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Risk factors for development of internal neoplasms in koi carp *Cyprinus carpio koi*

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ABSTRACT: Fish, like mammals, can be affected by neoplastic proliferations. As yet, there are only a very small number of studies reporting on the occurrence of tumours in koi carp *Cyprinus carpio koi* and only sporadic reports on the nature of the tumours or on risk factors associated with their development. Between 2008 and 2012, koi with abdominal swelling were examined pathologically: neoplastic lesions were diagnosed and classified histologically. We evaluated possible risk factors for the development of these internal neoplasms in koi carp in Switzerland, using an online 2-part questionnaire sent to fish keepers with koi affected by internal tumours and to fish keepers who had not previously reported any affected koi. Part 1 addressed all participants and focused on general information about koi husbandry and pond technical data; Part 2 addressed participants that had one or several case(s) of koi with internal tumour(s) between 2008 and 2012, and consisted of specific questions about affected koi. A total of 112 internal tumours were reported by the 353 koi keepers participating in the survey. Analysis of the obtained data revealed that tumour occurrence was significantly associated with the location (indoors vs. outdoors) and volume of the pond, frequency of water changes, origin of the koi, number of koi kept in a pond and the use of certain pond disinfectant/medication products. Our results contribute to the identification of possible risk factors, which in turn could help to establish prophylactic measures in order to reduce the occurrence of internal neoplasms in koi.

KEY WORDS: Koi carp · Neoplasm · Risk factor · Prophylactic measures · Early diagnostics

INTRODUCTION

The husbandry of koi *Cyprinus carpio koi* (the ornamental variety of common carp) as a hobby is gaining in popularity in Switzerland as well as in many countries worldwide. Koi can become very tame and live for many years, therefore they have high emotional value for the hobbyists (Carey & Judge 2000). Further, selected varieties can achieve high economic value.

According to an estimation based on the practical work of the authors' fish-specialized veterinary practice, there are about 2000 to 3000 koi keepers in Switzerland, with around 1000 of them keeping koi in specifically designed koi ponds (koi hobbyists). The remaining fish keepers keep a variety of fish species together with the koi, including species such as goldfish, golden orfe, rudd or sturgeon mostly in biotope-style ponds (owners of mixed populations).

The ponds are of variable sizes, depths and volumes, ranging from 1000 l up to 500 000 l, from basic to advanced filtration systems and from mud-bottomed ponds with abundant vegetation to clean, clear-watered 'sterile' basins.

Over the last 10 yr, the number of koi hobbyists in Switzerland has increased steadily (authors' obs.). Reasons for the increasing popularity include, amongst others, easier importation and better avail-

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ability of high quality Japanese koi and a higher level of professionalism in koi keeping in general; construction of koi ponds with advanced filter technologies (automatic cleaning of solids by drum filters) lead to improvements in water quality and a reduction in time and effort spent on maintenance. The koi hobby is mainly popular in the German-speaking part of Switzerland (see Fig. 1a). The authors' veterinary practice covers about 75% of koi hobbyists and therefore we believe that the results are a reasonably accurate representation of the core koi population in Switzerland. Most owners of mixed fish populations in biotope-style ponds are usually not interested in on-site health checks by fish veterinarians and therefore the health of these koi is mostly unknown.

As koi, like other animals, can suffer from diseases, the rising number of kept koi led to an increased demand for both pond and individual koi treatments by veterinarians specializing in fish.

Fish, like mammals, can be affected by neoplastic proliferations. Neoplasia is defined as uncontrolled proliferation and dispersal of autologous cells throughout the host body, resulting from accumulated DNA mutations (Martineau & Ferguson 2006). Some tumours in fish have been attributed to genetic factors (Meierjohann & Schartl 2006, Nairn et al. 1996); others were associated with a virus (Hanson et al. 2011, Coffee et al. 2013) or with environmental contamination (Fabacher & Baumann 1985, Baumann et al. 1990, Harshbarger & Clark 1990). However, the presence of tumours in koi populations has not yet been extensively studied. With sporadic case reports of tumours in koi worldwide being the only available literature on this topic (Knüsel et al. 2007, Sirri et al. 2010, 2012, Stegeman et al. 2010), data about prevalence or significance of neoplastic lesions in koi are still missing.

For the past 7 yr the authors' veterinary practice has offered on-site health checks for koi. During this period, a steadily increasing number of internal neoplastic lesions in koi have been observed. To gain more information about the nature of these internal neoplastic lesions, 117 koi with abdominal swelling were examined pathologically between 2008 and 2012. Tissue samples were examined, lesions were classified as neoplastic and the tumours were classified according to WHO classification (Kennedy et al. 1998, F. Ott Knüsel et al. unpubl. data). Tumours were mainly arising from the gonads with mesenchymal neoplasms being the most prominent finding (F. Ott Knüsel et al. unpubl. data).

Neoplastic lesions of internal organs are a particularly regular finding, with case numbers increasing over the last years (authors' obs.). Clinically, these fish present a sudden and abnormal increase in volume of the abdomen (often in late summer after spawning season) resulting in sluggish swimming behavior with uncontrolled spasms of hyperactivity. Sometimes koi with a greatly distended abdomen live for months or even 1 to 2 yr (authors' obs.).

Despite the relatively high number of these tumours among koi, to date there is no information available about possible causes of tumour induction or conditions which may predispose koi to tumour development. Consequently, there are no accepted prophylactic methods or treatments that could reduce the incidence of tumour development or methods which would facilitate early diagnosis of affected koi. In this study, our goal was to evaluate possible risk factors for the development of internal neoplasms using an online questionnaire, sent to keepers of koi affected by internal tumours and to owners of ponds with no reports of tumours in their fish as a control. Specifically, we sought answers to the following questions: (1) What is the frequency of internal tumours in the koi populations cared for by our veterinary practice? (2) Is there an association between pond characteristics and management and the occurrence of tumours? (3) Are there any typical clinical signs or behavioral patterns that can be associated with an early stage of tumour development which could be used for an early recognition of these tumours?

MATERIALS AND METHODS

Design of the questionnaire

To answer the above-mentioned questions, we developed an online questionnaire. The target populations were all clients of the authors' veterinary practice consisting of both koi hobbyists (n = 800) and keepers of mixed fish populations (n = 298).

First, a set of written questions was compiled and sent to 4 clients as a trial with the request to fill in the preliminary questionnaire and add suggestions of improvement or comments on the comprehensibility of the questions. After improvements were implemented, the online questionnaire was developed using the limesurvey software tool (www.limesurvey.org) and the link was sent to 50 clients as a second trial. After improving and finalizing the questionnaire, the corresponding link and a request to participate in the survey was sent to the clients of the veterinary clinic with available email addresses (approx. 650) and posted on the clinic homepage.

The final questionnaire consisted of 2 parts:

Part 1 focused on general information about koi husbandry and technical data of the pond; this part was to be filled in by all participants.

Part 2 addressed participants who had one or more cases of koi with internal tumour(s) between 2008 and 2012. It consisted of specific questions about affected koi and allowed separated data entry for up to 3 cases. The postal codes of all the participant locations were used to generate a demographic overview (see Fig. 1).

Statistics

The data were checked for consistency and distribution using conventional descriptive statistics such as means, medians, measures of spread and proportions by answer class, and were categorized for further analysis.

Fish keepers who did not report any koi with internal tumours were considered as control holdings whereas keepers with one or more koi diagnosed with internal neoplastic lesions were considered as case holdings.

The unit of analysis in the risk factor assessment was the holding. Facilities reporting multiple cases were counted up to a maximum of 3 times. Responses to all questions in Part 1 were statistically compared between case and control holdings using cross-tabulation with chi-square & Fishers Exact Test statistics (categorical variables) or 2-sample *t*-tests (parametric, non-parametric); depending on the format of the factor of interest.

Responses to questions in Part 2 (tumour cases) were summarized using frequency tables and basic descriptive statistics; no further statistical analyses were performed here. As not every question was answered by all koi owners, the total count varied from question to question; to compensate for this, the total number of responses per question was always set to 100 %.

The overall threshold level for statistical significance was $p < 0.05$. All analyses were done in NCSS 2008 (www.ncss.com).

RESULTS

Of the 650 individual koi keepers in Switzerland who were contacted, 353 took part in the survey (54 %). The demographic distribution of clients of the veterinary's practice and of the ponds participating in the survey is shown in Fig. 1 as frequency per community. Geographically, there was no accumulation of tumour cases compared to the distribution of the clients.

Of the 353 koi keepers, 74 reported a single koi with an internal neoplastic lesion, 10 had 2 koi with neoplastic lesions and 6 had 3 koi with internal neoplastic lesions. The total number of recorded cases with internal tumours was thus 112 (Fig. 1). The number of individual fish represented by this study was approx. 9300.

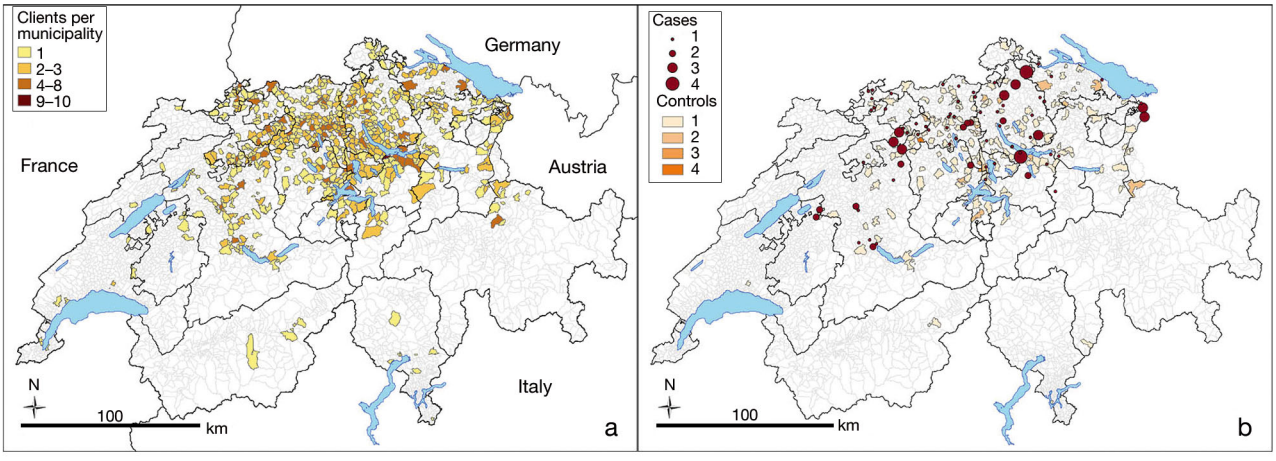

Fig. 1. (a) Distribution of clients keeping koi *Cyprinus carpio koi*, registered with the authors' veterinary practice in Switzerland: the patch color gradient denotes number of clients per community. (b) Distribution of koi ponds included in this study. Patches show communities with ponds that contain koi unaffected by tumours (controls): the color gradient denotes number of controls per community. Tumour cases are shown by red circles in proportion to outbreak magnitude

For the analysis of the individual koi tumour cases, each reported case was counted as a separate entity, therefore the sum of the individually registered responses was slightly higher than the number of responding koi keepers.

The results only represent the distribution of the tumour case series; there was no comparable information at the individual fish level available from healthy koi (controls).

Association of tumour status and pond management

The statistically evaluated results of Part 1 of the questionnaire are listed in Table S1 in the Supplement at www.int-res.com/articles/suppl/d114p199_supp.pdf.

For the relationship between tumour appearance and husbandry only the most important results are described below, with additional results presented in Table S1.

Influence of pond structure and maintenance

The tumour occurrence was significantly influenced by the pond location (outdoor/indoor), pond volume, and by the frequency of water changes. Significantly more tumour cases were seen in combined outdoor/indoor or indoor-only ponds, bigger ponds and ponds with higher frequencies of water change (Table S1).

Water temperature, pond depth, geographic location and altitude, turbidity, sun protection, filter system or source of water for the water exchange was not detected to have a significant influence on the occurrence of internal neoplastic lesions in this study (Table S1).

Influence of koi origin and husbandry

The breeder of the koi had a significant influence on tumour development, as did the number of animals kept. Koi from Breeder A were more prone to develop internal tumours than koi from other breeders. In ponds with more than 20 koi, neoplastic lesions were recorded more often (Table S1).

The country of origin of the koi had no significant influence on the tumour occurrence; however, the majority of the koi in this study were from Japan. The spawning habit and the presence of other fish species in the pond had no significant influence on the occurrence of internal tumours (Table S1).

Influence of medication

Koi from ponds which had received treatment (pond treatment) with praziquantel, formalin/malachite green or potassium permanganate in the 5 yr preceding the study showed a significantly higher likelihood of internal tumour development than koi from ponds with no treatment with any of the above mentioned medications in the past 5 yr (Table S1).

Pond treatment with nitroscanate, dye, sodium percarbonate, terbutryn, copper or separated treatment of individual animals (injections and dip treatments) had no significant influence on the occurrence of tumours during the past 5 yr (Table S1).

Description of koi tumour case series

The statistically evaluated results of Part 2 of the questionnaire are listed in Table 1.

The results reflect the diseased koi (n = 112) with no comparison to healthy animals.

Out of 83 koi with available information, 62% were 3 to 6 yr old (Fig. 2). Out of 92 koi, 28.5% showed the coloration red/orange+white and 18.5% the colora-

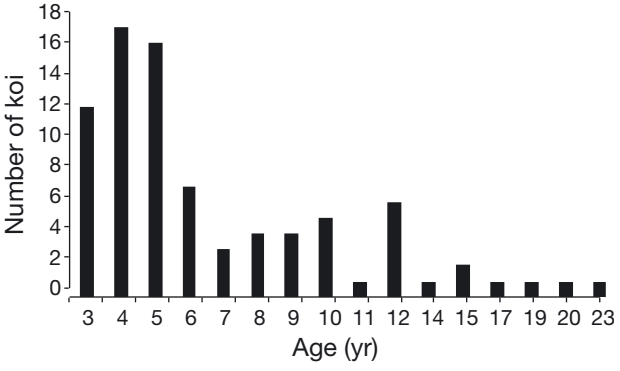

Fig. 2. Frequency distribution of the age (in years) of diseased koi *Cyprinus carpio koi* (n = 83)

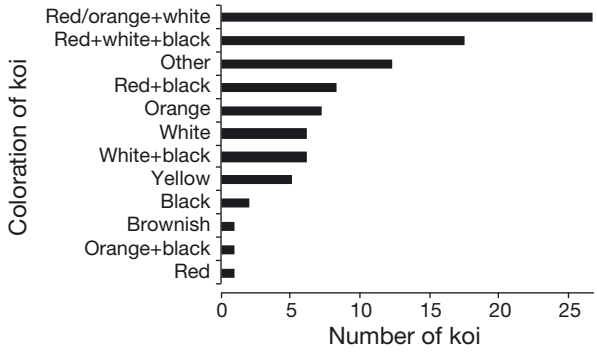

Fig. 3. Frequency distribution of coloration patterns in diseased koi *Cyprinus carpio koi* (n = 92)

Table 1. The statistically evaluated results of Part 2 of the questionnaire for diseased koi (n = 112) with no comparison to healthy animals. The full survey with all responses (diseased as well as healthy koi) can be found in Table S1 in the Supplement at www.int-res.com/articles/suppl/d114p199_supp.pdf

Questions	Variables	Categories	Number of valid answers	Total count per question	Value for cases in %
Tumour diagnosis during last 5 years?		Yes	112	112	100
Origin of the diseased koi					
Was the affected koi specifically bred or was it from a natural spawning in the pond?		Natural spawning	16	99	16.16
		Specifically bred	83		83.84
Country of origin?	Japan	Yes	62	112	55.36
		No	50		44.64
Country of origin?	Europe	Yes	5	112	4.46
		No	107		95.54
Country of origin?	Switzerland	Yes	23	112	20.54
		No	89		79.46
Exterior					
Did the diseased koi ever spawn?		The koi was male	17	96	17.7
		Never	16		16.6
		Rarely	5		5.2
		Regularly	7		7.2
		Don't know	51		53.3
Symptoms					
If the koi was purchased, how long before the symptoms showed?		More than 2 yr	47	76	61.8
		Less than 2 yr	28		36.8
		Don't know	1		1.4
How long before the death of the diseased koi was the tumour noticed/diagnosed?		0–1 yr	60	67	89.55
		More than 1 yr	7		10.45
Treatments					
Was the diseased koi treated individually in the last 5 years?	Injection	Yes	19	112	16.96
		No	93		83.04
	Dip treatment	Yes	7	112	6.25
		No	105		93.75
	None	Yes	68	112	60.71
		No	44		39.29
Was the tumour removed surgically?		Yes	13	112	11.61
		No	80		71.43
		No answer	19		16.96
If yes, what happened to the koi?		Died	19	24	79.16
		Died during surgery	3		12.5
		Don't know	1		4.16
		Koi survived surgery	1		4.16
Other fish					
Were there other fish in the pond affected with tumours?		Yes	13	90	14.4
		No	77		85.56

tion red+white+black (Fig. 3). Of the 112 koi, 55.3% originated from Japan.

The clinical sign most often seen prior to tumour diagnosis was abdominal distension (Fig. 4).

Most of the affected koi died within 1 yr of an internal neoplasm being diagnosed. In the majority of the cases, the diagnosis of a tumour was restricted to a single animal with no other koi affected in the previous 5 yr.

Most (60.7%) of the affected koi had received no specific single animal treatment; no association between individual treatment and occurrence of tumours could be established based on the present data.

DISCUSSION

Koi husbandry in Switzerland has been growing steadily for the last decade, which has coincided with an increase in the occurrence of spontaneous internal neoplasms (authors' obs.). At present it is not clear whether there is a true increase in tumour occurrence, or whether this is a result of more frequent pond-site health checks carried out by specialized fish veterinarians. The knowledge gathered in these health checks allowed us to perform the present study, which had the aims of assessing the frequency of tumour cases in the koi ponds, to deter-

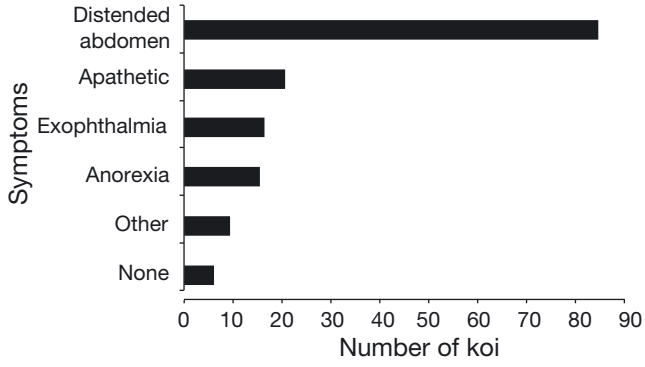

Fig. 4. Frequency of different clinical symptoms shown by diseased koi *Cyprinus carpio koi* (see 'Results: Description of koi tumour case series')

mine potential risk factors associated with tumour development and to establish early recognition methods of affected fish based on specific disease signs.

The number of fish included in this study was approx. 9300; 112 (1.2%) of these developed internal tumours in the years between 2008 and 2012. This equates to 22.4 (0.24%) tumour cases per year. To compare this number to other koi populations is difficult, as there are, to our knowledge, no published data about tumour incidence in koi available. Therefore our data can only be compared to incidence values available for other fish species. However, incidental tumour occurrence in other ornamental fish species is rare, according to our own diagnostic examinations. There is rarely more than a single case of tumour per year in other ornamental fish species, regardless of type or origin. Therefore, we interpret an incidence of 0.24% per year (as seen in the koi population in this study) as high.

Association of tumour status and pond management

Influence of temperature, pond structure and maintenance

Our data showed that significantly more tumour cases were detected in combined outdoor/indoor or indoor ponds only. As fish are poikilothermic animals, their metabolic rate is temperature-dependent (Evans et al. 2013) and metabolic rate increases with higher temperature. Indoor ponds are usually associated with a more constant and generally warmer temperature than outdoor ponds. Therefore, reduced body temperature and metabolic rate during winter months is normally not present for fish living in indoor ponds. Even in the absence of mutagens such as chemicals, viruses or radiation, the probability of spontaneous mutations increases with advancing age, leading to an increasing probability of spontaneous neoplasms (Martineau & Ferguson 2006). Aging is dependent on metabolic rate and oxidative DNA damage (Adelman et al. 1988, Hulbert et al. 2007). Therefore, with an increased metabolic rate due to water temperatures that are kept consistently high, regardless of season, the occurrence of spontaneous mutations might be accelerated. Over the last few years, it has become increasingly common for Japanese koi breeders to keep valuable koi in glasshouses during winter (authors' obs.) to maintain water temperature above 10 to 15°C. This enables the breeder to feed the koi all year round and achieve better growth and therefore larger fish to sell on the international koi market. The trend in the koi industry is to produce 2 yr old fish measuring more than 60 cm in length.

However, no data on tumour incidence in koi or common carp *Cyprinus carpio* from tropical countries with constantly high temperatures are available to support this hypothesis.

Our data showed an increased tumour occurrence in larger ponds. This may be correlated to the fact that koi hobbyists with larger ponds are likely to keep fast-growing, bigger and therefore more expensive fish, which may lead to more regular checks by a veterinarian and hence the increased probability of detection of any koi with tumours. It may also be correlated with the fact that larger ponds usually accommodate a larger number of fish.

Influence of koi origin and husbandry

Analysis of the country of origin of affected koi showed that there was a statistically significant accumulation of tumour cases in fish from one breeder in Japan. This could indicate a genetic influence on tumour development. Ishikawa & Takayama (1977) stated that ornamental carp with complex genetic histories developed ovarian neoplasms that may have been hereditary in nature. Those authors reported a survey of 21 ovarian neoplasms (dysgerminoma, theca cell tumour and granulosa cell tumour) in adult nishikigoi (= koi) from 1974 to 1976. Another possible cause for the accumulation of tumour cases from koi originating from one breeder could be peculiarities in the usage of medication, husbandry (e.g. high temperature during winter) or other environmental factors. The influence of certain chemicals on tumour occurrence is also significant and will be discussed in the next section.

The number of fish kept per pond also had a significant influence on tumour occurrence. In ponds with more than 20 koi, neoplastic lesions were recorded more often. The reason therefore may be that the increased likelihood of a tumour developing in a crowded pond is simply down to numbers — the higher the number of koi in a pond, the greater the probability of there being a koi affected by tumours.

Influence of medication

The increased incidence of tumours in fish kept in ponds treated with praziquantel, mixtures of formalin/malachite green (FMG) and potassium permanganate ($KMnO_4$) could be an indicator of a carcinogenic effect exerted by these chemicals.

According to Frohberg (1984) and Ali (2006), praziquantel has a very low toxicity and carcinogenicity in mammals. However, there is little information available on toxicity of praziquantel on fish (Mitchell & Hobbs 2007). The result regarding a possible tumour induction from praziquantel in this study could however be biased, because praziquantel was the only medication used against *Gyrodactylus* sp. and *Dactylogyrus* sp. by the veterinary practice in the years 2005 to 2010. As infestations with these flukes are a common nuisance in koi keeping, many ponds are dewormed once per year. Nitroscanate as an alternative substance to praziquantel was only used from 2010 onwards on a regular basis.

Malachite green (MG) is a known human carcinogen (Culp et al. 1999). Srivastava et al. (2004) describe the effects of MG on fish and certain mammals. They state that the toxicity of this dye increases with exposure time, temperature and concentration and that it causes carcinogenesis, mutagenesis, chromosomal fractures, teratogenicity and respiratory toxicity.

Formaldehyde can induce cancer in nasal tissues in rodents (Monticello et al. 1996, Schroeter et al. 2014). This chemical is part of many ornamental fish medications, mainly in combination with malachite green (FMG) and/or methylene blue to control fungi (*Saprolegnia* sp.) and unicellular parasites (e.g. *Ichthyophthirius multifiliis*, *Ichthyobodo necator* and others). Koi dealers in Switzerland often follow a quarantine disinfection protocol, whereby either FMG or similar medications containing MG are added to the quarantine tanks for 2 to 3 d after arrival. Also, recurrent exposure is likely because breeders, dealers, veterinarians and koi hobbyists frequently use FMG for antiparasitic and antifungal treatments in dips, short-time and long-time baths.

However, results in this study are based on clinical experience. Experimental studies are needed to confirm these results.

$KMnO_4$ is an oxidizing agent used for many years in aquaculture (both food and ornamental fish) for the treatment or prevention of diseases in fish, removal of parasites, and disinfection of fish tanks and aquariums (Lay 1971, Duncan 1974). $KMnO_4$ is potentially toxic to humans and other organisms (Kegley et al. 2010), including several fish species (Marking & Bills 1975, Cruz & Tamse 1989, Bills et al. 1993, Straus 2004, da Silva et al. 2006). As demonstrated by França et al. (2013) the prolonged use of $KMnO_4$ concentrations considered safe by literature for prophylaxis and treatment of diseases in fish farming (1.0 and 4.0 mg l^{-1}) may cause considerable changes to the health of exposed Nile tilapia *Oreochromis niloticus*, evidence of the toxic potential of $KMnO_4$ in certain fish species. $KMnO_4$ induced the destruction of red blood cells during a short period of exposure, indicating the direct action of its toxicity. However, carcinogenic activity has not been demonstrated.

According to our data, treatments of individual fish (such as injections and dip baths) had no significant influence on the occurrence of internal tumours. The appearance of fibrosarcoma at vaccination sites is well known in cats (Kass et al. 1993), and the intraperitoneal administration of oil-adjuvanted vaccines in Atlantic salmon *Salmo salar* can cause side effects including retarded growth (Midtlyng & Lillehaug 1998), granulomatous peritonitis, intra-abdominal adhesions and melanisation (Ferguson 2006). Millions of Atlantic salmon are vaccinated every year and harvested 2 to 3 yr later. The salmon are probably slaughtered before a visible tumour development is possible and furthermore, they are not routinely checked for the presence of tumours; however, the symptoms mentioned above could lead to tumour development. In contrast, in koi there seem to be no tendencies for intraperitoneal injections to cause tumour development.

It is difficult to compare our results regarding tumour development and medication with the literature on carp or other fish. This might be due to the high diversity of species in aquaculture and the ornamental fish industry. As already stated for praziquantel, it is possible that medications (especially FMC), which are shown to possibly predispose fish to tumour development, were used more often in comparison to the other medications and therefore our results could be biased. This study and our findings are meant to be exploratory. With regards to koi, literature on the

subject of tumours is generally restricted to case reports of single cases with hardly any available studies on the health status of populations.

Description of koi tumour case series

The country of origin of 55.3% of the diseased koi was Japan. The proportion of koi of specifically bred origin (trade koi) versus survivors of natural spawnings in hobbyists ponds ('Eurokoi') included in this study was around 6:1 (results not shown). Interestingly, the proportion of koi with tumour from specifically bred origin (83) versus the koi with tumour from natural spawnings (16) in this study was around 5:1, showing a slightly higher proportion of affected koi in the Eurokoi population than in the trade koi. The main difference between koi bred for the koi trade (Japan) and Eurokoi is that Eurokoi are not selected by professional breeders. It is likely that koi with external deformities or development disorders are rejected by the breeders in Japan at an early age while such fish survive in hobbyist ponds and develop internal tumours.

As shown in Fig. 2, 62% of the diseased koi were between 3 and 6 yr old. The relatively young age (carp species can live more than 30 yr, www.fishbase.org) is surprising and suggests a genetic predisposition rather than environmental influences. In contrast, findings in wild fish populations are more in favour of environmental factors playing a major role in tumour development; Hinton (1989) and Baumann et al. (1990) found that tumour-bearing wild fish reside in polluted areas near urban centres and are of advanced age.

We observed that koi with the colouring red/orange+white and red+white+black seemed to be more affected by internal tumours than koi with other colouring patterns (Fig. 3). The reason for this accumulation of tumours is unknown, but a possible reason could be that the varieties kohaku (red+white), showa (black+red+white) and sanke (red+black+white) are the classic and most expensive koi varieties in Japan and in the koi industry worldwide. There are several long-established specific bloodlines and therefore, the degree of inbreeding is probably higher than in other, more recent or less challenging varieties to breed (e.g. the unicolor varieties). These 3 classic koi varieties rank amongst the most popular koi kept by hobbyists in Switzerland and are therefore more likely to develop tumours by sheer numbers. Another possible reason could be a genetic predisposition of the red colour for internal tumours, similar to the predisposition for external growths/tumours of the skin of koi in dorsal areas and the eyes seen in koi with red/orange colouring (authors' obs.).

Clinical signs for early recognition

The most frequent symptom seen in koi affected by an internal tumour was a distended abdomen — sometimes grossly. However, this only appeared in an advanced stage of tumour development. Being the only regularly observed sign, early and simple recognition of a tumour seems to be difficult for the hobbyist and even for specialised veterinarians. Furthermore, a distended abdomen can also be a result of other conditions such as egg development in females or bacterial/viral infections inducing ascites. The use of ultrasound imaging might be useful for an early diagnosis.

In conclusion, the findings of the present study show that tumour occurrence in koi may be significantly influenced by several factors. The results might help to improve holding conditions, resulting in a reduced tumour occurrence in the future. As this study and our findings were of an exploratory character, we stress the importance of a designed study to gain further insights of possible risk factors for tumour development in koi. This study would have to include treatment data of (1) the koi breeders in Japan and other parts of the world, (2) of the koi importers/dealers in Switzerland and (3) of the koi hobbyists and their veterinarians.

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