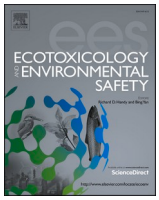
Ecotoxicology and Environmental Safety 253 (2023) 114651

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv



Residues of an anthelmintic veterinary drug (closantel) detected in red foxes (Vulpes vulpes) in Scotland

Marta Giergiel [a,*], Steve Campbell [b], Anna Giela [c], Elizabeth Sharp [b], Fabio Casali [d], Tomasz Śniegocki [a], Bartosz Sell [a], Piotr Jedziniak [a]

[a] National Veterinary Research Institute, Partyzantów 57 Street, 24–100 Puławy, Poland
[b] SASA, Roddinglaw Road, Edinburgh EH12 9FJ, United Kingdom
[c] The Scottish Government, Victoria Quay, Edinburgh EH6 6QQ, United Kingdom
[d] University of Strathclyde, 16 Richmond St, Glasgow G1 1XQ, United Kingdom

ARTICLE INFO

Edited by - Yong Liang

Keywords:
Closantel
Red fox
Sheep
Veterinary medicine residues
Environment contamination

ABSTRACT

The contamination of the environment by some veterinary medicines and their impact on wild animals is of increasing concern. However, there is a lack of information about their residues in wildlife. The sentinel animals most commonly used for monitoring the level of environmental contamination are birds of prey, and information on other carnivores and scavengers scarce. This study examined the livers from 118 foxes for residues of a range of 18 veterinary medicines (16 anthelmintic agents and 2 metabolites) used on farm livestock. The samples were collected from foxes, primarily in Scotland, shot during legal pest control activities conducted between 2014 and 2019. Closantel residues were detected in 18 samples, and the concentrations found ranged from 6.5 μgkg^{-1} to 1383 μgkg^{-1}. No other compounds were found in significant quantities. The results show a surprising frequency and level of closantel contamination, raising concerns about both the route of contamination and the potential impacts on wild animals and the environment, such as the potential for significant wildlife contamination to contribute to the development of closantel-resistant parasites. The results also suggest that red fox (*Vulpes vulpes*) could be a useful sentinel species for detecting and monitoring some veterinary medicine residues in the environment.

1. Introduction

The use of veterinary medicines such as antimicrobial and anthelmintic agents is common in livestock farming (Lemus et al., 2008; McKellar, 1997; Pedersen and Fenton, 2015; Zhao et al., 2020). Residues of medicines can remain in the tissues of treated animals and be excreted in their urine and faeces, there is also a spillage risk associated with some formulations, all of which could contaminate other fauna, soil and water (both surface and groundwater) (Cooke et al., 2017; McKellar, 1997; Mooney et al., 2021; Perkins et al., 2021). Such contamination could have impacts on non-target species or even find its way into the human food chain.

Prolonged or repeated contact with some toxic substances may lead to accumulation in the internal organs of animals (Heltai and Markov, 2012). This has for example been observed with contaminants such as heavy metals, organochlorides and anticoagulant rodenticides in foxes and birds of prey (Heltai and Markov, 2012; Gomez – Ramírez et al., 2014).

Although evaluation of the ecotoxicity of veterinary medicines is part of their legislative approval process, it may be difficult to predict indirect impacts on non-target animals and to the wider ecosystem (plants, invertebrates and others). An example of such unpredicted impact on non-target species is the toxicity of diclofenac (a non-steroidal anti-inflammatory drug) residues to vultures (Cuthbert et al., 2014; Galligan et al., 2021) and its subsequent impact on vulture populations, particularly in India. This illustrates the potential importance of drug residue monitoring, and of fully identifying and assessing environmental risk (Kuster and Adler, 2014). Concerns have been recognised for many

Abbreviations: VM, veterinary medicines; LOD, the limit of detection; WIIS, Wildlife Incident Investigation Scheme; "QuEChERS", (quick, easy, cheap, effective, rugged, and safe); SPE, a solid phase extraction method; LC-MS/MS, Liquid Chromatography - Tandem Mass Spectrometry; MRL, the maximum residue limit; UK, United Kingdom.

* Corresponding author.
E-mail address: marta.giergiel@piwet.pulawy.pl (M. Giergiel).

https://doi.org/10.1016/j.ecoenv.2023.114651
Received 11 June 2022; Received in revised form 8 February 2023; Accepted 11 February 2023
Available online 17 February 2023

years and some jurisdictions have acted to improve environmental assessment and monitoring processes, for example EU regulation 2019/6 (Regulation EU, 2019/6, 2018) which came into force on 28 January 2022 and replaced Directive 2001/82/EC (Directive, 2001/82/EC, 2001): efforts to improve processes will likely continue (Fabrega and Carapeto, 2020; Casa-Resino et al., 2021).

Wild animals can be a useful bioindicator of changes taking place in the environment (Egwumah et al., 2017; Espín et al., 2016), and sentinel animal species may provide early warning signals about the presence of contaminants and their potentially harmful effects, both directly to the environment, and indirectly to humans (Reif, 2011; Van der Schalie et al., 1999).

Monitoring, using wild animals, of a wide range of chemicals (e.g. organochlorine insecticides, PCBs, metals/metalloids, fungicides, flame retardants, anticoagulant rodenticides) has been widely practised around the world, particularly in Europe (Gjershaug et al., 2008; Gómez-Ramírez et al., 2011; Helander et al., 2008; Jaspers et al., 2006; Kenntner et al., 2003; Koskimies, 1989; Movalli et al., 2008; Sell et al., 2022; Van den Brink et al., 2003; Walker et al., 2008). The main subject of such studies has often been birds of prey, which hold a position in the food chain that can help to identify bioaccumulative contaminants, and due to their conservation importance individuals found dead are often collected and examined (Gómez-Ramírez et al., 2014; Badry et al., 2020).

In the UK, the Wildlife Incident Investigation Scheme (WIIS) investigates the deaths of wildlife and companion animals, primarily in situations where pesticide poisoning may have been a factor or where it is suspected that pesticides may be present. WIIS provides an opportunity to examine wild animals found dead, and to test for chemical residues. The results of these investigations in Scotland are published quarterly (Animal Poisoning Reports), they provide information about a range of substances that wild animals may have been in contact with and can detect low levels of contamination as well as levels with potentially harmful effects. However, WIIS may not be completely representative of the true extent of contamination in wild animals because a sampling bias for animals found dead makes it difficult to be confident about extrapolation of the findings to the whole population (Ruiz-Suárez et al., 2016). For this reason, the additional monitoring of selected species which are abundant in the environment, and which are sampled by a potentially less biased approach could provide valuable additional information about the extent of chemical exposure. A good example of such a potential species is the red fox.

The red fox occupies a diverse range of habitats, and has a very varied diet, because of this the fox population is potentially exposed to many toxic substances and thus may be a valuable source of information about environmental contamination. It has previously been used to monitor substances such as anticoagulant rodenticides and metals (Geduhn et al., 2015; Heltai and Markov, 2012; Tosh et al., 2011) and for similar reasons, it has also been used as a sentinel animal in the case of disease monitoring (Nemeth et al., 2016). Moreover, as animals at the top of the food chain, foxes may accumulate chemical residues from trophic levels below them through prey and scavenging. Foxes are commonly shot and trapped in Scotland and the rest of the UK for pest control purposes and thus there is an existing potential source of samples that could be used for environmental monitoring that may be less biased than the sourcing of samples for WIIS.

The aim of the current study was to examine fox livers for exposure to a range of anti-ecto- and endoparasitic agents which are used on farms in Scotland. There are no reports relating to the presence of veterinary drugs in wild animal tissues in Scotland and for that reason the results are potentially valuable and provide new information in that field.

2. Materials and methods

2.1. Sample sources

Foxes are regularly shot throughout the year by farmers, pest controllers and other land managers in the UK to protect both livestock and game animals. Carcasses of foxes shot for this purpose were donated by shooters for use in disease and pesticide monitoring. Between 2014 and 2019 foxes were collected from shooters in Scotland, the livers were removed in a laboratory, and a subsample taken and frozen at − 20 °C until required. Overall, 118 livers were sampled, 116 of these originated in Scotland and a further two were from a site in the North of England. These latter two were not intended to be tested but were processed due to a miscommunication, however we have included them in the results for completeness (see Fig. 2).

2.2. Analytes of interest

The analytes included in the examination were veterinary medicines from the group of anthelmintic and antiparasitic drugs for endoparasites (albendazole, clorsulon, closantel, doramectin, flubendazole, levamisole, mebendazole, moxidectin, oxfendazole, praziquantel, thiabendazole, triclabendazole), their metabolites (albendazole sulfoxide, fipronil sulfone) as well as for ectoparasites (avermectin B1a, coumaphos, dicyclanil, fipronil). Certified reference standards (purity $\geq$ 98%) of veterinary medicines were purchased from Qmx, Thaxted, England UK, Greyhound Chromatography & Chemicals, Birkenhead, Scotland UK, Sigma-Aldrich Company Ltd., Gillingham, England UK or LGC Ltd, Teddington, England UK.

2.3. Preparation of standard solutions

Stock standard solutions of all of the veterinary medicines were prepared in-house in methanol at concentrations of approximately 400 μgml^{-1}, purely as a convenient starting point to prepare the subsequent intermediate mixed stock solutions. Appropriate amounts of each stock were taken to make composite standard mixtures at concentrations of approximately 5 μgml^{-1} which could be stored at 4–7 °C for one year. From this, a mixed intermediate standard solution at 1 μgml^{-1} was prepared. The intermediate solution was used to prepare a series of solvent standards (ranging from 0.001 to 0.1 μgml^{-1}). The solvent standards were diluted 2-fold into 1 ml volumetric flasks using chicken liver matrix blank extract (extracted as described in Section 2.4) to obtain matrix standards from 0.0005 to 0.05 μgml^{-1}. The intermediate standard solution at 1 μgml^{-1}, the solvent and the matrix standards were stored at 4–7 °C and were available for use for up to 1 week.

2.4. Sample preparation

The samples were prepared according to the method described previously by Taylor et al. (2019). The liver samples were defrosted, chopped and 1.0 ($\pm$ 0.1) g of each was weighed into a plastic centrifuge tube. Then 5 ml of acetonitrile was added for extraction and the samples were vortex mixed for 1 min. Thereafter the commercial QuEChERS extraction salt packet (containing 4 g Na_2SO_4, 1 g NaCl) was added to the tube and after shaking for 1 min, the tubes were centrifuged for 5 min at approximately 2500 x g at room temperature. After that, a 3 ml aliquot of the supernatant was transferred to a 15 ml dispersive SPE tube (containing 50 mg of PSA, 150 mg of C18EC and 900 mg of anhydrous Na_2SO_4), the tube was vortex mixed and then centrifuged for 5 min at 3000 x g. The supernatant was filtered through 0.45 μm PTFE syringe filters into glass vials ready for LC-MS/MS examination.

2.5. LC-MS/MS analysis

Chromatographic analyses were performed using a Nexera X2 UPLC

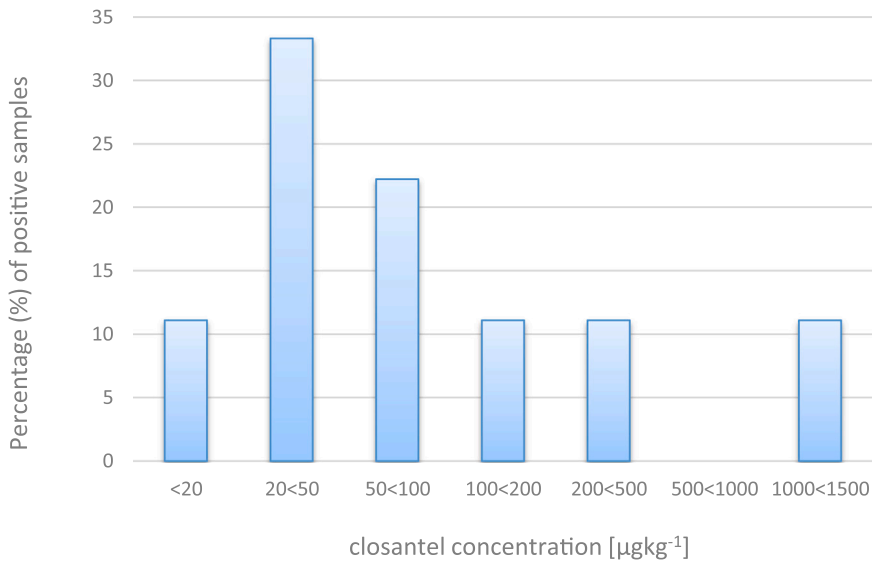


Fig. 1. The concentrations of closantel found in positive samples.

system coupled to a Shimadzu LCMS 8050 triple quadrupole mass spectrometer (Shimadzu UK Ltd., Milton Keynes). The chromatographic separation was performed using a Kinetex C18 UHPLC analytical column (2.6 μm, 50 ×4.6 mm) at 35 °C (Phenomenex, Torrance, USA),. The flow was set at 400 μlmin^{-1}. The injection volume was 5 μl. The total run time was 17 min and the gradient was programmed as in Table 1.

The limit of detection (LOD) was set at 5 μgkg^{-1} for all of the analytes. The samples were diluted in order to fit within the limits of the calibration curve. Acceptable recoveries fell within the range 60–140% with the mean being between 70% and 90% at low and high levels.

3. Results

Only one of the 18 medicines and metabolites being tested for was detected. The veterinary medicine Closantel was found above the limit of detection (LOD) in 18 (15.3%) of the examined samples (n = 118). The average concentration was 219 μgkg^{-1}, with the minimum concentration found being 6.5 μgkg^{-1} and the maximum level detected 1383 μgkg^{-1}. However the majority of the positive samples (55,6%) were in the range of 20 < 100 μgkg^{-1} (Fig. 1).

Positive samples were distributed widely across the country and came from 13 different geographic locations (Fig. 2) and between two and six positives were detected from each of the five study years.

4. Discussion

4.1. Closantel residues

Closantel was the only veterinary medicine detected in this study, to our knowledge it is the first time that closantel has been detected in wild animals in Scotland and we are not aware of similar results being found elsewhere Closantel is an anthelmintic veterinary drug from the salicylanilide group, which is used (often in combination with other active ingredients) in sheep and cattle to control liver fluke (Fasciola hepatica), roundworms such as *Haemonchus spp* and other endoparasites; it can also be used to treat some larval arthropod infestations such as nasal bot fly (*Oestrus ovis*) or mange mites such a *Psoroptes spp*. Although widely used in sheep and cattle in the UK it is not typically used on other livestock, pets or humans. Application to sheep and cattle in the UK is usually by oral 'drench' or parenterally by intramuscular or subcutaneous injection. There are also some 'pour on' products for external application on cattle (SCOPS, 2018; EMA, 2019).

Closantel was detected in 15.3% (n = 18) of the tested samples, and they were well distributed both through time and geographically, indicating that the occurrence was not due to a single incident. The frequency with which closantel was detected in the samples is notable relative to the other compounds tested for, none of which were detected, and raises questions about whether there is some factor unique to closantel, or the way it is used, that might account for this. However, we have no information on the relative levels of use of each compound. Liver concentrations of closantel averaged 219.3 μgkg^{-1}, however the range was wide and in two samples exceeded 1000 μgkg^{-1}. Given that the drug was not directly administered to foxes, > 1000 μgkg^{-1} could be considered high. For comparison, the maximum residue limit (MRL) imposed by the EU for closantel residues in the liver are 1000 μgkg^{-1} for cattle and 1500 μgkg^{-1} for sheep, however, these are limits below which it is considered acceptable to permit human consumption of the tissue and the concentrations in the immediate aftermath of treatment could be significantly higher. The most recent EU guidance is that a period of 107 days should be applied post-treatment to ensure that residues are below the MRL (EMA, 2014; EMA, 2019), implying a much higher starting point during typical treatments. Closantel has a relatively long biological half-life and is poorly metabolised, leading to much of a dose being excreted unchanged (Michiels et al., 1987).

Closantel is typically administered at a rate of 5 mgkg^{-1} via intramuscular injection to sheep and cattle and at a higher rate of 10 mgkg^{-1} via oral drench. If the absorption of closantel by the liver of a fox is similar to that of the ruminants then this could imply that some of the sampled foxes had a significant initial intake of closantel in the order of several mgkg^{-1}. Unfortunately, the time between ingestion of the closantel and sampling of the foxes tested is unknown and probably varied considerably. It is also likely that some foxes ingested closantel on several occasions and at different concentrations prior to the samples being taken. Thus, it is not possible to estimate the initial dosage that the foxes received.

4.2. Contamination routes

The red fox, while predominantly a hunter of small mammals, can exist on a very varied omnivorous diet, this allows it to be highly adaptable to many habitats and circumstances (Harris and Yalden, 2008). In some parts of Scotland sheep may form a significant component of the diet of foxes. This may comprise of lambs that have been predated, but mainly of scavenged lambs and adult sheep that have died of other causes (Hewson, 1984). Studies suggest that the overall impact of fox predation on lambs is likely to be low (<10% of overall mortality)

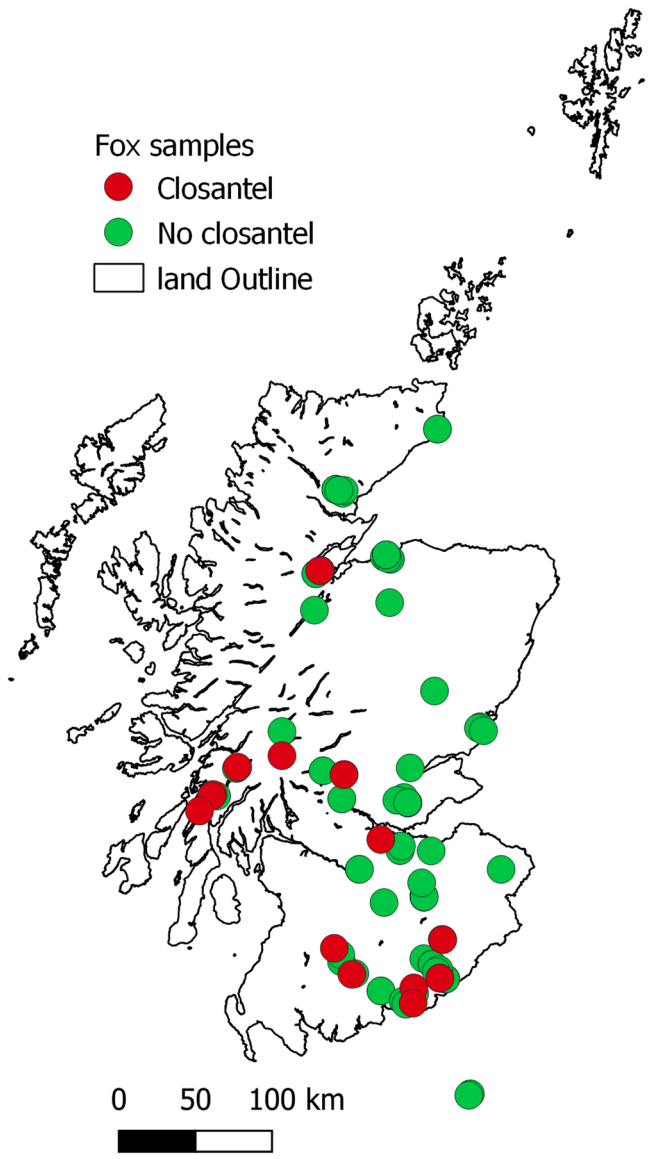


Fig. 2. Map of Scotland showing the origin of fox samples and in which samples closantel was detected.

Table 1
The chromatographic elution profile. Composition of mobile phase in channel A: methanol/H_2O 5/95 (5 mM ammonium acetate) and in channel B: methanol (5 mM ammonium acetate).

Time (minutes)	% of mobile phase in channel A	% of mobile phase in channel B
0.1	75	25
0.7	40	60
12.0	2	98
13.9	2	98
14.0	75	25
17.0	75	25

but can vary widely from farm to farm (Harris and Yalden, 2008). The scavenging of sheep and lamb carcasses, and the predation of lambs, are possible direct routes by which veterinary medicines could find their way into foxes. However, another potential direct route is medicines that have been spilled and which have contaminated either water or food items. Medicines might also be indirectly ingested as secondary contamination by the consumption of animals other than treated livestock that have themselves ingested the compounds, for example rodents in the vicinity of a farm.

Closantel is not classed as bioaccumulative Veterinary Medicines Directorate, 2019(defra.gov.uk) United Kingdom Veterinary Medicines Directorate, 2016., although it can persist in tissues of treated animals for many weeks, typically having an elimination half-life of 2–3 weeks (Michiels et al., 1987). A large proportion of administered Closantel is bound in blood plasma and quickly excreted; for oral dosing, this can be around 43% in the first 48 h (10% for intramuscular injection) and around 80% in total over several weeks (https://inchem.org/documents/jecfa/jecmono/v27je02.htm), in this situation the consumption of a treated animal in the first day or so after treatment seems likely to deliver the highest levels of contamination.

Alongside accidental spillage, the excretion of significant amounts of the chemical from treated livestock raises another possible route: i.e. eating or drinking from a source contaminated by faecal or urine deposits of livestock in the aftermath of treatment, or the direct consumption of such deposits. It is widely noted that the faecal matter of closantel treated animals is toxic to dung and soil invertebrates Veterinary Medicines Directorate, 2019(defra.gov.uk) indicating significant levels of the active ingredient can be present. Foxes could conceivably drink contaminated water or consume some contaminated soil while foraging for various food stuffs, however, the extent to which foxes might be directly coprophagic towards the faeces of sheep or cattle is unknown as we are unaware of any studies on this. Research has indicated that in some situations, foxes may be coprophagic on the faeces of domestic dogs (Waggershauser et al., 2022) however given the significant differences in composition between carnivore and herbivore faeces it is not certain that foxes would find adult ruminant faeces attractive. Macdonald (1987) reports observing a fox consuming the faeces of a young lamb, and it is known that some domestic dogs will eat ruminant dung, so the possibility exists that some, perhaps many, foxes may also exhibit this behaviour. Nevertheless, the consumption of faeces is likely to be difficult to detect by conventional dietary analysis and therefore could be significantly under-recorded, or even completely missed if it occurs.

Visual problems, up to and including blindness are a potential outcome of overdosing with closantel (Gill et al., 1999). In the case of sheep and lambs this and other sub-lethal effects of closantel poisoning could make them more susceptible to predators such as the fox. The consumption of tissue from livestock that have been excessively dosed is perhaps a route by which a fox could consume a high secondary dose. Negative effects may occur when the dose is from 2 to 6 times higher than recommended for kg body weight (Barlow et al., 2002). However, while accidental closantel poisoning of sheep has been recorded in some countries (Neha Rao et al., 2018; Rivero et al., 2015) the extent of closantel contamination found in foxes might imply a high frequency of overdosing by farmers if this were the cause, and there would likely be other evidence of such practices such as significant numbers of veterinary reports of closantel poisoning.

4.3. Impact of contamination

The toxic implications of closantel for foxes may be different than for ruminants; visual ailments are a common symptom of closantel poisoning among various species, including dogs (McEntee et al., 1995), however concentrations would likely have to be much higher than those detected to have significant negative impact on the animal. For example, tests on beagles determined that the 'no observable effect limit (NOEL) for closantel was a dosage of around 2.5 $mgkg^{-1}$ per day and even at rates of 10 and 0 $mgkg^{-1}$ per day it did not lead to any significant toxic outcomes in the animals (https://inchem.org/documents/jecfa/jecmono/v27je02.htm).

Carcasses in this study were not subject to detailed examination for signs of closantel (or other vet medicine) toxicity as they were primarily collected for other unrelated reasons. As previously noted, closantel

poisoning in various animals is often associated with blindness and vision problems (Bacellar-Galdino et al., 2020; Essabar et al., 2014; McEntee et al., 1995) and while nothing was observed during this study that suggested any of the animals sampled were suffering any visual impairments there were no specific examinations undertaken either, so effects such as characteristic ocular lesions that might be found with anatomopathological investigation would not have been detected.

It is worth considering that at certain levels, the presence of closantel in foxes may not be negative in terms of the outcome for the fox; there is even a possibility that at some concentrations it may be beneficial, helping to control or prevent some parasitic infections. However, the levels we detected varied considerably and while this may partly be due to different time periods since ingestion of the closantel, it is almost certainly also due to different levels of intake. It would seem reasonable to assume that some foxes will receive doses which are insufficient to significantly impact on parasite levels but which, along with higher doses in other individuals could help to select for closantel resistant parasites. Also, depending on the route of contamination the presence of the closantel in foxes may be indicative that other species could also be contaminated at levels where parasites may be subject to selection for resistant individuals. Parasite resistance to closantel has so far not been widely recorded, but possible signs of it have been detected in Barber's pole worm (*Haemonchus contortus*) and liver fluke (*Fasciola hepatica*) (Fairweather et al., (2020, 2015)).

5. Conclusion

Our results show that monitoring of veterinary drugs in the environment is potentially important (Boxall et al., 2004; Kümmerer, 2004) and the finding of widespread closantel residues illustrates that increased wildlife monitoring efforts should be considered (Kuster and Adler, 2014). This is the first time a veterinary medicine has been detected in the livers of wild foxes in the UK, and the number and distribution of positive samples suggests that it may not be an uncommon occurrence for closantel. The monitoring of birds of prey in various countries (Gómez-Ramírez et al., 2014; Badry et al., 2020) has been helpful in highlighting pollutant threats to wild animals; similar monitoring studies on red foxes which are also one of the main predators and scavengers and are similarly at the top of food chain in the UK could be of additional value. Red foxes inhabit a wide range of habitats and potentially encounter many different substances, and this could make them good sentinel animals, in addition carcasses are readily available as a by-product of pest control activities.

The contamination pathway of closantel and the effect of the contamination on foxes, the wider environment and parasite populations warrants further investigation.

Author contributions

Marta Giergiel: Conceptualisation, Investigation, Formal analysis, Data curation, Writing – original draft, Funding acquisition. **Steve Campbell:** Conceptualization, Methodology, Visualization, Investigation, Data curation, Writing – original draft. **Anna Giela:** Validation, Data curation, Writing – review & editing. **Elizabeth Sharp:** Methodology, Data curation, Writing – review & editing. **Fabio Casali:** Formal analysis, Data curation, Writing – review & editing. **Tomasz Śniegocki:** Methodology, Writing – review & editing. Bartosz Sell: Methodology, Writing – review & editing. **Piotr Jedziniak:** Supervision, Methodology, Writing – review & editing.

CRediT authorship contribution statement

Category 1.

Conception and design of study: Marta Giergiel; Steve Campbell, Piotr Jedziniak.

acquisition of data: Marta Giergiel; Anna Giela; Elizabeth Sharp; Steve Campbell; Fabio Casali,

analysis and/or interpretation of data: Marta Giergiel; Anna Giela; Elizabeth Sharp; Fabio Casali; Tomasz Śniegocki; Bartosz Sell.

Category 2.

Drafting the manuscript: Marta Giergiel; Steve Campbell; Anna Giela; Elizabeth Sharp; Tomasz Śniegocki; Bartosz Sell, Fabio Casali, Piotr Jedziniak.

revising the manuscript critically for important intellectual content: Marta Giergiel; Steve Campbell; Anna Giela; Elizabeth Sharp, Piotr Jedziniak;.

Category 3.

Approval of the version of the manuscript to be published (the names of all authors must be listed):

Marta Giergiel; Steve Campbell; Anna Giela; Elizabeth Sharp, Piotr Jedziniak; Tomasz Śniegocki; Bartosz Sell, Fabio Casali.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The authors do not have permission to share data.

Acknowledgements

The study was conducted during Marta Giergiel's research scholarship at Science and Advice for Scottish Agriculture (SASA) which was funded by the KNOW (Leading National Research Centre) Scientific Consortium "Healthy Animal – Safe Food", decision of Ministry of Science and Higher Education [Grant no. 05–1/KNOW2/2015]. We would like to thanks Claire Catherine Senior, Devanshi Shashikant Vyas for support in LC/MS-MS analysis and processing the data and Michael Jeffrey Taylor for opportunity and support during study. Thanks also to APHA (Animal and Plant Health Agency) and the various pest controllers who assisted with sample acquisition.

References

Bacellar-Galdino, M., Montiani-Ferreira, F., Somma, A.T., Vilani, R.G.D.C., Filho, I.R.B., 2020. Ophthalmic findings in sheep treated with closantel in Curitiba, Brazil. Vet. World 13, 860–864. https://doi.org/10.14202/vetworld.2020.860-864.

Badry, A., Krone, O., Jaspers, V.L.B., Mateo, R., García-Fernández, A., Leivits, M., Shore, R.F., 2020. Towards harmonisation of chemical monitoring using avian apex predators: Identification of key species for pan-European biomonitoring. Sci. Total. Environ. 731, 139198 https://doi.org/10.1016/j.scitotenv.2020.139198.

Barlow, A.M., Sharpe, J.A.E., Kincaid, E.A., 2002. Blindness in lambs due to inadvertent closantel overdose. Vet. Rec. 151, 25–26. https://doi.org/10.1136/vr.151.1.25.

Boxall, A.B.A., Fogg, L.A., Blackwell, P.A., Kay, P., Pemberton, E.J., Croxford, A., 2004. Veterinary medicines in the environment. Rev. Environ. Contam. Toxicol. 180, 1–91. https://doi.org/10.1007/0-387-21729-0_1.

Casa-Resino, I., Castuera, A.H., Elena R.C., Montejano, C.R., García, R.C., 2021. European legislation for veterinary medicines: Would a monograph system improve the environmental risk assessment? Integr. Environ. Assess. Manag. 17, 1274–1285. https://doi.org/10.1002/ieam.4431.

Cooke, A.S., Morgana, E.R., Dungait, J.A.J., 2017. Modelling the impact of targeted anthelmintic treatment of cattle on dung fauna. Environ. Toxicol. Pharmacol. 55, 94–98. https://doi.org/10.1016/j.etap.2017.07.012.

Cuthbert, R.J., Taggart, M.A., Prakash, V., Chakraborty, S.S., Deori, P., et al., 2014. Avian scavengers and the threat from veterinary pharmaceuticals. Philos. Trans. R. Soc. 369, 20130574. https://doi.org/10.1098/rstb.2013.0574.

Directive 2001/82/EC, 2001. Directive 2001/82/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to veterinary medicinal products. Off. J. Eur. Communities L311, 1–110. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32001L0082.

Egwumah, F.A., Egwumah, P.O., Edet, D.I., 2017. Paramount roles of wild birds as bioindicators of contamination. Int. J. Avian Wildl. Biol. 2, 194–200. https://doi.org/10.15406/ijawb.2017.02.00041.

European Medicines Agency EMA (2014) European public MRL assessment report (EPMAR) Closantel (bovine and ovine milk) – after the provisional maximum residue

limit (MRL). EMA/CVMP/751522/2013 Committee for Medicinal Products for Veterinary Use.
European Medicines Agency EMA (2019) Questions and answers on veterinary medicinal products containing 50 mg closantel per ml (as a single active substance) presented as solutions for injection for subcutaneous use in sheep. Outcome of a referral procedure under Article 35 of Directive 2001/82/EC (EMEA/V/A/126).
Espín, S., García-Fernández, A.J., Herzke, D., et al., 2016. Tracking pan-continental trends in environmental contamination using sentinel raptors-what types of samples should we use? Ecotoxicology 25 (4), 777–801. https://doi.org/10.1007/s10646-016-1636-8.
Essabar, L., Meskini, T., Ettair, S., Erreimi, N., Mouane, N., 2014. Harmful use of veterinary drugs: Blindness following closantel poisoning in a 5-year-old girl. Asia Pac. J. Med. Toxicol. 3, 173–175. https://doi.org/10.22038/APJMT.2014.3486.
Fabrega, J., Carapeto, R., 2020. Regulatory review of the environmental risk assessment of veterinary medicinal products in the European Union, with particular focus on the centralised authorisation procedure. Environ. Sci. Eur. 32, 99. https://doi.org/10.1186/s12302-020-00374-x.
Fairweather, I., Brennan, G.P., Hanna, R.E.B., Robinson, M.W., Skuce, P.J., 2020. Drug resistance in liver flukes. Int. J. Parasitol. Drugs Drug. Resist. 12, 39–59. https://doi.org/10.1016/j.ijpddr.2019.11.003.
Galligan, T.H., Mallord, J.W., Prakash, V.M., Bhusal, K.P., Alam, A., Anthony, F., Dave, R., et al., 2021. Trends in the availability of the vulture-toxic drug, diclofenac, and other NSAIDs in South Asia, as revealed by covert pharmacy surveys. Bird. Conserv. Int. 31, 337–353. https://doi.org/10.1017/S0959270920000477.
Geduhn, A., Jacob, J., Schenke, D., Keller, B., Kleinschmidt, S., Esther, A., 2015. Relation between Intensity of Biocide Practice and Residues of Anticoagulant Rodenticides in Red Foxes (Vulpes vulpes). PLoS One 10 (9), e0139191. https://doi.org/10.1371/journal.pone.0139191.
Gill, P.A., Cook, R.W., Boulton, J.G., Kelly, W.R., Vanselow, B., Reddacliff, L.A., 1999. Optic neuropathy and retinopathy in closantel toxicosis of sheep and goats. Aust. Vet. J. Apr. 77, 259–261. https://doi.org/10.1111/j.1751-0813.1999.tb11718.x.
Gjershaug, J.O., Kålås, J., Nygård, T., Herzke, D., Folkestad, A.O., 2008. Monitoring of raptors and their contamination levels in Norway. Ambio 37, 420–424. https://doi.org/10.1579/0044-7447(2008)37[423:moratc]2.0.co;2.
Gómez-Ramírez, P., Martínez-López, E., María-Mojica, P., León-Ortega, M., García-Fernández, A., 2011. Blood lead levels and δ-ALAD inhibition in nestlings of Eurasian Eagle Owl (Bubo bubo) to assess lead exposure associated to an abandoned mining area. Ecotoxicol 20, 131–138. https://doi.org/10.1007/s10646-010-0563-3.
Gómez-Ramírez, P., Shore, R.F., Van den Brink, N.W., Van Hattum, B., Bustnes, J.J.O., 2014. An overview of existing raptor contaminant monitoring activities in Europe. Environ. Int. 67, 12–21. https://doi.org/10.1016/j.envint.2014.02.004.
Gupta, S.K., Chaudhri, S.S., Poonia, J.S., 2003. Preliminary report of closantel resistance against Haemonchus contortus in sheep of western Haryana. Indian J. Anim. Sci. 73, 1024–1026.
Harris, S., Yalden, D., 2008. Mammals of the British Isles: handbook. Mammal Society.
Helander, B., Bignert, A., Asplund, L., 2008. Using raptors as environmental sentinels: monitoring the White-tailed Sea Eagle Haliaeetus albicilla in Sweden. Ambio 37, 425–431. https://doi.org/10.1579/0044-7447(2008)37[425:uraesm]2.0.co;2.
Heltai, M., Markov, G., 2012. Red fox (Vulpes vulpes Linnaeus, 1758) as biological indicator for environmental pollution in Hungary. Bull. Environ. Contam. Toxicol. 89 (4), 910–914. https://doi.org/10.1007/s00128-012-0755-z.
Hewson, R., 1984. Scavenging and predation upon sheep and lambs in west Scotland. J. Appl. Ecol. 21, 843–868 https://doi.org/10.2307/2405051. https://inchem.org/documents/jecfa/jecmono/v27je02.htm.
Jaspers, V.L.B., Covaci, A., Voorspoels, S., Dauwe, T., Eens, M., Schepens, P., 2006. Brominated flame retardants and organochlorine pollutants in aquatic and terrestrial predatory birds of Belgium: levels, patterns, tissue distribution and condition factors. Environ. Pollut. 139, 340–352. https://doi.org/10.1016/j.envpol.2005.05.008.
Kenntner, N., Krone, O., Altenkamp, R., Tataruch, F., 2003. Environmental contaminants in liver and kidney of free-ranging northern goshawks (Accipiter gentilis) from three regions of Germany. Arch. Environ. Contam. Toxicol. 45, 128–135. https://doi.org/10.1007/s00244-002-2100-8.
Koskimies, P., 1989. Birds as a tool in environmental monitoring. Ann. Zool. Fenn. 26, 153–166.
Kümmerer, K., 2004. Pharmaceuticals in the Environment — Scope of the Book and Introduction. In: Kümmerer, K. (Ed.), Pharmaceuticals in the Environment. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-09259-0_1.
Kuster, A., Adler, N., 2014. Pharmaceuticals in the environment: scientific evidence of risks and its regulation. Philos. Trans. R. Soc. B. 369, 20130587. https://doi.org/10.1098/rstb.2013.0587.
Lemus, J.A., Blanco, G., Grande, J., Arroyo, B., Garcıa-Montijano, M., Martínez, F., 2008. Antibiotics threaten wildlife: circulating quinolone residues and disease in avian scavengers. PLoS ONE 3 (1), e1444. https://doi.org/10.1371/journal.pone.0001444.
Macdonald, D., 1987. Running with the fox. Unwin Hyman, London.
McEntee, K., Grauwels, M., Clercx, C., Henroteaux, M., 1995. Closantel intoxication in a dog. Vet. Hum. Toxicol. 37, 234–236.
McKellar, Q.A., 1997. Ecotoxicology and residues of anthelmintic compounds. Vet. Parasitol. 72, 413–435. https://doi.org/10.1016/S0304-4017(97)00108-8.
Michiels, M., Meuldermans, W., Heykants, J., 1987. The metabolism and fate of closantel (Flukiver) in sheep and cattle. Drug. Metab. Rev. 8, 235–251. https://doi.org/10.3109/03602538708998307.
Mooney, D., Richards, K.G., Danaher, M., Grant, J., Gill, L., Mellander, P.E., Coxon, C.E., 2021. An analysis of the spatio-temporal occurrence of anthelmintic veterinary drug residues in groundwater. Sci. Total Environ. 769 (2021), 144804 https://doi.org/10.1016/j.scitotenv.2020.144804.
Movalli, P., Lo, Valvo, M., Pereira, M.G., Osborn, D., 2008. Organochlorine pesticides and polychlorinated biphenyl congeners in lanner Falco biarmicus feldeggii Schlegel chicks and lanner prey in Sicily, Italy. Ambio 37, 445–451. https://doi.org/10.1579/0044-7447(2008)37[445:opapbc]2.0.co;2.
Neha Rao, J.J., Parmar, D.B., Sadhu, A.I., Shah, Patel, D.M., 2018. Closantel Intoxication in a Sheep: case Study. Int. J. Curr. Microbiol. Appl. Sci. 7, 1445–1447. https://doi.org/10.20546/ijcmas.2018.710.160.
Nemeth, N.M., Campbell, G.D., Oesterle, P.T., Shirose, L., McEwen, B., Jardine, C.M., 2016. Red Fox as Sentinel for Blastomyces dermatitidis, Ontario, Canada. Emerg. Infect. Dis. 22, 1275–1277. https://doi.org/10.3201/eid2207.151789.
Novobilsky, A., Hoglund, J., 2015. First report of closantel treatment failure against Fasciola hepatica in cattle. Int J. Parasitol. Drugs Drug Resist 5, 172–177. https://doi.org/10.1016/j.ijpddr.2015.07.003.
Pedersen, A.B., Fenton, A., 2015. The role of antiparasite treatment experiments in assessing the impact of parasites on wildlife. Trends Parasitol. 31, 200–211. https://doi.org/10.1016/j.pt.2015.02.004.
Perkins, R., Whitehead, M., Civil, W., Goulson, D., 2021. Potential role of veterinary flea products in widespread pesticide contamination of English rivers. Sci. Total Environ. 755, Part 1, 143560.
Regulation (EU) 2019/6. (2018). Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC. Official Journal of the European Union, L4, 43–167. (EUR-Lex - 32019R0006 - EN - EUR-Lex (europa.eu).
Reif, J.S., 2011. Animal sentinels for environmental and public health. Public Health Rep. 126, 50–57. https://doi.org/10.1177/00333549111260S108.
Rivero, R., Matto, C., Soares, M.P., de Lourdes Adrien, M., 2015. Accidental and experimental Closantel intoxication in Uruguayan sheep. Pesq. Vet. Bras. 35, 599–604. https://doi.org/10.1590/S0100-736×2015000700001.
Ruiz-Suárez, N., Melero, Y., Giela, A., Henríquez-Hernández, L.A., Sharp, E., Boada, L.D., Taylor, M.J., Camacho, M., Lambin, X., Luzardo, O.P., Hartley, G., 2016. Rate of exposure of a sentinel species, invasive American mink (Neovison vison) in Scotland, to anticoagulant rodenticides. Sci. Total. Environ. 569–570, 1013–1021. https://doi.org/10.1016/j.scitotenv.2016.06.109.
SCOPS, Sustainable Control of Parasites, 2018. Know your Antihelminthic groups. Sustainable Control of Parasites, NSA, Malvern.
Sell, B., Śniegocki, T., Giergiel, M., Posyniak, A., 2022. White-Tailed Eagles' (Haliaeetus albicilla) Exposure to Anticoagulant Rodenticides and Causes of Poisoning in Poland (2018–2020). Toxics 10, 63. https://doi.org/10.3390/toxics10020063.
Taylor, M.J., Giela, A., Sharp, E.A., Senior, C.C., Vyas, D.S., 2019. A rapid multi-class, multiresidue UHPLC-MS/MS method for the simultaneous determination of anticoagulant rodenticides, pesticides and veterinary medicines in wild animals, pets and livestock. Anal. Methods 11, 1087–1101. https://doi.org/10.1039/C8AY02367K.
Tosh, D.G., McDonald, R.A., Bearhop, S., Lllewellyn, N.R., Fee, S., 2011. Does small mammal prey guild affect the exposure of predators to anticoagulant rodenticides? Environ. Pollut. 159, 3106–3112. https://doi.org/10.1016/j.envpol.2011.03.028.
United Kingdom Veterinary Medicines Directorate, 2016. PUBLICLY AVAILABLE ASSESSMENT REPORT FOR A VETERINARY MEDICINAL PRODUCT Closone 50 mg / ml Oral Suspension for Sheep (UK). UK/V/0575/001/DC.
Van den Brink, N.W., Groen, N.M., De Jonge, J., Bosveld, A.T.C., 2003. Ecotoxicological suitability of floodplain habitats in The Netherlands for the little owl (Athene noctua vidalli. Environ. Pollut. 122, 127–134. https://doi.org/10.1016/s0269-7491(02)00275-0.
Van der Schalie, W.H., Gardner, H.S., Bantle, J.A., De Rosa, Finch, R.A., Reif, J.S., Reuter, R.H., Backer, L.C., Burger, J., Folmar, L.C., Stokes, W.S., 1999. Animals as sentinels of human health hazards of environmental chemicals. Environ. Health Perspect. Apr. 107, 309–315 https://doi.org /10.1289/ehp.99107309.
Veterinary Medicines Directorate, 2019. SUMMARY OF PRODUCT CHARACTERISTICS, 2019 , NAME OF THE VETERINARY MEDICINAL PRODUCT Santiola 50 mg /ml solution for injection for cattle and sheep. AN: 004561/2019.
Waggershauser, C.N., Taberlet, P., Coissac, E., Kortland, K., Hambly, C., Lambin, X., 2022. Interspecific coprophagia by wild red foxes: DNA metabarcoding reveals a potentially widespread form of commensalism among animals. Ecol. Evol. 12, e9029 https://doi.org/10.1002/ece3.9029.
Walker, L.A., Shore, R.F., Turk, A., Pereira, M.G., Best, J., 2008. The predatory bird monitoring scheme: identifying chemical risks to top predators in Britain. Ambio 37, 466–471. https://doi.org/10.1579/0044-7447(2008)37[469:tpbmsi]2.0.co;2.
Zhao, H., Sun, R., Yu, P., Alvarez, P.J.J., 2020. High levels of antibiotic resistance genes and opportunistic pathogenic bacteria indicators in urban wild bird feces. Environ. Pollut. 266, 2. https://doi.org/10.1016/j.envpol.2020.115200.