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Veterinary pharmaceuticals and declining Cape Griffon Vulture (*Gyps coprotheres*) numbers: A potential threat to developing embryos

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ABSTRACT

Cape Vultures (*Gyps coprotheres*) are a vulnerable Old-World Vulture species in southern Africa. Of the numerous threats to their survival, malicious and accidental poisonings remain a major concern. Despite the dangers of poisonings little is however known about the more insidious effects of toxins on egg survival, despite the species known to have a long generational length. For this study, an extensive literature review focusing on veterinary pharmaceuticals was undertaken. Literature for vultures was scarce, with most studies focusing on the domestic chicken. Using information for domestic chickens, the risk was characterised from likely vulture exposure to production animal carcasses with residues of said drugs. From this various antibiotics, medetomidine and albendazole were identified with embryotoxic or teratogenic effects. We suggest that these drugs be tested to elucidate their dose-response relationship and/or mitigation measures to minimise vulture exposure.

1. Introduction

Vultures are long-lived birds known for their size, hooked beaks, bald heads and necks and exceptional eyesight. They are obligate scavengers and primarily feed on carrion and rarely, if ever, prey on live animals. The vulture family consists of old world (accipitrid) and new world (cathartid) vultures (Naidoo et al., 2011). New world vultures like the Turkey vulture (*Cathartes aura*) are found in and around the Americas, while old world vultures are found in Europe, Asia, and Africa. In their particular environments, vultures play an integral role in maintaining healthy ecosystems, by rapidly locating and devouring carcasses in combination with their highly acidic stomach content, they help curb and prevent the spread of pathogens (Den Heever et al., 2021). Vultures assist in regulating the numbers and composition of vertebrate scavengers and their declining numbers may lead to an increase in facultative scavengers like hyenas and jackals, resulting in prolonged carcass persistence in the environment, increasing the possibility of disease transmission viz. Vultures have been associated with decreasing the spread of diseases like anthrax, bovine tuberculosis, distemper, and rabies by decreasing contact between infected carcasses and other scavengers that come into close contact with humans, livestock, and other wildlife.

Despite their important role, vultures face many threats on a daily basis. Some of the most significant causes of declining CGV numbers described in literature include decreased availability of carrion, poisoning (both accidental and malicious poisoning), electrocution via powerlines, habitat loss and capture for use in the traditional medicine trade. Less frequent contributing factors include vultures flying into wind turbine blades, drowning in reservoirs, and being disturbed at their colonies. Vultures feed communally and in large numbers, making it possible to poison and kill a vast number of birds using a single poison-laced carcass, whether the purpose of the poisoned carcass is to control other scavengers or to kill vultures specifically, varies (Ogada et al., 2011). Recently, veterinary pharmaceuticals, like diclofenac, are being considered as a significant threat to declining vulture populations. Diclofenac has been implicated as the cause of severe population declines in Asian vulture species (Ogada et al., 2011), but very little to no research has been done on the sub-lethal effects of veterinary pharmaceuticals, more specifically their potential to cause toxicity in developing embryos of breeding CGV's.

This article aims to evaluate which veterinary pharmaceuticals may potentially induce toxicity in developing avian embryos, and to extrapolate the results from previous studies in other avian species to evaluate the potential risk these drugs pose to vulture embryos, whilst highlighting the gaps in our current knowledge and making suggestions for future research. For this review we also focused on the most likely route of exposure being through oral exposure in the food. With vultures being predominantly carrion eaters, exposure was limited to

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contaminants likely to occur from production animal carcasses, their most likely food source, prior to absorption and subsequent deposition into the forming egg within the oviduct. We also focus specifically on the Cape Griffon Vulture (*Gyps copotheres*) (CGV), which is endemic to only southern Africa. Currently they are classified as vulnerable and the number of mature birds is estimated at 9600–12800 globally (4810–6357 breeding pairs, with current population trends depicting a decline in their numbers (BirdLife International, 2021). Important to note for this review, is that the CGV's mate for life, nest on cliffs, and typically produce a single egg each year.

2. Materials and methods

A reference search was performed using Google Scholar, CABI and PubMed to find information on embryotoxicity or teratogenicity of veterinary pharmaceuticals in vultures or any other avian species. With scant information relating to vultures and drug-induced embryotoxicity being found, studies conducted in chickens and other bird species were also included, including articles on general toxic substances (no specific embryonic toxicity identified). Information from preclinical safety testing was however excluded when testing was undertaken only on mammals due to known interspecies differences especially drug pharmacokinetic differences between mammals and birds (Shore et al., 2014). The most useful articles were found by combining the specific names of commonly used veterinary pharmaceuticals with terms like: embryotoxicity, teratogenicity, chick development, hatchability, and reproductive toxicity. For each of the veterinary drugs identified, the following information was extracted: the species used for the study, the drug and doses used in the study, the day on which embryos were exposed to the drug, macroscopic (and microscopic if applicable) changes in the embryo caused by the drug, mechanism of embryotoxicity or teratogenicity of the drug, ~~and doses commonly used in animals~~. If the mechanism of embryotoxicity or teratogenicity of a specific drug had yet to be described in birds, the mechanism described in mice or rats was captured.

Following identification of potentially toxic pharmaceutical active ingredients, the potential risk posed by the chemical was evaluated based on the likelihood for exposure. For the route of exposure, the oral route of exposure was considered most important. With vultures being carrion eaters, drugs likely to accumulate in the tissues of animals, especially animals treated soon before death, were considered more likely to become bioavailable to the developing vulture embryo (Plaza et al., 2022). Literature was thus further limited to drugs used in livestock and with general exclusion of drugs used in small animal and human practice. While alternative exposure pathways, such as dermal and aerosol/inhalation, were considered of lower significance these were included, as a suspected case of pesticide poisoning was reported in literature for a lappet-faced vulture (*Torgos tracheliotus*) exposed to crop pesticide spraying in Israel (Ostrowski and Shobrak, 2001). Drugs identified as being only acutely toxic were excluded as these drugs would inherently cause death in adults before an impact on the embryo is possible.

When the actual concentration toxic to the embryo was identified during the review, the relative importance of the exposure concentration was compared to published egg residue concentrations (food safety) to ascertain of it was feasible to reach the published toxic concentrations. In instances where the egg concentrations were not available, hen plasma concentrations were used as a surrogate as previous studies have shown high similarity between hen plasma and egg white drug concentrations after treatment (Kan et al., 2000a and 2000b). With a general absence of information on the pharmacokinetics of identified drugs in vultures we determined the potential for vultures (5 kg) to be exposed to the dose reported in the identified chicken egg residue studies, by determining their exposure to drug residues in 1 kg of liver using the method from previous NSAID toxicity studies (Naidoo et al., 2009a). For the latter, toxicity was also considered likely if the estimated vulture exposure was within a tenth of the reported chicken dose, to account for interspecies differences. The latter was supported by the toxic dose of diclofenac in chickens also being tenfold higher than that seen in vulture.

3. Results

Veterinary pharmaceuticals with a potentially embryotoxic or teratogenic effect in the developing embryo.

3.1. The Quinolone Antibiotics

Enrofloxacin is a third-generation fluoroquinolone that is rapidly metabolised to ciprofloxacin. It has bactericidal antimicrobial activity and used cattle it used for the treatment of Bovine Respiratory Disease (BRD). Marbofloxacin is also fluoroquinolone antimicrobial with a wide spectrum of activity against Gram-positive and Gram-negative bacteria and *Mycoplasma* species used for the treatment of BRD and metritis-mastitis-agalactia syndrome in pigs. Enrofloxacin residues and residues of its metabolite, ciprofloxacin, have been found in Griffon vulture and Red kite (*Milvus milvus*) eggs in Spain. Embryos from eggs containing fluoroquinolone residues showed both macro- and microscopic lesions, especially affecting the tendons, articular cartilages, and growth plates (Lemus et al., 2009). A study conducted by Hruba et al. (2019) found that doses of 1, 10 and 100 μg enrofloxacin or marbofloxacin per 1 g egg weight caused significant abnormalities in the hatchability and development of chicken embryos, with reduction in hatchability due to embryo mortality, especially on the 13th day of incubation. Of these, 15–67% of hatchlings had joint deformities and 31–70% of chicks hatched one day early, with pre-term hatching associated with increased embryonic heart rates due to the cardiotoxic effects of fluoroquinolones. Of the two drugs, enrofloxacin appears to have a higher potential to induce adverse effects on the developing embryo when compared to marbofloxacin (Hruba et al., 2019). The specific mechanism by which fluoroquinolones induce embryotoxicity and teratogenicity in birds has not been described yet, but a possible mechanism has been described in rats using the drug norfloxacin, another fluoroquinolone antibiotic. It has been suggested that fluoroquinolones induce embryonic loss and malformations by inhibiting DNA gyrase and mitosis, with complete DNA damage resulting in foetal loss and partial damage causing malformations (Aboubakr et al., 2014).

3.2. Florfenicol

Florfenicol is a an amphenicol antimicrobial drug widely used in food-producing animals for its broad-spectrum, time dependent and bacteriostatic effect especially in the lungs. After a pharmacovigilance report of a decreased in hatchability in a flock of breeder fowls treated with florfenicol at 10 mg/kg, Al-Shahrani and Naidoo (2015), determined the effect of florfenicol on egg hatchability. Eggs collected from hens treated with 10, 20 and 30 mg/kg florfenicol for 5 days showed a mild decrease in fertility and hatchability after the 4th treatment, while eggs collected from hens treated with 60 and 90 mg/kg florfenicol showed a significant reduction in fertility as soon as 24 h after the first treatment, with hatchability dropping to 0%. Fertility returned to normal 4 days after the last treatment without abnormalities in any hatchlings. The reduced hatchability was associated with embryonic death on day 5 of incubation and correlated with the total concentration of florfenicol reached in the eggs, with $LC_{50} = 1.07$ μg/g. A second study evaluated the effect of florfenicol on embryonic growth and cardiovascular development by exposing laying hens to 50 mg/kg florfenicol for 5 days as well as exposing fertile chicken eggs to different doses of florfenicol. Florfenicol reduced egg hatchability in treated hens, most likely by inducing early embryonic death. Both groups of embryos (treated with 3.2 μg/g and 1.6 μg/g florfenicol respectively) showed abnormal development of the beak, wings, toes, joints, eye-pigmentation, and had

poorly developed hearts. Abnormalities were more severe in embryos exposed to a higher dose of florfenicol. A further study by Hu et al. (2020), showed significant reduction in day 7 embryonic weight in florfenicol treated embryos, leading them to speculate that florfenicol inhibited mitochondrial protein synthesis and downregulated the expression of vascular endothelial growth factor and fibroblastic growth factor 2 in the yolk sac membrane, thereby restricting vascular and embryonic development.

3.3. Doxycycline

Doxycycline is a semi-synthetic tetracycline antibiotic that has a broad spectrum of Gram-positive and Gram-negative bacteria and most *Mycoplasma* species. Tetracycline antibiotics are bacteriostatic and are used to treat anaplasmosis, heartwater, pneumonia and joint and navel ill in food-producing animals and strangles in horses. It is also widely used in the poultry industry to treat bacterial respiratory and gastrointestinal infections and tends to accumulate in the egg like other tetracyclines. Abbas et al. (2020) exposed 60 fertile chicken eggs to doses of doxycycline HCl ranging from 0.1, 0.5, 1.0, 1.5, 2.0 and 3.0 μg/100 μl distilled water/egg before the start of incubation (essentially 0.001 mg, 0.005 mg, 0.01 mg, 0.015 mg and 0.03 mg per egg, the weight of the eggs was not mentioned). Dead embryos were removed every 4 days and the remaining embryos were examined on day 12. Results showed a significant dose-dependent increase in embryo mortality and significant differences between the body weight, eye diameter, crown rump length, neck length, beak length, head diameter and the length of the humerus, radius and ulna, femur and fibula when compared to the control group. Various external abnormalities were also observed, affecting the eyes, head, limbs and organs with abnormalities becoming more severe and frequently observed as the dose increased. The mechanism of toxicity of doxycycline in the chick embryo is yet to be described, but studies conducted in rats and in human lymphocytes may suggest that the tetracycline antibiotics inhibit calcification when it is deposited in calcified tissues (Abd-Allah and Abd El-Rahman, 2020). Due to the slightly aromatic and polycyclic nature of doxycycline, it is also possible that it causes DNA damage by promoting the formation of reactive metabolites resulting in oxidative damage (Abd-Allah and Abd El-Rahman, 2020). In should also be noted that in contrast to the findings of Abbas et al. (2020), a study by Tavakkoli and Gooshki (2014) found that doses of 10 mg/kg egg-weight doxycycline did not have a significant lethal effect on the developing embryos of the Japanese quail.

3.4. Medetomidine

Medetomidine is an alpha-2 adrenergic receptor agonist used as a sedative, analgesic and premedication or anaesthetic adjunct in both small and large animals. Fertile chicken eggs exposed to medetomidine at 25 and 50 μg/egg on day 4 of incubation showed a dose-dependent reduction in the number of viable chick embryos on day 10 of incubation and an embryo lethality percentage of 53% in the 50 μg/egg dose group with no obvious macroscopic physical abnormalities (Mohammad et al., 2012). The same study evaluated the chicks exposed to 25 μg/egg in ovo 3 and 8 days after hatching and found it took both age groups significantly longer to move from the square of the open-field area, they showed reduced ambulation and less vocalisation in when compared to control groups of the same age, specifically in the 3-day old chicks. While the mechanism of toxicity is unknown, Mohammad et al., (2012) suggests that medetomidine induces behavioural changes in exposed chicks via its alpha-2 agonistic properties affecting central mechanisms, like the catecholamines controlling locomotion in the bird prenatally.

3.5. Fosfomycin

Fosfomycin is a synthetic phosphonic acid derivative antimicrobial drug that has rapid bactericidal activityand is used in poultry for the treatment of various bacterial infections like colibacillosis, fowl cholera and chronic respiratory disease and for the prevention of resistant bacteria in swine. Chicken embryos exposed to fosfomycin at doses of 160 and 320 mg/kg egg-weight showed significant physical abnormalities on day 18 of incubation. Embryos were also discoloured, stunted and under-developed with small wings and feet, incomplete feather formation and bulging and enlarged eyes (Tavakkoli et al., 2014). It has been suggested that fosfomycin may induce abnormalities in avian embryos due to its cytotoxic effects and negative impact on the central nervous system via its ability to cross the blood-brain-barrier in high concentrations (Tavakkoli et al., 2014). A second study proposed vascular alterations in the developing embryo as the mechanism for toxicity by altering the normal expression of specific genes needed for vascular development. It was further speculated that fosfomycin's affinity for VEGF-A proteins and ability to alter the angiogenic signalling pathway may result in vascular damage during embryonic development (Tavakkoli et al., 2019).

3.6. Trenbolone acetate

Trenbolone acetate is a synthetic anabolic-androgenic steroid, widely used as a growth promotor in beef feeder systems for improved production of muscle mass in animals. A study by Quinn, Jr. and Ottinger (2006) evaluated the effect of trenbolone in Japanese quail, with eggs exposed to 0.05, 0.5 or 50 μg trenbolone acetate on embryonic day 4. Chicks from treated eggs had smaller bursas containing fewer and smaller follicles when compared to the control group, with bursal alteration being retained until adulthood. They subsequently proposed that high levels of testosterone may prevent mesenchymal cells from differentiating properly, thereby leading to smaller bursas and follicles. This also led them to propose that trenbolone acetate affects the immune system, as proper development and maturation of the bursa is essential for B cell maturation, development of humoral immunity and the overall immunocompetence of the young bird. Testosterone may also impact IgG production and the normal isotype switch of IgM to IgG production by lymphocytes, essentially meaning that while birds may produce sufficient levels of antibodies, they are not specific to the pathogens they are exposed to and thus may be less effective.

When looking specifically at the impact of trenbolone acetate on the reproductive systems of Japanese quails in the same study, it appeared that males were more affected. Males from the 50 μg dose group only reached puberty a month after the control groups, while females were not affected. Males from the 5 and 50 μg treatment groups also had smaller cloacal glands than those of controls (45% and 55% smaller respectively), which may translate to less foam production by these glands during copulation and decreased fertilisation success. A significant reduction in copulatory behaviour was also noted in adult males that received treatment, with most treatment groups showing a 40% decrease in attempts to mount females and the 50 μg dose group showing a 73% reduction. Trenbolone seemed to primarily impact reproductive behaviour and induced very few changes in gonadal development or physiological reproductive measures (Quinn and Ottinger, 2006). While the mechanism is not fully understood, it is possible that endocrine distrupting chemicals (EDCs) impact the reproductive system by disrupting the formation of the hypothalamic-pituitary-gonadal (HPG) axis during embryonic development, thereby interfering with the later activation of this axis and altering the onset of puberty. Trenbolone also has the potential to alter the relative ratio of the concentration of testosterone to oestradiol, where this ratio is more important to the development of the HPG axis than the total concentration of either of these hormones (Quinn and Ottinger, 2006).

3.7. Albendazole

Albendazole is an antiparasitic agent falling under the benzimidazole

class of anthelmintics. Toxicity of albendazole in hens was confirmed at doses of 10, 40 and 80 mg/kg (10 mg/kg once and the others doses for 7 days). Eggs collected during treatment and 2 days post-treatment showed significantly reduced hatchability at 40 and 80 mg/kg (Moreno et al., 2018). Data regarding the impact of albendazole in birds is scarce and a mechanism of embryotoxicity has not yet been described, however possible mechanisms have been suggested based on studies conducted in other species and its mechanism in parasites. Carlsson et al. (2011) suggests that benzimidazoles are also capable of binding tubulin in mammalian cells as they do in helminths, which may lead to embryotoxicity. An additional mechanism may be the ability of albendazole to inhibit vascular endothelial growth factor.

3.8. Metronidazole

Metronidazole is a nitroimidazole antibiotic that has been used to treat trichomoniasis, giardiasis and amebiasis in veterinary medicine, along with bacterial infections caused by obligate anaerobes. Chicken eggs exposed to metronidazole at 25 mg/kg egg-weight on day 9, 10 and 11 (3 exposures per egg) of incubation and examined on day 18 showed stunted embryos with small feet and wings, altered feather formation and schistosomus reflexus (Laughton et al., 2005)). Histopathology showed hyperaemia of most organs, with the kidneys being the most severely affected (Mosallanejad et al., 2014). A second study looking at the impact of metronidazole on the skin and integument of chicken embryos at 50 and 100 mg/kg egg-weight on day 4 of embryonic development, showed small white nodules on the skin macroscopically, hyperkeratosis, epidermal detachment and degeneration of integument on histopathology at day 18 at the high dose (Tavakkoli et al., 2017). The mechanisms by which metronidazole causes brain, renal and skin lesions in the developing avian embryo have not been described and further research is needed.

3.9. Toxic veterinary pharmaceuticals determined to have low egg exposure potential from being either highly toxic or due to low exposure potential in the adult bird

The following drugs and chemicals were determined to have the potential to affect the developing embryos of birds, but were excluded based on the risk assessment criteria specified (Table 2). The

Table 1
Summary of veterinary pharmaceuticals that are potentially embryotoxic to developing avian embryos.

Drug name	Species used/ found in study	Dose used in study	Outcome	Ref.
Enrofloxacin and potentially ciprofloxaxin as a metabolite	Griffon vulture (Gyps fulvus) and Red kite (Milvus milvus) eggs	Griffon vulture eggs: enrofloxacin (7.14 ± 2.17 μg/ml, n = 10) ciprofloxacin (3.24 ± 1.91 μg/ml, n = 10) Red Kite eggs: enrofloxacin: 2.36 ± 0.79 μg/ml, n = 10; ciprofloxacin: 5.91 ± 1.27 μg/ml	macro- and microscopic lesions, especially affecting the tendons, articular cartilages, and growth plates	Lemus et al. (2009).
Enrofloxacin/ Marbofloxacin	Chicken (fertile eggs)	1, 10 and 100 μg enrofloxacin or marbofloxacin per 1 g egg weight on day 0 (yolk injection)	Embryo mortality leading to reduced hatchability and joint deformities.	Hruba et al. (2019)
Florfenicol	Chicken (hens and cockerels)	10, 20, 30, 60 and 90 mg/kg florfenicol for 5 days administered to hens by oral gavage.	Mild reduction in hatchability in low dose group after 4 days of treatment, significant reduction in hatchability after 24hrs in high dose groups.	Al-Shahrani and Naidoo, 2015
	Chicken (hens and fertile eggs)	50 mg/kg for 5 days in hens' food and 3.2 μg/g or 1.6 μg/g in fertile eggs (foetus injected after 24 h of incubation).	Hens: reduced hatchability of eggs Fertile eggs: abnormal development affecting the beak, wings, toes, joints and eye-pigmentation and poorly developed hearts.	Hu et al. (2020).
Doxycycline HCl	Chicken (fertile eggs)	0.001 mg, 0.005 mg, 0.01 mg, 0.015 mg and 0.03 mg per egg (day 0 and into the yolk)	Significant dose-dependent increase in embryo mortality, differences between body size and weight measurements when compared to controls and abnormal eyes, limbs, heads and organs.	Abbas et al. (2020)
Medetomidine	Chicken (fertile eggs)	25 and 50 μg/egg (After day 4 of incubation and into the air sac)	Increased embryo mortality in high dose group, low dose group was used to evaluate behavioural changes and caused decreased movement and vocalisation.	Mohammad, Faris and Al-Zubeady, 2012
Fosfomycin	Chicken (fertile eggs)	160 and 320 mg/kg egg-weight three times (dosed after 24 h of incubation and dropped in the shell membrane)	Embryos were discoloured, stunted and under-developed with small wings and feet, incomplete feather formation and bulging and enlarged eyes	Tavakkoli, Derakhshanfar and Gooshki (2014).
Trenbolone acetate	Japanese quails (fertile eggs)	0.05, 0.5 or 50 μg per egg (on day 4 of incubation, into the egg yolk)	Immune function: treated chicks had smaller bursas containing fewer and smaller follicles and adult birds had retained follicular alterations in their bursas. There did not appear to be a difference in the immune responses of adult birds.	Quinn, and Ottinger (2006)
			Reproductive system: Males from the 50 μg dose group only reached puberty a month after the control groups, while females were not affected. Males had smaller cloacal glands and a significant reduction in copulatory behaviour was seen.	Quinn, Lavoie and Ottinger, 2007.
Albendazole	Chicken (hens)	10, 40 and 80 mg/kg for 7 days in the feed	Fertility was not impacted at all, by dose or duration of treatment, but hatchability was significantly decreased in the 40 and 80 mg/kg treated groups.	Moreno et al., 2018.
Metronidazole	Chicken (fertile eggs)	25 mg/kg egg-weight 3 times (into chorioallantoic membrane at incubation days 9–11)	Stunted embryos with small feet and wings, altered feather formation and schistosomus reflexus	Mosallanejad et al. (2014).
		50 and 100 mg/kg egg-weight (into chorioallantoic membrane at incubation days 9–11)	The 100 mg/kg dose group had small white nodules on the skin macroscopically, and hyperkeratosis, epidermal detachment and degeneration of integument on histopathology. The 50 mg/kg dose group was normal.	Tavakkoli, Kheirandish and Moradi (2017).

Table 2
Drugs with reported toxic effects on the embryo, but determined to be low risk to the foetus.

Class	Drug name	Species used in study	Outcome (effects on bird embryo)	Mechanism of action (MOA)	Ref.
Chemotherapeutics	Cyclophosphamide	Rats, rabbits, mice, monkeys and chicks	Anophthalmia, microphthalmia, short limbs and abnormal beaks in one study. Smaller body size, everted viscera, twisted necks and similar lesions as mentioned above in another study.	Metabolites enter cells and induce damage by alkylating DNA, which leads to free radical production and induces apoptosis, affecting the growth and development of embryos.	Matalon et al. (2004)
	Doxorubicin	**Chicken (fertile eggs)**	**Decreased body weight and length, beak and eye abnormalities, short or curved limbs, everted viscera and various microscopic lesions in organs. Decreased hatchability.**	**Inhibits synthesis of DNA, causes damage to DNA and causes oxidative stress by promoting formation of reactive oxygen species – affects tumour cells and healthy cells like stem cells.**	National Toxicology Program, 2013; Abed, Ibraheem and Abbas (2020)
	Cisplatin	Chicken (fertile eggs)	Damaged ciliary epithelium causing decreased intraocular pressure, leading to bilateral microphthalmia	Binds to nuclear DNA and interferes with DNA transcription and replication that lead to cell death.	Fuertes et al. (2003)
	Actinomycin D	Chicken (fertile eggs)	Anomalous axial skeleton development and tail abnormalities.	Causes cytotoxicity by binding DNA and inhibiting RNA synthesis.	National Toxicology Program, 2013;
Non-steroidal Anti-inflammatory drugs	**Diclofenac**	Cape Griffon Vulture *(Gyps coprotheres)*	Visceral gout and acute renal failure. Renal failure leads to mortality. Microscopic lesions in the kidney, liver and spleen showing extensive deposition of uric acid crystals.	Inhibition of prostaglandin synthesis leads to the opening of the renal portal valve and causes venous blood to be shunted away from the cranial renal lobe, resulting in ischaemic renal necrosis. Death is associated with a prolonged period of severely reduced functionality in renal tubular secretion; nerual tube inhibition	Naidoo et al., 2009; Havenga et al. (2020)
	Aceclofenac	Drug administered to cattle and levels of diclofenac measured in tissues.	Was not directly tested on vultures but we can assume it will have the same or a similar effect as diclofenac.	Aceclofenac is rapidly metabolized to diclofenac when administered to cattle, therefore it has the same MOA.	Galligan et al. (2016)
	Ketoprofen	Cape griffon vultures *(Gyps coprotheres)*, African white-backed vultures *(G. africanus)*	Extensive visceral gout, microscopically there was widespread uric acid crystal deposition in the kidneys, liver, and spleen.	Only some vultures have CYP450 pharmacogenomic differences, meaning they lack an enzyme necessary to clear drugs like NSAID's. Decreased enzymes lead to the elimination pathway getting saturated and the drug having a longer half-life, making the drug toxic in a similar way to diclofenac. This explains why not all vultures exposed to ketoprofen experience toxicity.	Naidoo et al., 2009
Insecticies	Organophosphates (general), methamidophos, carbafuran, aldicarb	Cape vultures *(Gyps coprotheres); Bateleur eagles (Terathopius ecaudatus); African white-backed vultures (Gyps africanus),*	Acute toxicity and death in most cases. Specific pathology is not seen in acute pesticide toxicity and diagnosis is dependent on history, clinical signs observed and analytical data.	Irreversible (OP's) or reversible (carbamates) inhibition of acetylcholinesterase at nerve synapses and neuromuscular junctions and subsequent accumulation of acetylcholine at these sites. This leads to cholinergic overstimulation manifesting and muscarinic, nicotinic, and central signs. Death is usually due to paralysis of respiratory muscles.	Gupta (2014),
	Chlorpyrifos (organo-phosphate) and cypermethrin (pyrethroid)	Fertile eggs from Rhode Island Red chickens	Severe embryonic skeletal malformations, beak deformities, microphthalmia, anophthalmia, wry neck, craniorachischisis (brain and spinal cord remain open), micromelia and umbilical hernias. Abnormalities worsened with increasing doses.	Both insecticides are teratogenic on their own, but in combination they work synergistically. Chlorpyriphos slows the enzymes responsible for metabolising cypermethrin and enhances its toxicity. Vertebral defects are associated with a decrease in acetylcholinesterase and disruption of the cholinergic system. A lack of acetylcholine impacts the proliferation, differentiation, and migration of target cells during embryonic development and may give rise to malformations.	Uggini et al., 2010
	Imidacloprid	Chicken (fertile eggs)	Abnormal craniofacial osteogenesis. Increase in embryo mortality and neural tube defects associated with increased dose.	Imidacloprid disrupts Neural Crest Cell (NCC) development and leads to abnormal craniofacial osteogenesis as follows: Inhibits NCC's proliferation, apoptosis, production, migration, and differentiation through repression of	Wang et al. (2016)

(*continued on next page*)

Table 2 (*continued*)

Class	Drug name	Species used in study	Outcome (effects on bird embryo)	Mechanism of action (MOA)	Ref.
				various genes on the developing neural tube and by altering the expression of certain adhesion molecules.	
	Permethrin	Chicken (fertile eggs)	Various head, limb, eye and jaw defects, decreased body weight and decreased crown-rump length.	Decreased body weight may be due to permethrins ability to induce enzymatic and mitochondrial changes, causing impaired cellular metabolism. Dysregulation of dopaminergic neurons, increased oxidative stress and inflammatory responses in the brain induced by permethrin may be responsible for head, eye and jaw defects.	Curtis et al. (2021)

1 - Drugs that are not used in production animals and thus unlikely to result in high exposure in vultures; 2 - Drugs that are highly toxic in vultures with resulting in low liklihood of bioavailability to the chick due to female death; 3 - when exposure results from topical exposure which can occur during crop spraying (Please note that this does not preclude this category from being toxic if oral exposure was to unintentionally result)

chemotherapeutic agents were excluded due to low probability of exposure as this class is most frequently used in small animal practice with proper waste management that preclude their entry into the vulture food chain, such as at landfills. Furthermore, many of these drugs, including doxorubicin and actinomycin D, have low oral bioavailability (Mealey, 2004), making ingestion of treated carcasses a low-risk exposure pathway for vultures. In contrast, the non-steroidal anti-inflammatory drugs (NSAID's) were excluded as they are highly toxic to vultures, with mortalities reported in adult vultures as soon as 30 h following single exposure (Naidoo et al., 2009). Nonetheless, it should be noted that Ertekin et al. (2019) reports neural tube defects in chicken embryos following diclofenac exposure, which could be indicative that long term exposure to very low doses of diclofenac could potentially cause chick defects.

The barbiturates were ~~also~~ excluded as they are also lethal to adult vultures shortly after exposure, with large numbers of vultures often found dead around euthanized animal carcasses. Vultures tend to be exposed to ~~the~~ barbiturates when they consume carcasses of pets or livestock that were euthanized and incorrectly disposed of in the veld or in landfills, or when they ingest barbiturate contaminated bait, either intended for them specifically or for the control of other predators. Scavengers like vultures are especially likely to consume high doses of barbiturates, as the drug concentrates in highly vascularised organs like the liver and spleen after death, organs often favoured by vultures (Wells, Butterworth and Richards, 2019). Pentobarbital, phenobarbital, barbital and thiopental were detected in bait in Spain, with pentobarbital being the most commonly found barbiturate. Eurasian griffon vultures (*Gyps fulvus*) were most frequently intoxicated (Herrero-Villar et al., 2021).

The organophosphates and carbamates were excluded due to their lethality in adult vultures following acute exposure. Vultures are exposed to them when consuming pesticide contaminated bait intended to kill jackals, feral dogs, or other livestock predators; or more recently, vultures are directly targeted by ivory poachers to limit detection of the poachers' location by the their circling overhead (Ogada, Botha and Shaw, 2015); while the other insecticides were excluded because, as mentioned previously, cases where aerosol inhalation of pesticides have directly impacted vultures are not commonly reported (Ostrowski and Shobrak, 2001).

3.9.1. Risk assessment using available residues concentrations

Following the evaluation of the drugs with identified chicken egg

Table 3
Risk assessment based on drug concentrations reported to be toxic in relationship to report egg residue concentrations and estimate vulture exposure doses.

Drug	Egg Residues				Potential for egg toxicity based on residues	Likely Vulture Exposure			Ref.
	Hen Dose (mg/kg)	Egg white (ug/g)[a]	Lowest Toxic Dose[c]	Residue: Toxic Conc		Liver Conc (mg/kg)[c]	Vulture exposure (mg/kg)	Dose comparison	
Enrofloxacin	10	2	1.57 µg/ml;	1.27	likely	10	2	One tenth	Cornejo et al. (2012); (EMA, 2023)
Marbofloxacin	1.52	0.26	1 µg/g egg	0.26	unlikely	3.14	0.628	One tenth	Errecalde et al. (2021); (EMA, 2023)
Florfenicol	20	0.328	1.6 µg/g egg	0.21	unlikely	4.1	0.82	Below	Filazi et al. (2014); (EMA, 2023)
Doxycycline	11.5	0.15	!0.02 ug/g egg	7.50	likely	0.01771	0.003542	Well below	Kan et al. (2000a) and (2000b); Landoni and Errecalde (1992)
Fosfomycin	40	24[b]	160 ug/g egg	0.15	unlikely			Unknown	Dieguez et al. (2011)
Albendazole			40 mg/kg per bird			20	4	One tenth	Bistolette et al. (2013)
Metronidazole	60	2.2	25 ug/g egg	0.09	unlikely	3.576	0.7152	Below	Youssef et al. (2013);Pan et al. (2017)

[d]Concentration reported for cattle with the exception of metronidazole which is for pigs. The dose comparision column compares the chicken residue dose to the estimated vulture exposure – values reported as one tenth indicate the potential to be toxic when one accounts for interspecies differences.

[a] Egg size set at average of 50 g for dose calculation when the dose was reported per egg rather than per gram. Egg estimated 60% white; 30% yolk;

[b] No egg concentration was available. The indicated value is plasma concentration in the hen;

[c] The lowest toxic dose was obtained was the values reported in Table 1.

toxicity, the reported toxic concentration was compared to the actual exposure concentrations that could be expected in the chicken egg following treatment at recommended doses (Table 3). Despite a large list of drugs identified in Table 1, residues concentration information was available for enrofloxacin, marbofloxacin, florfenicol, doxycycline, fosfomycin, and metronidazole. Of these 6 drugs, enrofloxacin and doxycycline were flagged as likely reaching toxic concentrations. When the exposure dose used in the chicken residue studies were compared to likely exposure concentrations, which would occur as residues in the meat, and taking into account interspecies differences, enrofloxacin, marbofloxacin and albendazole were flagged as potentially toxic to vulture eggs. Of these albendazole and enrofloxacin were identified as the most likely to be toxic.

4. Discussion

Intensive livestock farming makes use of a wide range of veterinary pharmaceuticals to treat and prevent diseases, often using more than one drug at a time. Sick animals are often treated shortly before death, and in countries such as Spain it has been found that these carcasses are disposed of via carcass dumps (also known as vulture restaurants in some countries) (Lemus et al., 2009). As a result, vultures may likely be exposed to doses much higher than the minimum residue limits (MRL's) prescribed for each drug. The deaths of millions of vultures on the Asian subcontinent highlighted the risks posed by these pharmaceuticals and the specific influence that species-specific constraints in metabolism can have on the overall susceptibility of a species to a specific drug (Naidoo et al., 2009). While the impact of pharmaceuticals in terms of mortalities is described for many chemicals (Shore et al., 2014), few studies consider the subtle sub-lethal side effects such as behavioural changes or immunocompetence.

With vultures being such long-lived birds, they are also potentially exposed to many different pharmaceuticals and other chemicals throughout their lifetime, which could be deposited in the egg and have a direct impact on the developing avian embryos, as well as having a synergistic effect when multiple chemicals are involved. Despite the potential to harm, it was immediately evident that there is limited information available on the impact of veterinary drugs on developing embryos, with many of the available studies performed in other avian species, most commonly the domestic chicken. We also found no prior studies describing the chemicals that may be present in vulture eggs under natural conditions in South Africa. Further, in addition to a drug having the potential to adversely impact the embryo, the concentration of exposure is another factor to consider viz. not all drugs pose an equally significant threat to developing embryos, as some drugs are used more frequently or in a wider range of species thereby increasing the chance of exposure. In vultures this is a further unknown variable as the concentration vultures are exposed to under field conditions depends greatly on how much tissue they consume, which tissues they consume and how long after treatment they consume tissues from treated animals. Therefore, we cannot assume that they are exposed to high enough doses to induce embryotoxicity.

From the various studies reviewed, the fluoroquinolones were the most frequently detected antibiotics across vulture species (Plaza et al., 2022). Lemus et al. (2009) described macro- and microscopic lesions following exposure of the developing vulture embryo, moist likely from consumption of drug residues in cattle tissue. Due to the frequent use of fluoroquinolone antibiotics, and confirmed exposure and embryotoxicity, this group of veterinary pharmaceuticals arguably poses the most significant threat to developing vulture embryos. From the other drugs identified their level of use in South Africa do raise concern. For doxycycline, the concern will be its widescale use due to its free availability over the counter in South Africa as well the close relationship between the toxic dose and the likely concentrations reached within the egg. Another drug of concern is trenbolone acetate which is commonly used in the beef feedlot industry. Unfortunately, we could find no information on the likely concentrations of trenbolone that could occur in the egg to quantify the risk. Nonetheless with the drug being toxic to the embryo at very low concentration (0.001 μg/g of egg) together with significant concentrations present in cattle liver (0.042 mg/kg)(JECFA, 1989), it is likely that toxic concentrations will be present in the egg. Lastly, medetomidine while not frequently used in production animals, is frequently used in combination with other drugs for the immobilisation and capture of wildlife in South Africa (Citino et al., 2001) and thus represents a potential exposure pathway for vultures. Besides interference with embryonic development, medetomidine poses another problem as the drug appears to cause sedation of the hatching chick which could potentially interfere with pipping or feeding soon after hatching.

In addition to the likelihood of exposure, the ability of the drug to accumulate prior to toxicity was considered. Of the drugs evaluated, while both florfenicol and albendazole were identified as likely toxic, they required repeat exposure for the toxic effect to be noted. With vulture~~s~~ feeding approximately every 3 days, their feeding frequency should limit the potential of toxicity on the assumption that not every carcass fed upon is contaminated (Naidoo et al., 2009). Lastly, fosfomycin and metronidazole were regarded as being of very low concern as the potential for exposure was low. While fosfomycin was identified as having an embryotoxic effect (Tavakkoli et al., 2019), it is exclusively used in the poultry industry in South Africa. The low bioavailability of the drug together with poultry not featuring as a common food source for vultures in South Africa due to sanitary measures, renders the chance of exposure ~~is~~ highly unlikely. Likewise, while metronidazole has the ability to induce embryotoxicity and teratogenicity, the non-use of the product in production animals due to human safety concerns also limits the potential for vulture exposure. However, with the drug being used off-label in the equine industry, the uncontrolled disposal of equine carcasses could pose a concern in areas where equine meat is fed to vultures (Mosallanejad et al., 2014).

While we focus on the likelihood of exposure above, the stage of embryonal development impacted by the drug should also be considered e.g., developing embryos were exposed to fosfomycin and metronidazole repeatedly on different days of embryonic development. This poses the question whether the drug itself, the repeated exposure, or the exposure on specific days of embryonic development caused the embryotoxicity. Medetomidine and trenbolone acetate studies exposed embryos to these compounds on day 4 of embryonic development, and fluoroquinolones and doxycycline HCl exposure was prior to the start of incubation. When evaluating the likely exposure in vultures, it is important to consider whether these drugs will impact embryonic development at any point in time, or if they only bring about embryotoxicity when administered on certain days of embryonic development because they disrupt a specific developmental stage. We suggest that studies where hens were exposed to the drug, as in the case of florfenicol and albendazole, would be the better predictors of toxicity as it simulates the true exposure that would occur under natural conditions.

5. Conclusion

In conclusion, this article clearly shows the potential of veterinary pharmaceuticals to affect the developing embryos of vultures and therefore impact overall population numbers and the conservation of this critically important species. It also highlights the large gaps in our knowledge and the great need for further research in this field to fully understand the risks and implications of veterinary pharmaceuticals on breeding vultures. For this, programmes monitoring concentrations of chemicals in infertile eggs need to be established. Once drug residues in vulture eggs have been quantified, in-ovo studies can be performed in fertile chicken eggs using the same concentrations and possibly different combinations of veterinary pharmaceuticals to evaluate whether they can induce embryotoxicity or teratogenicity.

Contribution to the publication

LW: undertook the literature review and wrote the draft manuscript; **VN** - Conceptualised the study, reviewed the manuscript, was academic supervisor to **LW**.

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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

No data was used for the research described in the article.

References

Abbas, S., Eman, F., Ullah, R., Niaz, S., Haleem, S., 2020. Embryotoxicity of doxycycline HCl in Gallus domesticus: morphometric changes and external anomalies. Indian J. Anim. Res. 1, 4.

Abd-Allah, E.R., Abd El-Rahman, H.A., 2020. Influence of doxycycline administration on rat embryonic development during organogenesis. J. Biochem. Mol. Toxicol. 35 (1) https://doi.org/10.1002/jbt.22613.

Abed, A.R., Ibraheem, A.F., Abbas, H.A., 2020. Embryogenic effects of Doxorubicin in chicken embryo. Curr. Top. Pharmacol. 24 (24), 99–104.

Aboubakr, M., Elbadawy, M., Soliman, A., El-Hewaity, M., 2014. Embryotoxic and teratogenic effects of norfloxacin in pregnant female albino rats. Adv. Pharmacol. Sci. 1–6.

AL-Shahrani, S., Naidoo, V., 2015. Florfenicol induces early embryonic death in eggs collected from treated hens. Article 13 BMC Vet. Res. 11 (1), 1–7. https://doi.org/10.1186/s12917-015-0536-0.

BirdLife International (BirdLife International 2021. IUCN Red List of Threatened Species: Gyps coprotheres. [online] IUCN Red List of Threatened Species. Available at: ⟨https://www.iucnredlist.org/species/22695225/197073171#taxonomy⟩ [Accessed 31 Mar. 2022].

Bistoletti, M., Alvarez, L., Lanusse, C., Moreno, L., 2013. Disposition kinetics of albendazole and metabolites in laying hens. J. Vet. Pharmacol. Ther. 36 (2), 161–168.

Carlsson, G., Patring, J., Ullerås, E., Oskarsson, A., 2011. Developmental toxicity of albendazole and its three main metabolites in zebrafish embryos. Reprod. Toxicol. 32 (1), 129–137.

Citino, S.B., Bush, M., Grobler, D., Lance, W., 2001. Anaesthesia of roan antelope (Hippotragus equinus) with a combination of A3080, medetomidine and ketamine. J. South Afr. Vet. Assoc. 72 (1), 29–32. https://doi.org/10.4102/jsava.v72i1.605.

Cornejo, J., Lapierre, L., Iragüen, D., Cornejo, S., Cassus, G., Richter, P., San Martin, B., 2012. Study of enrofloxacin and flumequine residues depletion in eggs of laying hens after oral administration. J. Vet. Pharmacol. Ther. 35 (1), 67–72.

Curtis, G.H., Nogueiro, S., Schneider, S., Bernhofer, M., McDermott, M., Nixon, E., Perez, K.N., Reeve, R.E., Easterling, M.R., Crespi, E.J., 2021. Trans-ovo permethrin exposure affects growth, brain morphology and cardiac development in quail. Environ. Toxicol. 36 (7), 1447–1456. https://doi.org/10.1002/tox.23141.

Den Heever, L.V., Thompson, L.J., Bowerman, W.W., Smit-Robinson, H., Shaffer, L.J., Harrell, R.M., Ottinger, M.A., 2021. Reviewing the role of vultures at the human-wildlife-livestock disease interface: an African Perspective. J. Raptor Res. 55 (3).

Dieguez, S., Soraci, A., Tapia, O., Carciochi, R., Peŕez, D., Harkes, R., Romano, O., 2011. Determination of antibiotic fosfomycin in chicken serum by liquid chromatography-tandem mass Spectrometry. Liq. Chromatogr. Relat. Technol. 34, 116–128.

EMA, The European Agency for the Evaluation of Medicinal Products. Available online at ⟨https://www.ema.europa.eu/en/medicines/field_ema_web_categories%253Aname_field/Veterinary/ema_group_types/ema_document-maximum_residue_limits_report⟩. Accessed July 2023.

Errecalde, C., Urzúa Pizarro, N.F., Prieto, G., Luders, C., Liboa, R.A., Gramaglia, R.A., 2021. Disposition and residues of marbofloxacin in eggs of laying hens. Adv. Pharmacol. Clin. Trials 6 (1), 000185.

Ertekin, T., Bilir, A., Aslan, E., Koca, B., Turamanlar, O., Ertekin, A., Albay, S., 2019. The effect of diclofenac sodium on neural tube development in the early stage of chick embryos. Folia Morphol. 78 (2), 307–313. https://doi.org/10.5603/fm.a2018.0080.

Filazi, A., Sireli, U.T., Yurdakok, B., Aydin, F.G., Kucukosmanoglu, A.G., 2014. Depletion of florfenicol and florfenicol amine residues in chicken eggs. Br. Poult. Sci. 55 (4), 460–465.

Fuertes, M., Castilla, J., Alonso, C., Pérez, J., 2003. Cisplatin biochemical mechanism of action: from cytotoxicity to induction of cell death through interconnections between apoptotic and necrotic pathways. Curr. Med. Chem. 10 (3), 257–266. https://doi.org/10.2174/0929867033368484.

Galligan, T.H., Taggart, M.A., Cuthbert, R.J., Svobodova, D., Chipangura, J., Alderson, D., Prakash, V.M., Naidoo, V., 2016. Metabolism of aceclofenac in cattle to vulture-killing diclofenac. Conserv. Biol. 30 (5), 1122–1127. https://doi.org/10.1111/cobi.12711.

Gupta, R.C. 2014. MSD Veterinary Manual. Available at: ⟨https://www.msdvetmanual.com/toxicology/insecticide-and-acaricide-organic-toxicity/carbamate-insecticides-toxicity⟩ [Accessed 10 Jun. 2022].

Havenga, L., Naidoo, V., Wolter, K., Duncan, N., Groenewald, H.B., 2020. Does the renal portal valve exist in a raptor species? A study aimed at further evaluating the mechanism of toxicity of diclofenac in vultures. Anat. Histol. Embryol. 49 (6), 836–841. https://doi.org/10.1111/ahe.12590.

Herrero-Villar, M., Sánchez-Barbudo, I.S., Camarero, P.R., Taggart, M.A., Mateo, R., 2021. Increasing incidence of barbiturate intoxication in avian scavengers and mammals in Spain. Environ. Pollut. 284, 117452 https://doi.org/10.1016/j.envpol.2021.117452.

Hruba, H., Abdelsalam, E.E.E., Anisimov, N., Bandouchova, H., Havelkova, B., Heger, T., Kanova, M., Kovacova, V., Nemcova, M., Piacek, V., Sedlackova, J., Vitula, F., Pikula, J., 2019. Reproductive toxicity of fluoroquinolones in birds. BMC Vet. Res. 15 (1) https://doi.org/10.1186/s12917-019-1957-y.

Hu, D., Meng, F., Cui, Y., Yin, M., Ning, H., Yin, Z., Chen, L., Ge, Y., Liu, S., 2020. Growth and cardiovascular development are repressed by florfenicol exposure in early chicken embryos. Poult. Sci. 99 (5), 2736–2745. https://doi.org/10.1016/j.psj.2020.01.007.

JECFA, 1989, Summary of the evaluation performed by the Joint FAO/WHO expert committee of food additives (JECFA) on trenbolone acetate, available online at ⟨www.Inchem.org⟩, accessed July 2023.

Kan, C.A., Petz, M., 2000a. Residues of veterinary drugs in eggs and their distribution between yolk and white. J. Agric. Food Chem. 48 (12), 6397–6403.

Kan, C.A., Petz, M., 2000b. Residues of veterinary drugs in eggs and their distribution between yolk and white. J. Agric. Food Chem. 48, 6397–6403.

Landoni, M.F., Errecalde, J.O., 1992. Tissue concentrations of a long-acting oxytetracycline formulation after intramuscular administration in cattle. Rev. Sci. Et. Tech. Off. Int. Des. Epizoot. 11, 909-909.

Laughton, K.W., Fisher, K.R.S., Halina, W.G., Partlow, G.D., 2005. Schistosomus reflexus syndrome: a heritable defect in ruminants (online). Anat. Histol. Embryol. 34 (5), 312–318. https://doi.org/10.1111/j.1439-0264.2005.00624.x.

Lemus, J.Á., Blanco, G., Arroyo, B., Martínez, F., Grande, J., 2009. Fatal embryo chondral damage associated with fluoroquinolones in eggs of threatened avian scavengers. Environ. Pollut. 157 (8–9), 2421–2427. https://doi.org/10.1016/j.envpol.2009.03.013.

Matalon, S.T., Ornoy, A., Lishner, M., 2004. Review of the potential effects of three commonly used antineoplastic and immunosuppressive drugs (cyclophosphamide, azathioprine, doxorubicin on the embryo and placenta). Reprod. Toxicol. 18 (2), 219–230. https://doi.org/10.1016/j.reprotox.2003.10.014.

Mealey, K.L., 2004. Therapeutic implications of the MDR-1 gene. J. Vet. Pharmacol. Ther. 27 (5), 257–264. https://doi.org/10.1111/j.1365-2885.2004.00607.x.

Mosallanejad, S.S., Tavakkoli, H., Derakhshanfar, A., Salandari, S., 2014. An experimental study of the systemic alteration of nitroimidazoles in the middle stage of embryonic development. Int. J. Adv. Biol. Biomed. Res. 2 (5), 1468–1474.

Naidoo, V., Wolter, K., Cromarty, D., Diekmann, M., Duncan, N., Meharg, A.A., Taggart, M.A., Venter, L., Cuthbert, R., 2009a. Toxicity of non-steroidal anti-inflammatory drugs to Gyps vultures: a new threat from ketoprofen. Biol. Lett. 6 (3), 339–341. https://doi.org/10.1098/rsbl.2009.0818.

Naidoo, V., Wolter, K., Cuthbert, R., Duncan, N., 2009b. Veterinary diclofenac threatens Africa's endangered vulture species. Regul. Toxicol. Pharmacol. 53 (3), 205–208. https://doi.org/10.1016/j.yrtph.2009.01.010.

Naidoo, V., Wolter, K., Espie, I., Kotze, A., 2011. Vulture rescue and rehabilitation in South Africa: an urban perspective. J. South Afr. Vet. Assoc. 82 (1), 24–31.

National Toxicology Program NTP NTP Monograph Developmental Effects and Pregnancy Outcomes Associated With Cancer Chemotherapy Use During Pregnancy. 2013. [online] Available at: ⟨https://ntp.niehs.nih.gov/ntp/ohat/cancer_chemo_preg/chemopregnancy_monofinal_508.pdf⟩ [Accessed 23 May 2022].

Ogada, D., Botha, A., Shaw, P., 2015. Ivory poachers and poison: drivers of Africa's declining vulture populations (online). Oryx 50 (4), 593–596. https://doi.org/10.1017/s0030605315001209.

Ogada, D.L., Keesing, F., Virani, M.Z., 2011. Dropping dead: causes and consequences of vulture population declines worldwide. Ann. N. Y. Acad. Sci. 1249 (1), 57–71.

Ostrowski, S., Shobrak, M., 2001. Pesticide poisoning in a free-ranging lappetfaced vulture (*Torgos tracheliotus*). Vet. Rec. 149 (13), 396–397. https://doi.org/10.1136/vr.149.13.396.

Pan, Y., Yi, J., Zhou, B., Xie, S., Chen, D., Tao, Y., Qu, W., Liu, Z., Huang, L., Yuan, Z., 2017. Disposition and residue depletion of metronidazole in pigs and broilers. Sci. Rep. 7 (1), 7203.

Plaza, P.I., Wiemeyer, G.M., Lambertucci, S.A., 2022. Veterinary pharmaceuticals as a threat to endangered taxa: mitigation action for vulture conservation. Sci. Total Environ. 817, 152884 https://doi.org/10.1016/j.scitotenv.2021.152884.

Quinn Jr., M.J., Ottinger, M.A., 2006. Embryonic effects of androgen active endocrine disrupting chemicals on avian immune and reproductive systems. J. Poult. Sci. 43 (1), 1–11. https://doi.org/10.2141/jpsa.43.1.

Shore, R.F., Taggart, M.A., Smits, J., Mateo, R., Richards, N.L., Fryday, S., 2014. Detection and drivers of exposure and effects of pharmaceuticals in higher vertebrates. Philos. Trans. R. Soc. B: Biol. Sci. 369 (1656), 20130570 https://doi.org/10.1098/rstb.2013.0570.

Tavakkoli, H., Gooshki, S.N., 2014. The effect of doxycycline on the viability of the quail embryo during incubation period. Int. J. Adv. Biol. Biomed. Res. 4 (8), 2390–2394.

Tavakkoli, H., Derakhshanfar, A., Gooshki, S.N., 2014. Toxicopathological lesions of Fosfomycin in embryonic model. Eur. J. Exp. Biol. 4 (2), 63–71.

Tavakkoli, H., Kheirandish, R., and Moradi, Z., 2017. Histopathological Evaluation of the Effect of Metronidazole on the Embryo Skin and Integument Tissues using a Chicken Embryo Model. Journal of Kerman University of Medical Sciences, [online] 24(3), pp.190–199. Available at: ⟨http://jkmu.kmu.ac.ir/article_50710_8759ab9e9fe076d9c41e8a3a92e7c80d.pdf⟩ [Accessed 25 May 2022].

Tavakkoli, H., Attaran, R., Khosravi, A., Salari, Z., Salarkia, E., Dabiri, S., Mosallanejad, S.S., 2019. Vascular alteration in relation to fosfomycin: In silico and in vivo investigations using a chick embryo model. Biomed. Pharmacother. 118, 109240 https://doi.org/10.1016/j.biopha.2019.109240.

Uggini, G.K., Patel, P.V., Balakrishnan, S., 2010. Embryotoxic and teratogenic effects of pesticides in chick embryos: a comparative study using two commercial formulations. Environ. Toxicol. 27 (3), 166–174. https://doi.org/10.1002/tox.20627.

Wang, C., Wang, G., Wang, X., Liu, M., Chuai, M., Lee, K.K.H., He, X.-S., Lu, D., Yang, X., 2016. Imidacloprid exposure suppresses neural crest cells generation during early chick embryo development. J. Agric. Food Chem. 64 (23), 4705–4715. https://doi.org/10.1021/acs.jafc.6b01478.

Wells, K., Butterworth, A., Richards, N., 2019. A review of secondary pentobarbital poisoning in scavenging wildlife, companion animals and captive carnivores. J. Vet. Forensic Sci. 1 (1), 1–15. https://doi.org/10.32473/jvfs.v1i1.128307.

Youssef, R.M., El-Dlebshany, A.E., Ragab, M.A., El-Kimary, E.I., 2013. Monitoring of metronidazole and its metabolite residues in eggs of two strains of hens using HPLCA-DAD. J. Liq. Chromatogr. Relat. Technol. 36 (14), 2044–2058.