 cases for koi, for which information on water temperature was reported

strength of the association between the presence of a particular clinical sign and a CEV infection were calculated (Figure 3). When cases from carp were considered separately, apathy was significantly associated with clinical disease related to a CEV infection while in koi, apathy and enophthalmia were associated with CEV infection (Figure 3). Clinically affected farmed carp or koi belonged to all age classes, from K_1

TABLE 3 Clinical signs reported from carp and koi cases submitted for the carp edema virus survey from 2015 to 2016

Clinical sign	Farmed carp Neg./Pos.*	Ornamental koi Neg./Pos.*	Sum Pos.*/Total	% CEV positive	*p*
Anorexia	2/21	37/45	66/105	62.9	.001
Apathy	2/24	24/29	53/79	67.1	<.001
Edema	1/4	9/8	12/22	54.6	1.000
Enophthalmos	1/23	17/16	39/57	68.4	.002
Exophthalmos	0/1	9/5	5/15	40.0	1.000
Lying on the bottom	0/7	30/27	34/64	53.1	.570
Gill necrosis	1/17	19/23	40/60	66.7	.030
Gill swelling	1/18	17/19	37/55	67.3	.010
Mucosal detachment	1/11	13/13	24/38	63.2	.240
Skin haemorrhages	2/11	33/34	45/80	56.3	.150
Skin ulceration	3/11	33/34	27/64	42,2	.270
With clinical signs	4/32	85/91	123/212	58.0	nt
Mortality	3/30	52/40	70/125	56.0	nt
Without clinical signs	10/3	27/8	11/48	22.9	nt
Mortality	1/1	11/2	3/15	20.0	nt
No information	8/16	86/31	47/141	33.6	nt
Sum	22/51	198/130	181/401	45.1	

*Negative/Positive for genetic material of CEV.

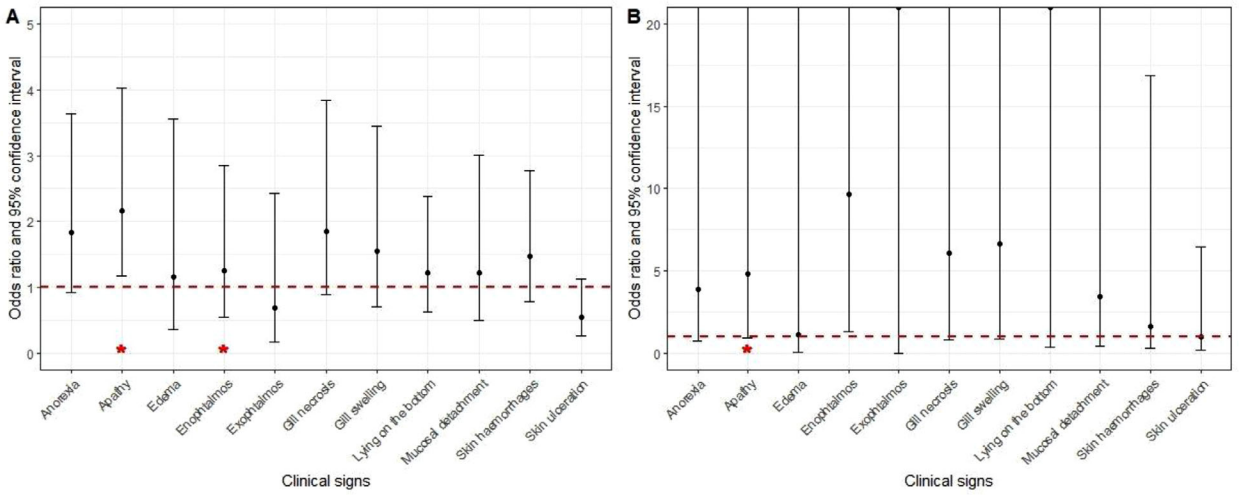


FIGURE 3 Associations between the presence of a particular clinical sign of disease and the detection of carp edema virus specific genetic material. Shown is the odds ratio and the span of the 95% confidence interval of the association as calculated on data provided from (A) 199 koi and (B) 75 carp cases (different scale, in some cases the upper confidence limit is outside the limit of the figure). The red dashed line shows the OR of 1, and the red stars show statistically significant associations

(first year of production) to K_4 (4^{th} year of production with oversized >2 kg market fish) and from older spawners.

In 130 cases with clinically affected farmed carp or ornamental koi, mortality was observed. In 75 cases (56%, 95% confidence interval 49.2–66.2%), an infection of CEV was confirmed by PCR and in these cases, mortality rates varied between a few individuals and up to 100% of the population. However, mortality was also reported in 15 cases without the fish developing further clinical signs of a disease and in fish specimens from 3 of these cases a presence of CEV genetic material was confirmed (Table 3). When the virus-specific DNA load in gills was considered, in most cases where clinical signs of a disease were not reported or in a low percentage only, the fish specimens were PCR-positive with a low virus load of 10 to 1000 copies of CEV-specific DNA per 250 ng DNA (Table 4; Figure 4). In most fish from cases with a

TABLE 4 Presence of clinical signs and virus load in cases of carp and koi submitted for the carp edema virus survey from 2015 to 2016

	Farmed carp				Ornamental koi			
	Clinical signs				Clinical signs			
Cases	with	without	not reported	total	with	without	not reported	total
CEV positive	32	3	16	51	91	8	31	130
Virus load								
1–1000	4	1	1	6	37	4	18	59
1000–10,000	4	0	1	5	28	2	11	41
10,000–1,000,000	15	1	6	22	15	0	2	17
not reported	9	1	8	18	21	3	4	28
CEV negative	4	10	9	23	85	27	86	198

high morbidity and mortality, a high virus load was detected in 10,000 copies of CEV-specific DNA per 250 ng of DNA or higher. This was particularly seen in cases reported in farmed carp (Figure 4).

3.4 | Spatial and temporal distribution of molecular variants of carp edema virus isolates

When the amplicons received from the CEFAS's end-point PCR were sequenced and compared to CEV-specific DNA sequences deposited in the GenBank, the presence of CEV was confirmed in these samples and a considerable variation was observed in the nucleotide sequence of the amplified fragment. A phylogenetic analysis confirmed that sequences obtained from CEV isolates present in farmed carp clustered into one clade (genogroup I) and isolates from koi clustered into a second clade (genogroup II, Figure 5(a),(b)). This observation is in line with the results from previous phylogenetic analyses (Zrnčić et al., 2020).

Isolates from the genogroup II displayed a considerable degree of diversity. Some CEV isolates obtained from koi during the current survey, like KSD-137-16, KSD-161-16 or KSD1-15 (Figure 5(a)) formed a clade together with the sequence J-CyPP-3, originally isolated from koi in Japan by Oyamtasu et al. (1996), the sequences C-CD-2016 and C-WJ2016 from China, and a sequence isolated from koi in the UK (UK_R083), suggesting an Asian origin of this variant. In the present study, genetically identical isolates from this variant were also found in samples collected in different regions of Germany between 2010 and 2016, and the presence of this variant was associated with mortality ranging from 5% up to 50% (Figure 5(a)). The majority of isolates from koi included in the present survey formed a second clade, together with isolates from koi in the UK (e.g., UK_R004, UK_R082, UK_m141), Poland (e.g., PL_112-2015, PL_687_2014, PL_55-2013), but also in China (C_A3) and Korea (Korea_KSWS2-2, Figure 5(a)). In the present survey, several isolates from this clade shared identical nucleotide sequences. For instance, isolates with a nucleotide sequence identical with that from KSD-114-16, KSD-10-11 or KSD-07-10 were repeatedly isolated from koi sampled in various areas throughout Germany during several years (Figure 5(a)). In particular, isolates identical with KSD-07-10 were associated with variable morbidity and mortality.

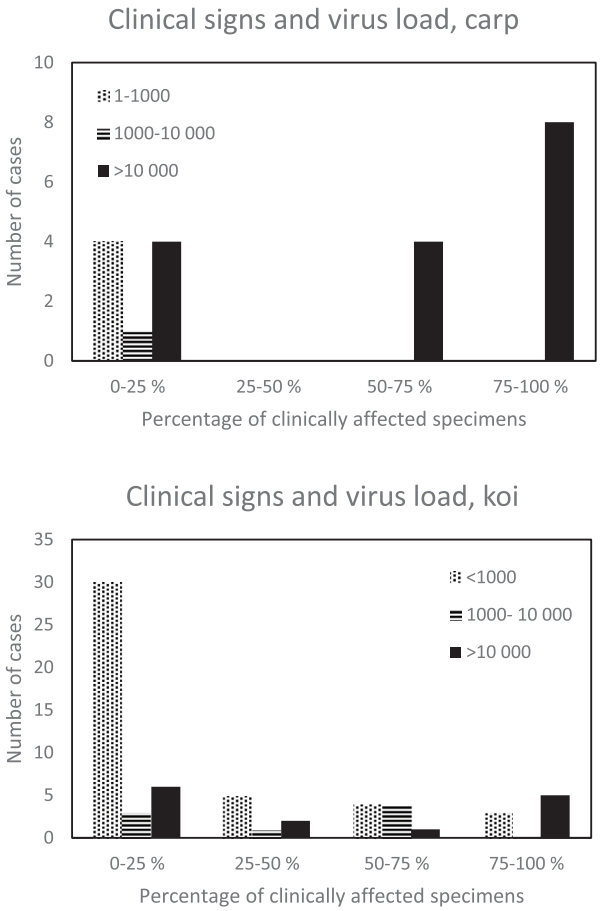
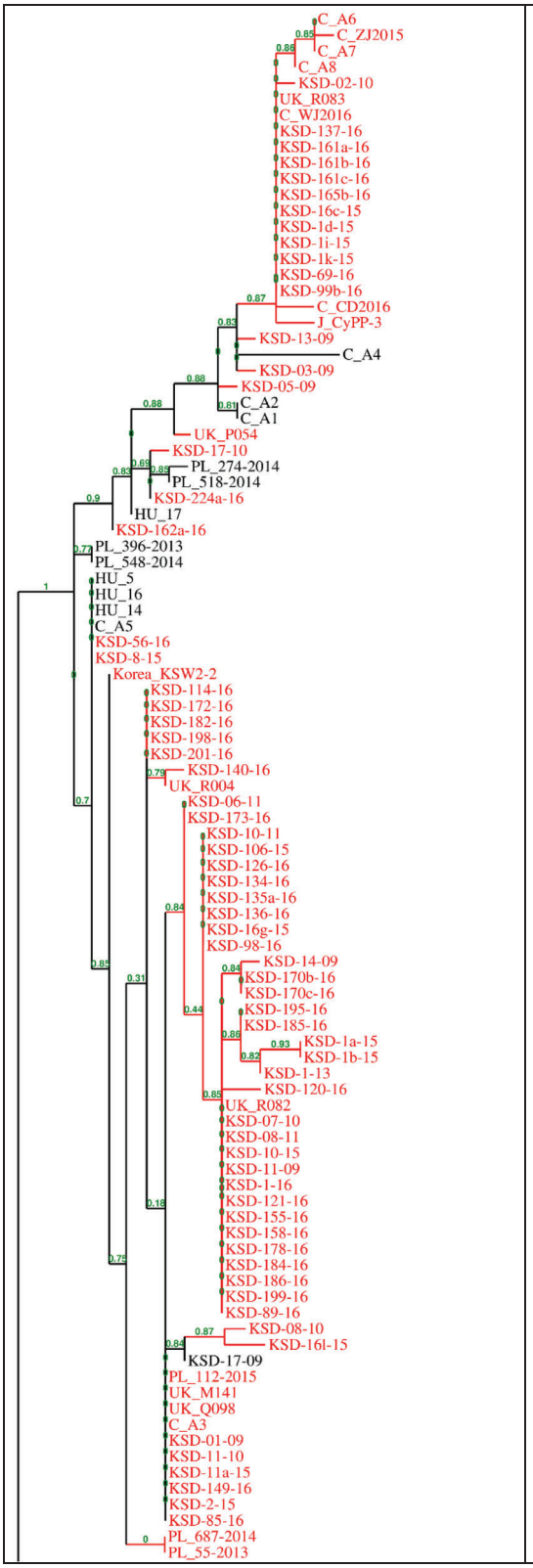


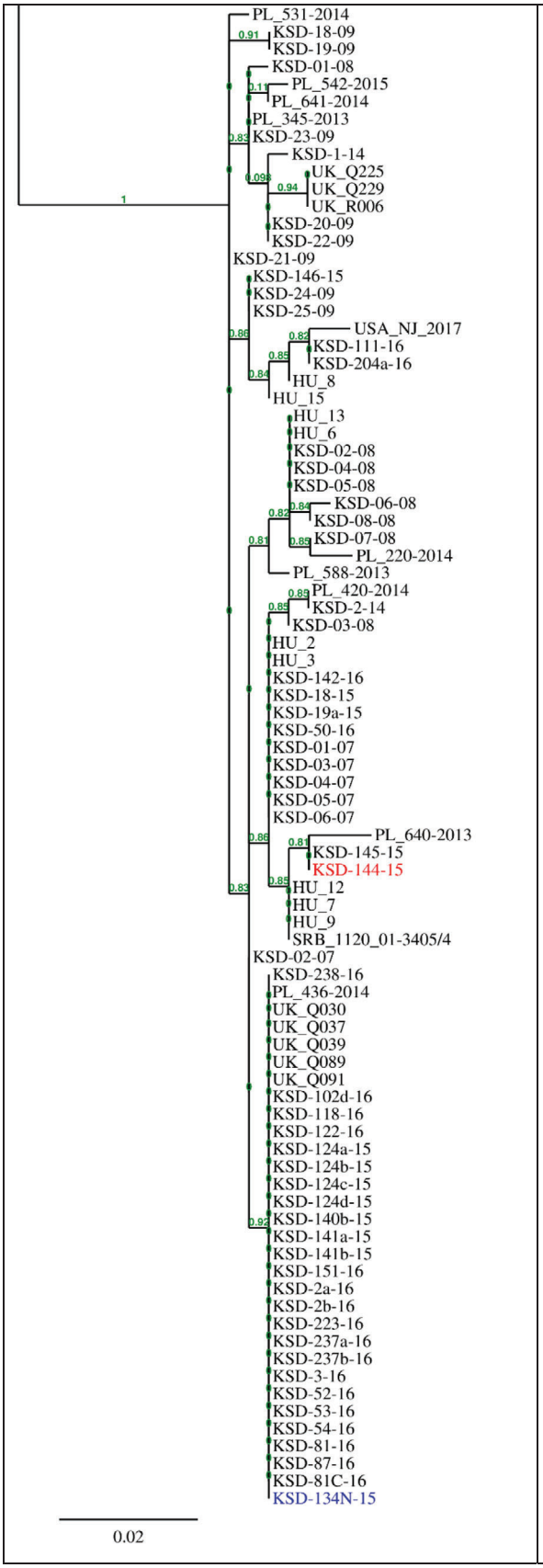
FIGURE 4 Presence of clinical signs in cases of farmed carp and ornamental koi PCR positive for carp edema virus (CEV) in Germany in relation to virus load during a survey in 2015/2016. The virus load was determined as copy number of CEV-specific DNA per 250 ng DNA isolated from gill tissue from suspected specimens. The percentage of clinically affected specimen is given as reported in the survey form

While infections with isolate KSD-178-16 or KSD-161-15 from this variant were associated with high morbidity, cases of clinical disease were not reported for koi in which the presence of the genetically identical isolates KSD-08-11 or KSD 1–16 was confirmed. This suggests



Sample Number	Geographical Origin	Mortality rate
C_A6	China	
C_ZJ2015	China	
C_A7	China	
C_A8	China	
KSD-02-10	**Lower Saxony**	-
UK_R083	United Kingdom	
C_WJ2016	China	
KSD-137-16	**Bavaria**	5%
KSD-161-16	-	-
KSD-165-16	**Lower Saxony**	90%
KSD-16c-15	**Saxony-Anhalt**	-
KSD-1-15	**Thuringia**	30%
KSD-69-16	**Saxony-Anhalt**	-
C_CD2016	China	
J_CyPP-3	Japan	
KSD-13-09	**Lower Saxony**	80%
C_A4	China	
KSD-03-09	**Lower Saxony**	-
KSD-05-09	**Lower Saxony**	-
C_A2	China	
C_A1	China	
UL_P054	United Kingdom	
KSD-17-10	**Lower Saxony**	0%
PL_274-2014	Poland	
PL_518-2014	Poland	
KDS-224-16	**Lower Saxony**	0%
HU_17	Hungary	
KDS-162-16	**Lower Saxony**	-
PL_396-2013	Poland	
PL_548-2014	Poland	
HU-5	Hungary	
HU_16	Hungary	
C_A6	China	
KSD-56-16	**North Rhine-Westphalia**	8%
KSD-8-15	**Schleswig-Holstein**	50%
Korea_KSW2-2	Korea	
KDS-114-16	**Lower Saxony**	-
KSD-172-16	**Lower Saxony**	-
KSD-182-16	**Lower Saxony**	16,7%
KSD-198-16	**Hesse**	0%
KSD-201-16	**Hesse**	-
KSD-140-16	**Bavaria**	10%
UK_R004	United Kingdom	
KSD-06-11	**Hamburg**	0%
KSD-173-16	**North Rhine-Westphalia**	25%
KSD-10-11	**Mecklenburg-West Pomerania**	0%
KSD-106-15	**Bavaria**	-
KSD-126-16	**Rhineland-Palatinate**	15%
KSD-134-16	**Bavaria**	-
KSD-135-16	**Lower Saxony**	0%
KSD-136-16	**Hesse**	0%
KSD-16-15	**Saxony-Anhalt**	-
KSD-98-16	**Lower Saxony**	-
KSD-14-09	**Lower Saxony**	0%
KSD-170-16	**Lower Saxony**	92,1%
KSD-195-16	**Schleswig-Holstein**	25%
KSD-185-16	**Bavaria**	3%
KSD-1-15	**Thuringia**	30%
KSD-1-14	**Lower Saxony**	0%
KSD-120-16	**Bavaria**	20%
UK_R082	United Kingdom	
KSD-07-10	**Saxony-Anhalt**	-
KSD-08-11	**Lower Saxony**	0%
KSD-10-15	**Lower Saxony**	-
KSD-11-09	**Lower Saxony**	-
KSD-1-16	**Bavaria**	0%
KSD-155-16	-	-
KSD-158-16	**Bavaria**	12,5%
KSD-178-16	**Hesse**	93%
KSD-184-16	**Bavaria**	2,5%
KSD-186-16	**Bavaria**	3%
KSD-195-16	**Bavaria**	13%
KSD-88-16	**Bavaria**	10%
KSD-08-10	**Lower Saxony**	-
KSD-161-15	**Bavaria**	80%
KSD-17-09	**Saxony**	-
PL_112-2015	Poland	
UK_M141	United Kingdom	
UK_Q098	United Kingdom	
C_A3	China	
KSD-01-09	**Lower Saxony**	-
KSD-11-10	**Lower Saxony**	-
KSD-11-15	**North Rhine-Westphalia**	3%
KSD-149-16	**Hesse**	0%
KSD-02-15	**Lower Saxony**	0%
KSD-85-16	**Lower Saxony**	0%

FIGURE 5 (a,b) Phylogenetic analysis of the nucleotide sequence of a fragment of the gene encoding the carp edema virus p4a core protein, geographical location of isolation and proportion of clinically affected specimens in the population. The isolates from this survey are denoted in bold. This analysis includes sequences obtained from ornamental koi (denoted in red), grouped in genogroup II (5a) and farmed carp (denoted in black), grouped in genogroup I (5b) from the present survey. In addition, samples were included from previous studies performed in different geographical areas and deposited the in GenBank. The branch length is proportional to the number of substitutions per site. Color code: Mortality rate: green: 0%, yellow: 1–39%, orange: 40–69%, 70–100%, - = not specified



Sample Number	Geographical Origin	Mortality rate
PL_531-2014	Poland	
KSD-18-09	**Saxony**	-
KSD-19-09	**Saxony**	-
KSD-01-08	**Bavaria**	-
PL_542-2015	Poland	
PL_641-2014	Poland	
PL_345-2013	Poland	
KSD-23-09	**Saxony**	0%
KSD-1-14	**Saxony**	80%
UK_Q225	United Kingdom	
UK_Q229	United Kingdom	
UK_R006	United Kingdom	
KSD-20-09	**Saxony**	-
KSD-22-09	**Saxony**	-
KSD-21-09	**Saxony**	-
KSD-146-15	**Baden-Württemberg**	25%
KSD-24-09	**Saxony**	-
KSD-25-09	**Saxony**	-
USA_NJ_2017	USA	
KSD-111-16	**Saxony**	10%
KSD-204-16	**Saxony**	5%
HU_8	Hungary	
HU_15	Hungary	
HU_13	Hungary	
HU_6	Hungary	
KSD-02-08	**Bavaria**	-
KSD-04-08	**Bavaria**	-
KSD-05-08	**Bavaria**	-
KSD-06-08	**Bavaria**	-
KSD-08-08	**Bavaria**	-
KSD-07-08	**Bavaria**	-
PL_220-2014	Poland	
PL_588-2013	Poland	
PL_420-2014	Poland	
KSD-2-14	**Saxony**	8%
KSD-03-08	**Bavaria**	-
HU_2	Hungary	
HU_3	Hungary	
KSD-142-16	**Hesse**	60%
KSD-18-15	**Lower Saxony**	-
KSD-19-15	**Lower Saxony**	-
KSD-50-16	**Saxony**	-
KSD-01-07	**Bavaria**	-
KSD-03-07	**Bavaria**	-
KSD-04-07	**Bavaria**	-
KSD-05-07	**Bavaria**	-
KSD-06-07	**Bavaria**	-
PL_640-2013	Poland	
KSD-145-15	**Baden-Württemberg**	40%
KSD-144-15	**Baden-Württemberg**	10%
HU_12	Hungary	
HU_7	Hungary	
HU_9	Hungary	
SRB_1120_01_3405/4	Serbia	
KSD-01-07	**Bavaria**	-
KSD-238-16	-	-
PL_436_2014	Poland	
UK_030	United Kingdom	
UK_Q037	United Kingdom	
UK_Q039	United Kingdom	
UK_Q089	United Kingdom	
UK_Q091	United Kingdom	
KSD-102-16	**Saxony**	0%
KSD-118-16	**Thuringia**	80%
KSD-122-16	**Saxony**	75%
KSD-124-15	**Saxony**	-
KSD-140-15	**Saxony**	30%
KSD-141-15	**Saxony**	5%
KSD-151-16	**Baden-Württemberg**	30%
KSD-2-16	**Saxony**	50%
KSD-223-16	**Lower Saxony**	0%
KSD-237-16	**Bavaria**	-
KSD-3-16	**Saxony**	-
KSD-52-16	**Saxony**	10%
KSD-53-16	**Saxony**	10%
KSD-54-16	**Saxony**	10%
KSD-81-16	**Lower Saxony**	-
KSD-87-16	**Saxony**	60%
KSD-81-16	**Lower Saxony**	-
KSD-134-15	**Saxony**	0%

FIGURE 5 Continued

that individual variants of CEV were not associated with increased virulence (Figure 5(a)).

The phylogenetic analysis of CEV variants of genogroup I from farmed carp revealed the presence of several variants of CEV as well, however, with a lower genetic diversity (Figure 5(b)). Genetically identical isolates from this genotype were recorded for carp in different areas of Germany, but also for other European carp populations, including Hungary, Poland and the UK. Some variants, like the genetically identical isolates KSD-01-07 as well as KSD-06-07 were isolated from carp sampled in a particular region during a short period, but these were also recorded in carp in other regions of Germany or from carp in other European countries at a later time-point, e.g. HU_2, HU_3, PL-2-14 (Figure 5(b)). The isolates genetically identical with KSD-238-16 were recorded in various regions of Germany as well as in Poland (PL_436-2014) and the UK (UK-Q030 to UK_Q091, Figure 5(b)). Infections with the genetically identical isolates KSD-118-16 and KSD-122-16 were associated with morbidity and mortality in up to 89% of the population, while infections with the genetically identical isolates KSD-223-16 or KSD-134-15 were not associated with clinical disease. This also suggests that individual variants were not associated with increased virulence (Figure 5(b)).

4 | DISCUSSION

CEV was initially reported as originating from various populations of ornamental koi in Japan (Ono et al., 1986; Oyamatsu et al., 1997) in the USA (Hedrick et al., 1997) and in several European countries (Way et al., 2017). Recently, however, this virus was identified in populations of farmed common carp in China, the main producer of common carp globally (Luo et al., 2020; Zhang et al., 2017), and in several major European producers like Poland (Matras et al., 2017), Hungary (Adamek, Baska, et al., 2018), the Czech Republic and Slovakia (Matějíčková et al., 2020), Serbia (Radosavljevic et al., 2018), Italy (Marsella et al., 2021), Croatia (Zrnčić et al., 2020) and Germany (Bachmann & Keilholz, 2016). Except for Japan, where the presence of CEV has been more intensively studied on farms from several regions of the country (Oyamatsu et al., 1997), the other reports describe disease outbreaks in a single population or in a limited number of populations, and do not include an estimation of the spread and the impact of the infection on carp farming or the koi trade. The present study comprises about 651 fish samples originating from a total of 401 cases, submitted for disease diagnostics during a period of approx. two years from regions all over Germany. A presence of CEV-specific DNA was confirmed in a substantial proportion of these samples. Even though the samples were not collected according to a coordinated plan, the results underline that CEV is present in many populations of both koi and farmed carp throughout Germany. In addition, the analysis of archived sample material of carp from previous studies concerning the presence of CyHV-3 or of samples from ornamental koi suspected of being infected with CyHV-3 revealed that CEV had already been present in carp or koi stocks in Germany several years before it was first detected in 2014 (Jung-Schroers et al., 2015). Importantly, the virus was detected in gill samples from koi or carp collected during suspected KHVD outbreaks, which had been found negative for CyHV-3. As CEV and CyHV-3 induce similar clinical signs of a proliferative gill disease, our results show that an infection with CEV could have been confused with KHVD caused by CyHV-3-infection in certain cases (Adamek et al., 2019).

The screening of a higher number of samples collected during two subsequent years (2015–2016) also gave some indication of a seasonal pattern in the presence of a CEV infection and the disease associated with this pathogen. In particular, in farmed carp, CEV was frequently detected in samples submitted in spring and early summer, during April until June, and a second peak occurred in October and November. This correlates with an observation on farms regarding mortality events in carp populations during this time of the year occurring at the end of the hibernation period and the early ongrowing phase when water temperature increases (Bachmann & Keilholz, 2016; Way & Stone, 2013; Zrnčić et al., 2020). In many cases, the aetiology of these "winter kills" or "spring losses" has remained unresolved until now, in particular because an infection with CyHV-3 or spring viraemia of carp virus (SVCV) could be excluded (Zrnčić et al., 2020). Importantly, screening of archival samples from spring carp mortality syndrome (SCMS) described in the UK in the 1980s and 1990s showed high prevalence of CEV (Way et al., 2017). Also, in the Netherlands, the SCMS cases were linked with CEV (Haenen et al., 2016). With the current results, the hypothesis that an infection with CEV might contribute to unresolved losses in spring gains further support with the observation that most clinical cases associated with CEV were observed in carp populations at a temperature range between 8 and 12°C. This water temperature is frequently measured in carp ponds in Germany in April and early May when these mortality events occurred.

In contrast to a biphasic pattern of CEV-positive cases on carp farms in spring and autumn in ornamental koi, CEV-positive specimens were mainly detected in samples submitted from April to August. A larger proportion of positive samples was observed in April, but the infection was also regularly detected in samples submitted between May and July. When the information on water temperature at the time of sampling was considered, clinical disease related with a CEV infection occurred in ornamental koi more frequently at a water temperature ranging between 18 and 22°C, which is measured in ponds in Germany in early summer. Thus, these data suggest that a clinical manifestation of the disease related with a CEV infection occurs in carp at a lower temperature compared to ornamental koi. This might be due to biological differences between CEV variants from genogroup I, which are mainly detected in farmed carp (Way et al., 2017), and isolates from genogroup II, which are mainly recorded from ornamental koi (Way et al., 2017). However, in the present survey, no carp samples were submitted during July and a few samples only in August. In contrast to this, during 2017 and 2020, CEV-related losses were observed in several carp populations in Saxony in July as well (Böttcher, personal observation). These personal observations and the detection of CEV in two samples in August, which in the present study were collected from clinically affected carp, indicate that farmed carp may also be clinically infected with CEV at a higher temperature. In contrast to carp aquaculture, the majority of koi are traded for purposes of stocking

garden ponds in late spring, early summer. Therefore, the development of clinical disease might be observed more often during this period of time. However, to clarify a seasonal occurrence of CEV-related disease, a systematic collection of samples would be required. Likewise, further experimental studies would be needed in order to analyse a correlation between temperature and replication as well as virulence of CEV from different genogroups, because this aspect cannot be verified solely based on field samples.

In populations of both varieties of common carp, clinical signs associated with a CEV infection included gill pathology, like swollen gills and gill necrosis, apathy and anorexia. In the present survey, other clinical signs were also reported in diseased farmed carp or ornamental koi. Nonetheless, these were not significantly related with the presence of a CEV infection. In populations of carp or koi with a high virus load, clinical signs and mortality were observed in a larger proportion of fish. In contrast to populations with less than 10^3 CEV-specific DNA copies, where clinical signs of a disease were recorded in a small proportion of specimens only, populations which were PCR-positive with virus copies of 10^4 and higher suffered from clinical disease or mortality to a greater extent. In particular, in farmed carp, the development of a disease was related with a high load of CEV-specific DNA-sequences in gills. Therefore, in particular in carp, CEV should be considered as a primary cause of disease. This view is supported by results from previous experimental infections performed by Oyamastu et al. (Oyamatsu et al., 1997) and Adamek et al. (Adamek, Oschilewski, et al., 2017). In both studies, clinical disease and mortality could be induced in naive specimen by intraperitoneal injection of a gill homogenate from diseased specimens or by cohabitation with diseased carp. These experimental studies also underlined that carp or ornamental koi suffering from an infection with CEV were susceptible to secondary infections by pathogenic bacteria, such as flavobacteria (Adamek, Teitge, et al., 2018). However, when the bacterial infection was cleared by antibiotic treatment, the clinical disease induced by CEV could not be eradicated as well (Adamek, Teitge, et al., 2018). This underlines the virulence of a CEV infection for carp and ornamental koi. In order to understand the importance of CEV infection for carp farming and the spread of this pathogen in the carp population, a systematic study would be needed.

In European carp aquaculture, fish from different species from the native fish fauna migrate into production or hibernation ponds or are raised in polyculture with carp. This includes cyprinid species such as tench (*Tinca tinca*), bighead carp (*Hypophthalmichthys noblis*) or grass carp (*Ctenopharyngodon idella*), rudd (*Scardinius erythrophthalmus*) and roach (*Rutilus rutilus*) as well as European perch (*Perca fluviatilis*), pikeperch (*Sander lucioperca*) or northern pike (*Esox lucius*). In addition, goldfish (*Carassius auratus*) and golden ide (*Leuciscus idus*) are frequently traded together with koi and kept as ornamental fish in garden ponds. Experimental studies previously confirmed that clinically affected koi or common carp were able to transmit the infection to naive carp by cohabitation (Adamek, Oschilewski, et al., 2017). This would suggest that clinically affected koi or carp shed infective virus particles, which in turn could transfer the infection to specimens from other fish species as well. In the current survey, gill samples from various fish species, which cohabitated the same water body with clinically affected koi or carp were analysed for the presence of CEV-specific DNA sequences. In these samples, CEV DNA was either not found, or it was found in low copy numbers only. Comparable results were obtained in an extensive experimental study (Matras et al., 2019), in which fish from different fish species were kept together with carp PCR-positive for CEV. In this earlier study, either no or very low levels of CEV-specific DNA (Cq levels of 34 to 43) were detected in gill samples as well (Matras et al., 2019). In addition, CEV- specific DNA could not be detected in gill samples from different fish species from a natural lake in Minnesota, USA where CEV-related mortality in carp was observed (Tolo et al., 2021). CEV DNA also could not be detected in gill samples of invasive round goby (*Neobobius melanostomus*) from various locations in Poland and Germany (Jin et al., 2020). In particular, the results of Matras's study (Matras et al., 2019) and the present data suggest that CEV primarily infects carp and koi and that replication of the virus most likely does not occur in other fish species. Thus, according to our assessment, fish from other species than common carp might only play a minor role in the spread of CEV through passive transfer of the virus.

Comparison of the nucleotide sequences of a fragment of the P4a gene of CEV amplified by the PCR used in the present study revealed that koi and carp were PCR-positive for different genetic variants of CEV, as has been established in previous studies (Matras et al., 2017; Way et al., 2017). Generally, two genogroups of CEV with a considerable degree of genetic difference of 6–10% between the lineages were found in koi and carp (Matras et al., 2017; Way et al., 2017). The isolates from koi and carp obtained in our study could be divided into these two main genogroups in which several genetic variants could be recognized. In line with previous studies, the German virus isolates from koi clustered with genetic variants of the CEV genogroup II from koi and carp from Asia and isolates from farmed carp clustered with variants of the genogroup I. Variants from genogroup I have previously been found in European farmed carp but were also reported in USA waters (Lovy et al., 2018; Padhi et al., 2019). Recently, an additional genogroup III was proposed for CEV isolates from Austria (Soliman et al., 2019). In the current analysis, none of the isolates were classified into this proposed genogroup III. In addition, we could not replicate the results from Soliman et al. (Soliman et al., 2019). However, as already described by Zrncic et al. (Zrnčić et al., 2020), we found that for the sequences belonging to this newly proposed genogroup III and deposited in the GenBank, the nucleotide sequences were entered in reverse order. Thus, the existence of the proposed genogroup III is questionable and should be confirmed by sequencing other larger fragments of the CEV genome.

The ornamental fish trade was shown to be a significant factor for spreading other contagious fish viruses to Europe. Imported viruses were linked with severe mortality epizootics of for example CyHV-3 in koi in several European countries (Haenen et al., 2004), Cyprinid herpesvirus 2 (CyHV-2) in goldfish in Germany and the Netherlands (Adamek et al., 2017; Ito et al., 2017) or infectious spleen and kidney necrosis virus (ISKNV) in several ornamental fish species in Germany (Jung-Schroers et al., 2016). Taking a closer look at the CEV variants isolated from koi and carp in the present study revealed that several variants of the genogroup II clustered together with isolates recorded

in koi in Japan or China. Our phylogenetic analysis revealed that isolates from the same variant or from closely related variants had been previously recorded in koi in Poland, the UK or Hungary as well (Figure 5(a)). In the present study, CEV-positive koi were detected in the ornamental fish stock of wholesalers and retailers. Furthermore, in an earlier study, CEV of genogroup II was detected in eight out of 12 koi consignments from Asia, mainly in specimens showing no clinical signs (Adamek et al., 2016). This underlines that the supposition that CEV can be spread through the koi trade is correct, and that these isolates represent Asian variants which are imported to Europe including Germany via the international koi trade. Further genogroup II variants were recorded in several unrelated cases from different regions all over Germany as well as from the UK and Poland during the entire survey period. The presence of isolates from the same genetic variant in koi from various geographical locations and during different years suggests that these cases are related, by import from the same sources in Asia or by an intra-European trading relationship.

CEV variants from the genogroup I are currently restricted to populations of farmed carp in Europe (Zrnčić et al., 2020), with the exception of several cases, where genogroup I isolates were recorded from common carp in USA waters (Lovy et al., 2018; Padhi et al., 2019). This could be related to the fact that carp were introduced to USA from Germany in the 19th century. The CEV genogroup I could have been introduced at that time or during several later introductions of common carp from Europe to the USA (Lovy et al., 2018). The phylogenetic analysis of the CEV genogroup I isolates from carp obtained in the present study revealed that genetically identical isolates were obtained from carp populations in different regions of Germany and from carp in several European countries, like Poland, the UK or Hungary (Adamek, Baska, et al., 2018; Matras et al., 2017; Way et al., 2017) as well. The presence of identical isolates in these different cases suggest that these cases might be related, most likely by a trading relationship between carp breeders and a spread in the absence of surveillance programmes on virus infections for these consignments.

Meanwhile, it is more difficult to correlate particular CEV variants with mortality events in koi or carp populations. For instance, isolate KSD-178-16 from genogroup II induced high morbidity and mortality. However, it shared the sequence with several isolates from 2010 to 2016 from various regions in Germany from which low morbidity and mortality were reported. Likewise, the isolates KSD 118-16 and KSD-122-16 induced high mortality in carp populations in Saxony and Thuringia, while additional isolates with an identical nucleotide sequence were not associated with severe clinical signs. This suggests that more severe clinical signs might not be caused by certain variants with increased virulence, but could be caused by all CEV variants depending on the constitution of the fish and the husbandry conditions. Until recently, the nucleotide sequence of a short segment of the p4a encoding gene was known and could be amplified and aligned in the current study. Meanwhile, large parts of the complete genome sequence of a CEV isolate from koi became available (Mekata et al., 2021), which could be used for a more thorough analysis of genes, which possibly contribute to virulence. Furthermore, the mortality could also depend on the genetic makeup of the carp population (Adamek, Oschilewski, et al., 2017) or infection with additional pathogens (Adamek, Teitge, et al., 2018). Cohabitation experiments of CEV-infected carp with naive carp from different strains demonstrated that CEV can be transmitted from diseased to previously non-infected carp and can induce a severe disease in specimens from certain strains (Adamek, Oschilewski, et al., 2017),while other strains were far less susceptible to the development of the disease. This was also shown for other viruses in common carp including CyHV-3 and SVCV (Adamek, Matras, et al., 2019; Dixon et al., 2009; Piačková et al., 2013). Nonetheless, CEV has to be regarded as a serious infectious agent rather than an opportunistic pathogen affecting weakened specimens.

The data of the present communication show a relatively high presence of CEV in koi and carp populations in various regions of Germany and that the infection can be associated with outbreaks of a severe disease. Currently, KHVD caused by CyHV-3, is considered the most dangerous pathogen for carp (OIE, 2019). According to the official statistics of the Federal Research Institute for Animal Health (Friedrich-Loeffler-Institut) in Germany, there were 67 and 53 KHVD outbreaks in 2015 and 2016, respectively (TSIS-TierSeuchenInformationsSystem, 2021). Even though the data on the presence of CEV in koi and carp from the present study were collected on a different basis than the entries in the official statistics for a notifiable disease, the data could suggest similar widespread distribution of CEV and CyHV-3 in carp and koi populations in Germany. On the other hand, results from UK show a much higher presence of CyHV-3 when compared to CEV in common carp (Cano et al., 2021). In Croatia, as a consequence of the mitigation efforts focused on CyHV-3, this pathogen has been successfully eradicated from carp populations, while CEV is presently causing mortalities in Croatian carp stocks (Zrnčić et al., 2020). The one redeeming fact for KSD is the ease of treating clinical signs of this disease in ornamental fish by supplementing water with salt. Unfortunately, this approach is impossible to implement in intensive carp farming within large earth ponds or natural lakes. Furthermore, it could lead to persistent subclinical infections, which could increase the viral spread. However, at present, it is unresolved whether CEV persists in infected specimens or how the infection remains in the populations. These issues need to be investigated in experimental studies and by closely observing affected field populations.

5 | CONCLUSIONS

CEV is highly prevalent in German common carp and koi stocks. Therefore, in disease diagnostics, the presence of this virus should be monitored and always considered for differential analyses of an epizootic in common carp suffering from gill disease. As the virus induces pathological changes of the gills, the infection might be mistaken for an infection with CyHV-3, which should be borne in mind during disease investigations. However, disease outbreaks of CEV mainly occur in spring and early summer at lower water temperatures than those usually observed in clinical outbreaks of KHVD. The finding that CEV variants with similar or identical nucleotide sequences are detected in samples from koi collected over a period of several years from

different geographical locations in Asia and Europe imply intense trading of koi between these countries without taking necessary precautions to avoid the spread of a viral infection. Spreading of CEV might be further facilitated by the fact that a relatively high virus load was found even in koi without clinical signs of the disease. Likewise, in farmed carp, CEV variants with identical nucleotide sequences were isolated from populations in various areas of Germany and from different European countries. This also suggests that intensive trade relations within Germany and with various European countries led to the spread of this virus infection. Therefore, even if CEV is not listed as a relevant carp pathogen by OIE, regular testing of carp populations for the presence of CEV could prevent further spreading of the infection and mortality associated with this infection. This is of particular importance because until today it is unclear whether CEV persists in infected populations and could be spread through moving asymptomatic fish. Furthermore, with a high prevalence in open environments, complete eradication of the pathogen will be extremely difficult.

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ETHICAL STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as animals were not subjected to a regulated experimental procedure. The sampling was performed according to best veterinary practice, in accordance with rules and regulations valid for sample collection for diagnostic purposes in Germany.

DATA AVAILABILITY STATEMENT

The data is available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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