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Alpha-chloralose poisoning in cats and dogs in Norway - a project

Alpha-chloralose poisoning in cats and dogs in Norway – a project

Content

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Summary

Due to many cases of diseased cats and dogs at Veterinary clinics in Norway where poisoning with the mouse poison alpha-chloralose was a possible diagnosis, the Norwegian Veterinary Institute, with support from the Norwegian Environment Agency, initiated a collection of material from relevant cases. Cats and dogs that were clinically ill or dead with alpha-chloralose poisoning as a suspected cause were included in the project. The purpose was to get more information about alpha-chloralose poisoning in cats and dogs in Norway.

During the collection period, 37 cases were investigated; 4 dogs and 33 cats. The reason why most cases of poisoning were registered among cats is believed to be that they are more sensitive to this toxin than dogs, probably related to a reduced ability to excrete the substance in the kidneys. Cats eat mice to a greater extent than dogs, and it is likely that cats can be poisoned with alpha-chloralose by eating poisoned mice.

Poisoning with alpha-chloralose causes ataxia and disorientation, often with excitation. The animals get reduced body temperature and at higher doses, a state of anaesthesia occurs. The prognosis is good if the animals come home and receive supportive treatment. Usually they recover after 2-3 days. For animals that are not found in time and remain in a poisoned state outside in cold environments, the prognosis is characterised as poor.

Background

During 2019, there were many cases of diseased cat and dogs at Veterinary clinics in several parts of Norway, where the suspicion was directed at alpha-chloralose poisoning. Alpha-chloralose as a mouse poison had become available from 2015 and preparations with alpha-chloralose gradually took over the market after restrictions in 2018 on the sale and use of mouse and rat poison containing anticoagulants.

However, there was no opportunity to confirm the suspicion of alpha-chloralose poisoning by chemical analysis. The Norwegian Veterinary Institute (NVI) established an analysis method, and from late autumn 2019, the NVI was able to analyse samples from animals with a suspicion of alpha-chloralose poisoning. In collaboration with the Norwegian Environment Agency, a project was established where veterinarians/veterinary clinics were offered a free examination of samples from dogs and cats if there was a suspicion of alpha-chloralose poisoning. The offer comprised analysis for alpha-chloralose in samples of blood serum and urine from clinical cases or autopsy with analysis of alpha-chloralose in tissue samples. In order to be offered a free examination, the submission of the material had to be approved by the NVI.

The purpose of the project was to get more information about alpha-chloralose poisoning in cats and dogs in Norway, including the source of poisoning, disease development, symptoms, treatment and prognosis. It was also a goal to be able to compare alpha-chloralose poisoning in dogs and cats, as well as assess the possibility of secondary poisoning by eating poisoned mice.

Materials and methods

Animals and samples

From December 2019 to June 2020, samples were received from 37 cases; 4 dogs and 33 cats. The cases came from various parts of Norway, and were received from veterinary clinics/veterinarians together with information about clinical signs and treatment.

Eight of the cases were cats that had been killed or found dead and sent for autopsy at the NVI. From these cats, samples were mainly taken from liver, kidney, brain and stomach contents, and from some animals also spleen and small intestine contents.

From the remaining cases, submitted samples were examined by chemical analysis only. In one case various organs were received from a euthanized cat, while the other samples came from live animals treated at the clinics. From most cases, both blood serum and urine were received, but from a few cases either serum or urine was received. From some cases the material also comprised stomach contents.

Method of analysis

The method used by Centro Regional Anti-Doping in Italy was established at the NVI [1]. The method involves the extraction of alpha-chloralose α- [R-1,2-O- (2,2,2-trichloroethylidene) -α-D-glucofuranose] from the samples with acetonitrile (blood serum) or chloroform (urine, liver, kidney, stomach contents and brain). The compound was analysed by high performance liquid chromatography - mass spectrometry. HPLC: Agilent 1200 series high performance liquid chromatography, Agilent Technologies, Santa Clara, CA, USA. MS: G6470A Triple Quadrupole, Agilent Technologies.

Urine samples were divided into two equal sub-samples, one was incubated with the enzyme ß-glucuronidase. Total alpha-chloralose was the alpha-chloralose released after enzyme treatment in addition to the free alpha-chloralose in the sub-sample. Glucuronic acid conjugates correspond to the difference between total and the free alpha-chloralose, which was determined in the other sub-sample. A detailed method description can be found in the NVI's method collection of the quality assurance system.

Results and discussion

Alpha-chloralose concentrations in the samples

A great variation was observed in the alpha-chloralose concentration in the different samples between the cases, especially in blood serum, urine and stomach contents. In most cases, the concentration of alpha-chloralose in urine was significantly higher than in serum, while some animals had alpha-chloralose at approximately the same level in serum and urine. A probable explanation for the variation is differences in the dose of alpha-chloralose ingested and the point of time of sampling in the poisoning phase.

In the tissue samples, there were generally much lower alpha-chloralose concentrations than in the urine. The material is not suitable for looking at animal species differences in alpha-chloralose concentrations because samples from only four dogs were examined.

In the cats where several samples were analysed, the concentrations of alpha-chloralose in tissue samples and body fluids were compared (Figure 1). Such a comparison does not necessarily give a complete picture of the kinetics and concentrations in different tissues since the sampling times (in relation to the intake of alpha-chloralose) vary from individual to individual. However, the results reflect the findings from individuals where several samples were examined (Annex Table 1).

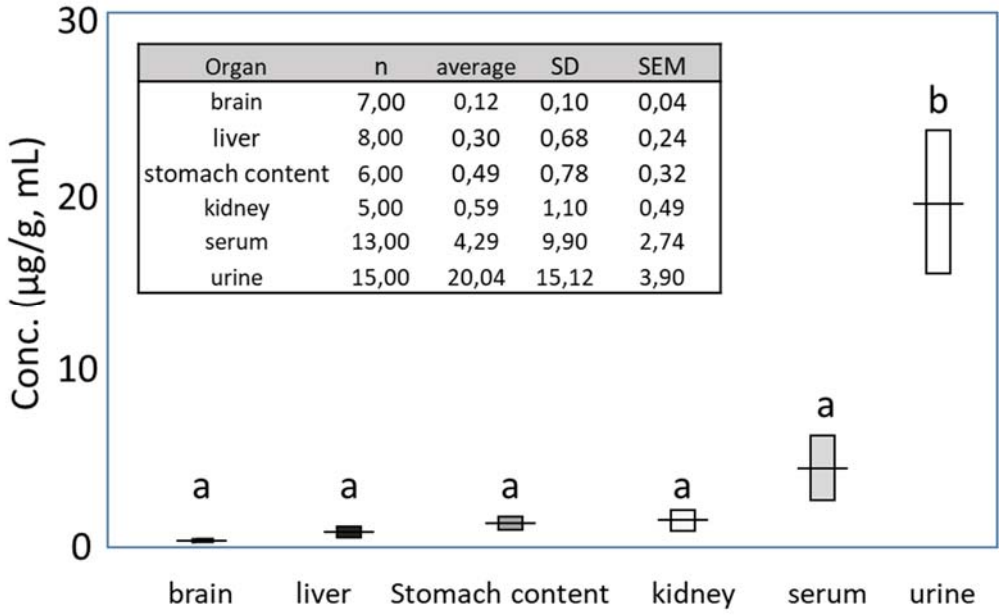

Figure 1: Concentrations of alpha-chloralose in various organs (µg/g) and body fluids (µg/mL) from cats. Organs/body fluids with different letters indicate significant differences (Tukey Kramer HSD).

Difference between animal species

The distribution between dogs and cats in the material reflects that far fewer dogs than cats appear to be poisoned with alpha-chloralose. It would have been desirable to have more dogs in the project, but the material presented here was what available during the collection period.

The suspicion of alpha-chloralose poisoning was confirmed in 34 of the 37 cases - in all four dogs and in 30 of 33 cats (91 %). All dogs recovered after treatment, while 22 of the cats (73 %) with detected alpha-chloralose in the body, recovered.

Of the eight cats with alpha-chloralose poisoning who died or were euthanized, three were found to have an underlying serious illness at autopsy, and one probably had a complicating neurological disorder. Some of the cats were euthanized (because the owner wanted it), even though it had been medically possible to save them.

The main explanation for more common alpha-chloralose poisoning among cats than dogs may be that cats are more sensitive to alpha-chloralose than dogs. LD_{50} values (lethal dose in 50 % of tested animals) are stated to be 600 mg/kg body weight in dogs and 100 mg/kg body weight in cats [2].

From previously reported poisonings in humans, it is stated that alpha-chloralose is excreted in urine, mainly after conjugation with glucuronic acid [1, 3-4]. Conjugation with glucuronic acid is an important metabolic process that increases the excretion of several compounds including drugs and toxins via urine or bile.

In a human case of poisoning, the excretion of glucuronic acid conjugate by alpha-chloralose was seven times higher than free alpha-chloralose [1]. Metabolism and drug excretion vary between species, so human knowledge cannot be directly transferred to dogs and cats.

Cats, unlike dogs, lack certain enzymes (UDP-glucuronosyltransferases; UGT) that catalyse conjugation with glucuronic acid, thereby leading to reduced excretion of substances secreted by this mechanism. Examples of substances that are not, or to a small extent, conjugated in cats are carprofen, propofol, acetaminophen and benzoic acid. Benzoic acid is often used as a preservative in foods and feeds. Fatal

poisonings in cats have occurred after consuming cat food preserved with benzoic acid [5, 6]. It has not been possible to obtain information from the literature on whether cats lack or have a reduced ability to metabolise alpha-chloralose in the form of glucuronic acid conjugates.

In this project, therefore, urine samples were analysed both freely and indirectly for conjugated alpha-chloralose by determining UGT conjugates after deconjugation with ß-glucuronidase (total alpha-chloralose). The results showed that there was little conjugated alpha chloralose in cat urine. On average, the concentration of free and total alpha-chloralose in cat urine was virtually the same, 18.6 and 20.0 µg/mL, respectively. In some samples of cat urine, however, indication of a conjugation were found - up to 47 %. There was also a clear linear relationship between free and total alpha chloralose in cat urine with a correlation coefficient of 0.96 (Figure 2; Annex Table 2). This indicates that most cats lacked or had little ability to metabolise alpha-chloralose in the form of glucuronic acid conjugates.

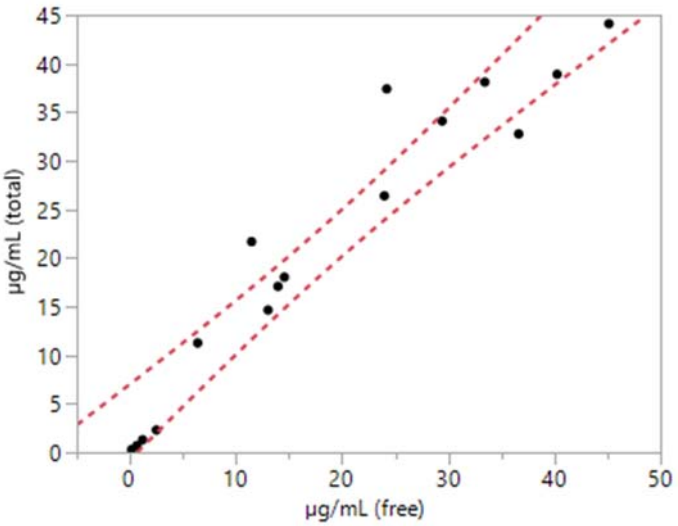

Figure 2: Linear relationship between free and total alpha-chloralose in urine from cats (n = 18); µg/mL(total) = 3.121 + 0.973* µg/mL (free). Correlation coefficient (Pearson) r = 0.96.

Urine from only one dog was examined, and 69 % of alpha-chloralose was conjugated with glucuronic acid. This indicates that dogs have a higher conjugation rate than cats and can therefore more easily excrete alpha-chloralose. However, there may also be other, unknown, biological mechanisms behind cats' susceptibility to alpha-chloralose.

On the other hand, cats are usually far more picky and less experimental in eating unfamiliar food than dogs. Bait with alpha-chloralose seems to be an exception. In several cases of alpha-chloralose poisoning, the cat has eaten the bait.

Cats catch and eat mice to a greater extent than dogs, and this may also be an explanation for the incidence of poisoning in cats. Some of the cats in the material had eaten mice, but the collected material did not prove that eating of alpha-chloralose-poisoned mice was the cause of the poisoning.

Secondary exposure

Whether ingestion of mice which contain alpha-chloralose may poison cats and dogs (secondary exposure) has been assessed via the following calculation:
Alpha-chloralose is found in preparations with 40 g of active substance per kg. A 20 gram mouse can eat 2 grams of dried food per day. If a hungry mouse eats 2 grams of bait, it ingests 80 mg of alpha-chloralose. Perhaps the mice may not be able to eat 2 grams of bait before they are poisoned. In any case, however, 1 gram (40 mg of alpha-chloralose) should be possible. This is far more than LD_{50} for alpha-chloralose in mice (300 mg/kg; 6 mg/20 g mice), but in this context LD_{50} is less relevant; Because it takes some time for

the symptoms of intoxication after oral exposure to appear, mice are likely to be able to eat more than LD_{50}.

If an average cat (approx. 3 kg body weight) eats a mouse with 40 mg alpha-chloralose, it will ingest 13 mg/kg body weight. Mice that have eaten a dose equivalent to LD_{50}, 6 mg/20 g body weight, will cause the 3 kg cat to ingest 2 mg/kg. Cats can also eat several mice in a short time and thus get a higher dose. Ingestion of 3-4 mice that are easy to catch because they are poisoned with alpha-chloralose is probably realistic for a cat. If each mouse gives the cat a dose between 2 and 13 mg/kg, four mice will give a dose of between 8 and 50 mg/kg. This is still below the LD_{50} for cats observed under animal test conditions, but such doses can still be fatal to the cat because it may be clinically affected with the risk of, among other things, heat loss, disorientation and possible anaesthesia. The outcome of poisoning with the substance will depend on the ambient temperature, and there will also be individual variations in sensitivity.

The calculation indicates that cats are likely to be poisoned and die from eating mice exposed to alpha-chloralose. Because LD_{50} in dogs is higher than in cats, and dogs usually have significantly greater body weight and eat mice less often, dogs are less likely to be poisoned by such secondary exposure.

Observed symptoms

The symptoms of alpha-chloralose poisoning as described in the anamneses from the investigated cases (enclosed in the version of this report written in Norwegian) and what is known from the literature can be summarized as follows: Alpha-chloralose causes ataxia and disorientation, often with excitation, and at higher doses an anaesthesia occurs. The usual poisoning develop in that animals become unsteady, start to tremble, get twitches (especially noticeable in the ears or more widespread in the head). They often show hyperesthesia and in some cases seizures or drooling occur. The pupils are usually small, the heart rate is reduced and they have a lowered body temperature. Poisoned animals can become comatose. The effect lasts for many hours, but dogs and cats that receive supportive therapy such as infusion fluid, help to maintain normal body temperature, and any antispasmodic/anti-anxiety/sedative such as diazepam, in most cases appear to be recovered after 2-3 days.

Good prognosis following treatment

This study indicates that dogs and cats that are poisoned with alpha-chloralose but receive veterinary supportive treatment, usually recover. Animals that remain in a poisoned state in cold environments have a poor prognosis. The relatively long anaesthesia including lowered body temperature is likely to pose a high risk of death for poisoned animals who do not return home and thus do not obtain supportive treatment.

References

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Annex tables

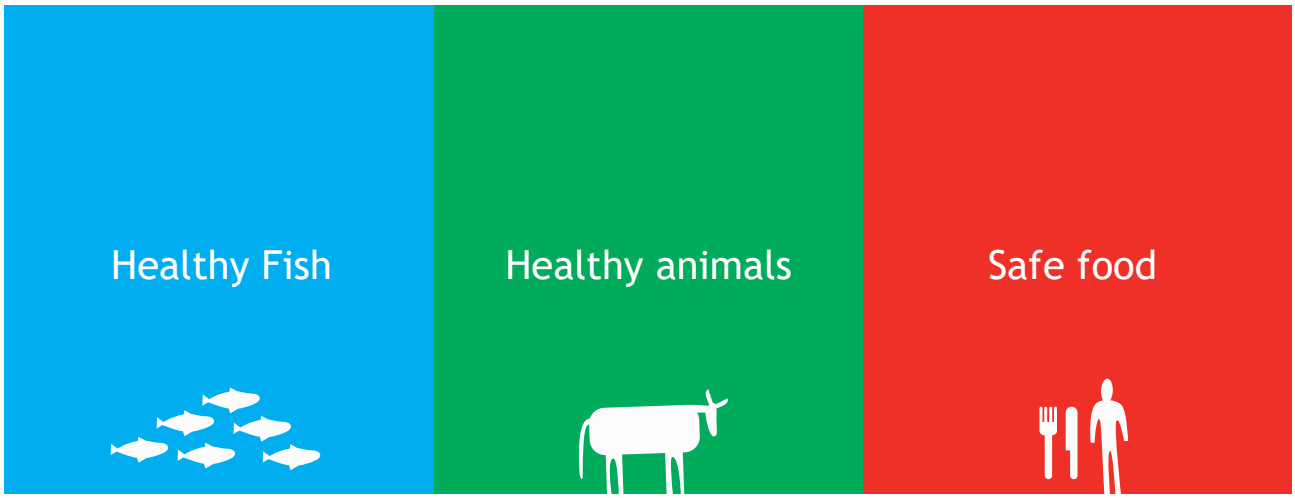
Table 1. Concentrations of alpha-chloralose in tissues (µg/g) and body fluids (µg/mL) from cats from which several samples were collected.

ID-no.	Matrices							
	Urine	Serum	Liver	Kidney	Spleen	Brain	Stomach content	Small intestine
2020-21-6	11.3	0.17					0.08	
2020-21-7	1.3	0.32						
2020-21-8	0.3	0.17						
2020-21-15	18.0	0.23						
2020-21-17	0.4	0.76						
2020-21-18	32.8		0.04	0.07	0.04		0.30	
2020-21-25	37.4	0.32	0.02	0.09		0.13		
2020-21-26	2.3	0.29						
2020-21-27	38.9	0.31						
2020-21-31	17.1	0.23						
2020-21-34	44.1	0.25					0.08	
2020-21-35	14.6	0.31						
2020-04-10054	26.4		1.97			0.29	2.04	5.72
2020-21-40	34.1	30.6						
2020-21-41	21.7	21.8						
2020-04-15565			0.10	0.06		0.03	0.03	0.03
2019-04-58339			0.08			0.06		
2019-04-58340			0.06			0.06	0.38	
2020-04-9584			0.01	2.56		0.03		
2019-0455236			0.11	0.17		0.21		

Table 2. Concentrations of alpha-chloralose (free and conjugated AC) in urine samples from 19 cats and 1 dog. Conjugated alpha-chloralose was measured by indirect determination of UGT conjugates after ß-glucuronidase deconjugation.

ID-nr.	Free AC µg/mL	Total AC µg/mL	Conjugated AC µg/mL	% Conjugated AC
2020-21-6	6.4	11.3	4.87	43
2020-21-7	1.2	1.30	0.10	6
2020-21-8	0.2	0.28	0.12	44
2020-21-9	*	0.47		
2020-21-15	14.6	18.0	3.48	19
2020-21-17	*	0.36		
2020-21-18	36.6	32.8	0.00	0
2020-21-25	24.2	37.4	13.2	35
2020-21-26	2.5	2.30	0.00	0
2020-21-27	40.3	38.9	0.00	0
2020-21-31	14.0	17.1	3.12	18
2020-21-34	45.1	44.1	0.00	0
2020-21-35	13.0	14.6	1.62	11
2020-04-10054	24.0	26.4	2.41	9
2020-21-40	29.4	34.1	4.67	14
2020-21-41	11.5	21.7	10.2	47
2020-21-42	33.4	38.1	4.66	12
2020-20-51	0.66	0.70	0.04	6
2020-21-30 (dog)	0.19	0.69	0.50	69
Mean (for cats)	**18.6**	**20.0**	**3.07**	**17**

* Too little urine to analyse free alpha-chloralose.



Scientifically ambitious, forward-looking and collaborative- for one health!

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