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Determination of fluoroquinolone antibiotic residues in the plasma of Eurasian griffon vultures (*Gyps fulvus*) in Spain

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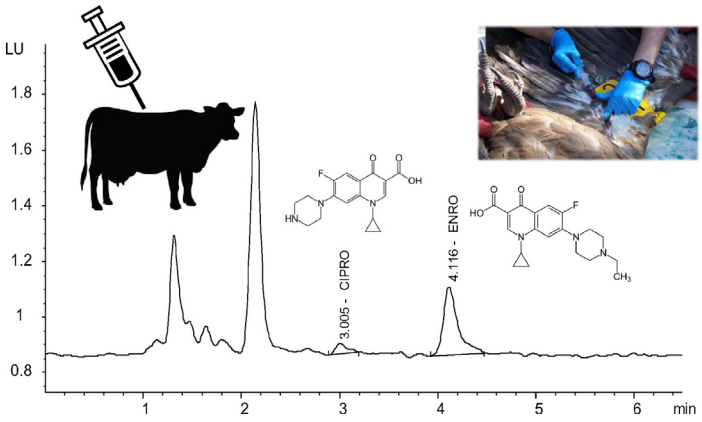
HIGHLIGHTS

- Quinolone residues were detected in plasma of Eurasian griffon vultures (*Gyps fulvus*).
- Differences in the exposure between areas can be explained by the carrion management.
- Vultures in areas providing intensive dead livestock are more exposed to quinolones.
- Protocols are needed to improve control of toxic veterinary drugs presence in carrion.

GRAPHICAL ABSTRACT



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ABSTRACT

Due to the possible toxicological impact, the accumulation of pharmaceuticals in wildlife as a consequence of human practices is of growing concern. The consumption of carrion at feeding stations – the so-called 'vulture restaurants' – with no management of the veterinary drugs it contains may expose scavengers to pharmaceuticals. To demonstrate this, we analyzed plasma from Eurasian griffon vultures (*Gyps fulvus*) originating from two different areas of Spain for antibiotics such as enrofloxacin and ciprofloxacin, its primary metabolite. Quinolone residues were detected in about 65% ($n = 106$) of birds, of which 15.1% (16/106) had quantifiable amounts of

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enrofloxacin (0.049 ± 0.102 μg/mL) and 5.7% (6/106) of ciprofloxacin (0.009 ± 0.007 μg/mL). The differences in exposure between the two sampled areas are attributable to different types of carrion management: the vultures that fed in areas with a high density of dead livestock (supplied directly to feeding stations) were more prone to exposure than those that sought food in areas where carcass availability is more unpredictable. Our findings are evidence that vultures have access to medicated livestock and that there are quantifiable levels of livestock antibiotics in vulture plasma. However, the vultures analyzed in this study had maximum antibiotic concentrations of only 0.4 μg/mL, much less than the concentrations used in the clinical treatment of scavengers and a level that is probably too small to cause intoxication.

1. Introduction

Avian scavengers have provided important ecosystem services to humans for millennia and of all such services the elimination of animal debris has had the greatest known impact (Moleón et al., 2014). However, the increase in certain anthropogenic activities (e.g. illegal poisoning, the use of veterinary pharmaceuticals, changes in habitats, and sanitary policies) is currently affecting vulture populations negatively worldwide and thus also the ecosystem services they provide (Margalida et al., 2010; Markandya et al., 2008; Oaks et al., 2004; Ogada et al., 2012).

In Europe, the most important vulture populations (>90%) are found in Spain. Recent changes in sanitary policies in this country in light of the outbreak of bovine spongiform encephalopathy (Donázar et al., 2009b; Margalida et al., 2010) have led to shortages in the amount of available carrion in the ecosystem. Although Spanish vultures are a rare exception in that they are one of the few Old World vulture populations that are on the increase, impacts on the quality of their food supplies could affect their conservation and population dynamics (Donázar et al., 2009a; Margalida and Colomer, 2012). In this sense, prior to the changes in sanitary policies (i.e. before 2005), the quantity of animal biomass removed from the wild was estimated to represent over 80% of the available food biomass (Cortés-Avizanda et al., 2010; Donázar et al., 2010; Margalida and Colomer, 2012; Margalida et al., 2014a). Thus, the greatest impact of any change in food quality is expected to occur at individual and population levels in areas in which supplementary feeding constitutes the main food resource for avian scavengers. Several studies have documented changes in behaviour, demographic parameters, dietary shifts and population trends (see review in Donázar et al., 2009a). Nonetheless, information about the health status of vulture populations is still scarce and contradictory (Blanco et al., 2016; http://retractionwatch.com/category/by-author/jesus-angel-lemus).

The Eurasian griffon vulture (*Gyps fulvus*) is one of the most sensitive avian species to reductions in food supplies (Margalida and Colomer, 2012), and any such reduction may lead to a shift in diet (Donázar et al., 2010). The drop in the amount of food provided by extensive livestock herds has led to an intensification of the consumption of food by vultures at predictable feeding stations and rubbish dumps, as well as the consumption of carcasses of wild ungulates (Donázar et al., 2010; Margalida et al., 2011). Given these trends, it is necessary to assess whether this reduction in the consumption of carrion derived from extensive grazing has increased the likelihood of ingesting carrion from medicated animals. Enrofloxacin (ENRO) and its primary metabolite, ciprofloxacin (CIPRO), are fluoroquinolones that provide rapid bactericidal action in livestock against a wide variety of clinically important bacterial organisms (Papich and Riviere, 2009). After treatment with ENRO, CIPRO can be found in tissues at levels of 7–55% of total residue (EMA/MRL/388/98-FINAL). It is therefore logical to assume that CIPRO may be detected in vultures that feed on the carcasses of animals treated with ENRO, even though CIPRO is not sold in Spain as a treatment for livestock. Data on the prevalence of antibiotics in vultures are unreliable and are even thought in some cases to have been fabricated (http://retractionwatch.com/category/by-author/jesus-angel-lemus). A recent study has revealed the presence of fluoroquinolones residues in plasma in a high proportion of griffon vulture nestlings from central Spain (Blanco et al., 2016). Thus, our goal was, first, to test for the presence of quinolone antibiotic residues in the plasma of griffon vultures and to measure their concentrations. We then compared the presence of quinolones in vultures sampled from two areas, each with different carrion management practices: Navarra (NAV), in which carrion is managed mainly in feeding stations, and Catalonia (CAT), where there are fewer feeding stations for griffon vultures but more unpredictable food resources provided by extensive grazing.

2. Materials and methods

2.1. Study area and animals

The study was carried out in northern Spain, an area with important griffon vulture populations (1116 pairs in CAT and 2783 in NAV; Del Moral, 2009) that are on the increase. Vultures feed mainly on the cadavers of domestic ungulates (*Ovis aries*, *Capra hircus*, *Bos taurus*, *Equus caballus*, *Sus scrofa* var. dom., see Donázar, 1993) and, to a lesser degree, on wild ungulates (mainly *Rupicapra pyrenaica*, *Cervus elaphus* and *Sus scrofa*, see Margalida et al., 2011). Carrion is generated by natural mortality, hunting and other causes. While the main source of carrion in NAV are domestic ungulates disposed of in supplementary feeding stations, its presence is more unpredictable in CAT. In NAV, there are 10 official supplementary feeding stations in which cadavers are delivered regularly for griffon vultures but only six in CAT. In this latter area, the presence of extensive livestock herds that are semi-stabled most of the year guarantees spatially widespread food resources that are far less predictable in appearance.

A total of 106 griffon vultures were captured between 2011 and 2012 in two feeding stations, 61 in CAT (434463.38 E, 4658381.15 N) and 45 in NAV (625671.50 E, 4689727.54 N) (Fig. 1). Food was supplied regularly at both feeding stations managed by local authorities: in CAT the feeding station is located in a rubbish dump and the carrion includes the lungs and carcasses of pigs purchased from slaughterhouses, while in NAV the feeding stations depend on animal carcasses provided by nearby authorized farms.

The capture method used in both areas was a portable trap with fixed side panels, with 10 × 10 cm mesh nets as a roof and self-locking swinging doors (dimensions 5.2 × 5.2 m). Once captured, vultures were physically restrained and blood samples were collected from the ulnar wing vein. Disposable 10-mL syringes fitted with 21 G × 1″ (25 mm) needles were used. Four millilitres of each sample were placed in a commercial tube containing tripotassium ethylenediaminetetraacetic acid (EDTA K3) as an anticoagulant and were kept in a portable icebox until they reached the laboratory. After hematologic studies, samples were centrifuged at 340*g* for 10 min, and plasma was obtained within 24 h of collection and frozen at −20 °C.

2.2. Analysis of plasma samples

The determination of ENRO and CIPRO plasma concentrations was carried out by the Servei d'Anàlisi de Fàrmacs - Departament de Farmacologia, Terapèutica i Toxicologia (Drug Analysis Service) of the Universitat Autònoma de Barcelona.

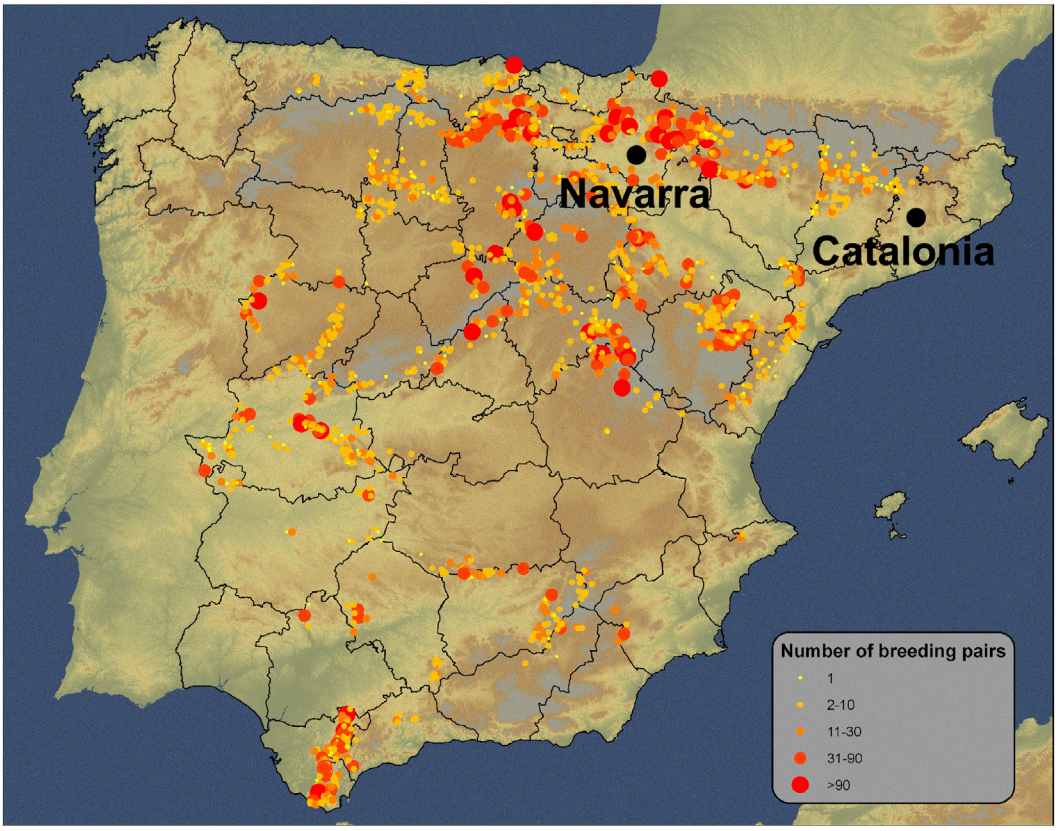

Fig. 1. Map indicating the location of the two study areas, as well as the population status and distribution of the Eurasian griffon vulture in Spain (modified from Del Moral, 2009). Locations: CAT (434463.38 E, 4658381.15 N), NAV (625671.50 E, 4689727.54 N). Short title of legend: Origin of studied vultures.

2.2.1. Reagents

Acetonitrile (Fisher, HPLC grade, ref. A/0626/17), phosphoric acid H_3PO_4 (Sigma, ref. 30417), dibutylammonium (J. T. Baker, ref. 8578) NaOH were obtained (Fisher, ref. S/4920/60) and used to prepare working solutions. Pure ENRO (Sigma, ref. 17849, batch 0001369030, purity 98.7%) and CIPRO (Sigma, ref. 17850, batch 0001396108, purity 99%) standards were used.

2.2.2. Standard solutions

0.1 mg/mL standard solutions of CIPRO and ENRO were prepared in 0.1 M water solution of NaOH. 0.01, 0.001, 0.005 and 0.0001 mg/mL solutions of ENRO and CIPRO were prepared by dilution with NaOH 0.1 M.

Calibration curve preparation: for the validation of the technique and for each day of sample analysis, calibration curves were constructed by spiking blank plasma samples with different standard volumes in order to obtain a 5-level calibration curve with a 0.005 μg/mL–2.5 μg/mL range for ENRO and a 0.005 μg/mL–0.1 μg/mL range for CIPRO.

Quality control sample preparation: each day of problem sample analysis a set of three quality control samples were prepared by spiking blank plasma samples with 0.025 μg/mL of CIPRO and 0.1 μg/mL of ENRO.

2.2.3. Extraction

200 μL of thawed plasma were placed in 1.5 mL Eppendorf tubes. For the calibration and control samples, the corresponding standard solution volume was added. Then, 300 μL of ice-cold acetonitrile was added and samples were mixed in a vortex shaker for 2 min. The samples were centrifuged at 14,500 rpm for 5 min at 4 °C. The supernatant was placed in a 2 mL HPLC glass vial, capped and placed in the autoinjector of the HPLC system. 3 μL of sample were injected.

2.2.4. HPLC analysis

An Agilent 1100 series system was used that consisted of a degasser (G1322A), a quaternary pump (G1311A), a Peltier refrigerated autoinjector (G1329A), a column oven (G1316A) and a fluorometric detector (G1321A). A C_{18} reverse phase column (Kromasil 200 × 4 mm, 5-μm particle size) was used. The flow rate was 1.5 mL/min. The mobile phase was composed of acetonitrile, a 0.1% H_3PO_4/0.05% dibutylammonium solution (pH = 2.3) and water in an isocratic mode at a proportion of 12:44:44. The detection was performed using a fluorescence detector set at an excitation wavelength of 278 nm and an emission wavelength of 440 nm. At these conditions the retention times of CIPRO and ENRO were 3.0 min and 4.0 min, respectively.

2.3. Analytical method validation

2.3.1. Linearity

For the validation of the analytical method the linearity of the calibration curve was studied in the range between 0.005 μg/mL–2.5 μg/mL for ENRO and 0.005 μg/mL–0.1 μg/mL for CIPRO. An ANOVA test of the linear regression of the calibration curve was conducted.

2.3.2. Precision and accuracy

The precision, expressed as the coefficient of variation (C.V., %), was obtained by applying the experimental procedure to three sample replicates, on three different days (interday precision) and on the same day (intraday precision), for three concentrations of the analytes (0.005, 0.025 and 0.1 μg/mL for CIPRO and 0.005, 0.1 and 2.5 μg/mL for ENRO). The accuracy is expressed as the relative error (R.E., %) obtained by applying the experimental procedure to three sample replicates, on the same day, for three concentrations of the analytes, as was done for the precision estimates. The critical value for the C.V. and R.E. was 15%.

2.3.3. Limit of detection and limit of quantification

The Limit of Detection (LOD) was established using the standard deviation of the response (S.D.) and the slope of the calibration curve (S), following the formula:

$$LOD = 3.3 \times S.D./S.$$

The value of S.D. was calculated from 10 blank plasma samples (10 European griffon vultures in captivity without antibiotic treatment). The area at the retention times of CIPRO and ENRO were measured, and the standard deviation resulting from these 10 areas was the value used as the S.D. The slope was that obtained in the linearity study. The Limit of Quantification (LOQ) was considered to be the lowest concentration of the calibration curve, which is accepted if the following conditions are met: the analyte response at the LOQ should be at least five times the response compared to the plasma blank response; and the analyte peak (response) should be identifiable, discrete, and reproducible with acceptable precision and accuracy.

2.3.4. Specificity

The interferences in the retention time of CIPRO and ENRO were studied in all the plasma blank samples analyzed during the validation study.

2.3.5. Stability of ENRO and CIPRO

The stability of ENRO and CIPRO in working solutions at storage conditions (−20 °C) was studied in the 0.005 mg/mL secondary solutions. Two sample sets were stored at −20 °C (±5 °C) and analyzed 2 and 7 days after preparation (one sample set in each period).

The stability in stored samples was assayed on 15 blank samples spiked with 0.025 μg/mL of CIPRO and 0.1 μg/mL of ENRO. After preparation the samples were stored at −20 °C (±5 °C). Sets of three plasma samples were analyzed 12, 37, 110, 230 and 387 days after preparation. In each cycle, the ENRO and CIPRO concentrations of samples were determined by interpolation in a calibration curve, which was prepared and analyzed on the same day of analysis. The stability was determined by comparing the mean concentration obtained with the nominal concentration. The results were expressed as R.E. (%), following the formula:

$$R.E. = (CalculatedConc. - NominalConc.) \times 100/NominalConc.$$

The final extracts from the linearity study were re-analyzed 24 h after extraction, to quantify ENRO and CIPRO. During this time, the extracts were maintained in the chromatographic vials, in the autosampler system, at approximately 4 °C. The stability of the analytes in the processed samples was determined calculating the mean concentration obtained at each concentration and comparing it with the initial assay result. The results were expressed as R.E. (%), as for the stability in stored samples.

2.4. Statistical analyses

We used the Chi-square test to test whether differences exist between the two areas in the number of individuals in which antibiotics were present. The concentration levels in plasma (only for ENRO due to the small sample size for CIPRO) were tested with the Mann-Whitney *U* test. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Validation of the analytical method

The chromatographic profiles of standard solutions of CIPRO and ENRO are shown in Fig. 2. As can be observed, there is a clear separation between the two antibiotics. A plasma blank sample and a calibration sample are shown in Fig. 3, both extracted and processed as incurred samples. There was no interference of peaks in the retention times of CIPRO and ENRO in blank samples, and both antibiotics can be distinguished from the base line.

Linearity was established from 0.005 μg/mL to 0.1 μg/mL for CIPRO and from 0.005 μg/mL to 2.5 μg/mL for ENRO. The correlation coefficient (r) of the regression was more than 0.999, thereby suggesting a good relationship between the response and the amount of analytes present in the samples. The intraday precision and accuracy for CIPRO were 5.4% and 5.4% for the 0.005 μg/mL concentration, 3.4% and 3.4% for the 0.025 μg/mL concentration and 1.3% and 1.0% for the 0.1 μg/mL concentration. The intraday precision and accuracy for ENRO were −2.9% and 0.9% for the 0.005 μg/mL concentration, 1.7% and 6.7% for the 0.1 μg/mL concentration and −1.8% and 4.9% for the 2.5 μg/mL concentration. All the values were within the acceptance criteria of ±1.5% for accuracy and 15% for precision. The interday precision for CIPRO ranged between 9.7% at 0.025 μg/mL concentration and 3.1% for the 0.1 μg/mL concentration. For ENRO those results ranged between 11.9% for the 0.005 μg/mL concentration and 4.0% for the 2.5 μg/mL concentration. The LOD was established at 0.0010 μg/mL for CIPRO and 0.0006 μg/mL for ENRO. The LOQ for CIPRO and ENRO were both 0.005 μg/mL and the ratio of responses between LOQ and LOD were, respectively, 5 and 15. Finally, the stability of the analytes in the vulture plasma under storage conditions (−20 °C) was demonstrated at up to 387 days.

3.2. Analysis of samples

The results of the CIPRO and ENRO quantification in vulture plasma from birds sampled in Catalonia and Navarra are shown in Table 1. As shown, some of the samples contained both analytes. The concentrations found were in most cases close to the LOD of the technique, thereby indicating that there were only very low levels of the studied quinolones in the plasma of these birds. Chromatograms from a negative sample (A), a sample between LOD and LOQ (B) and two positive samples (C and D) are shown in Fig. 4.

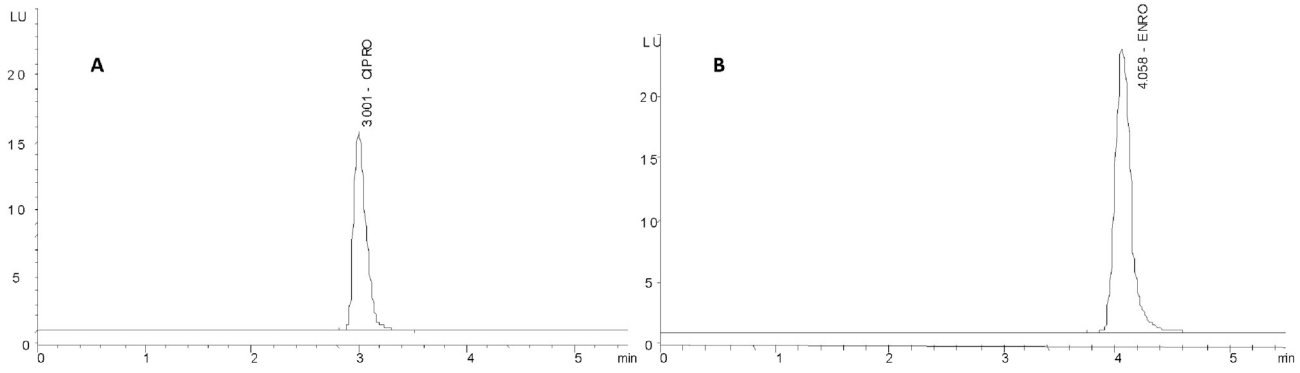

Fig. 2. Chromatograms corresponding to an injection of 3 μL of a 0.001 mg/mL of CIPRO (A) and ENRO (B).

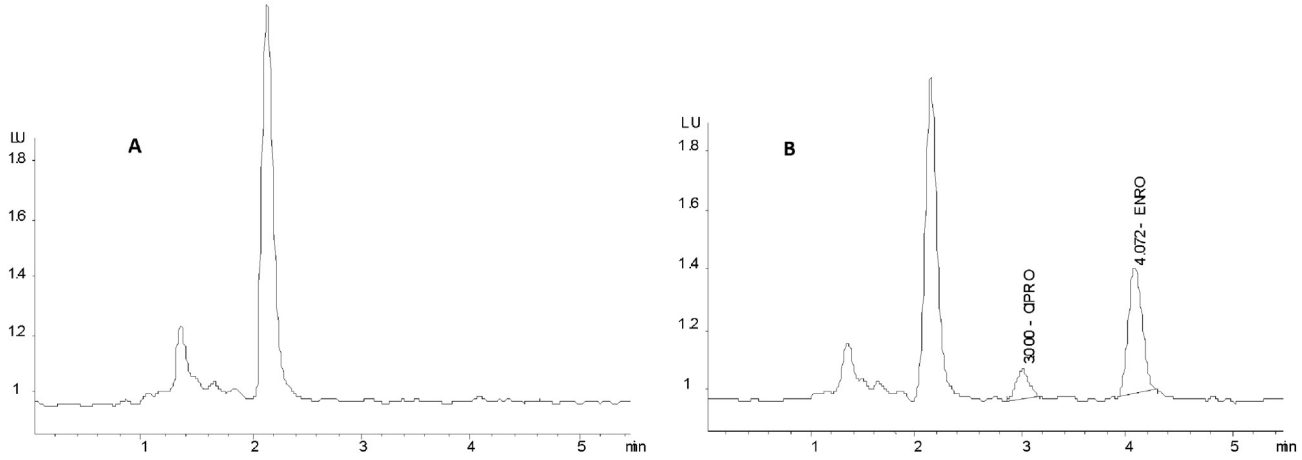

Fig. 3. Chromatograms corresponding to blank plasma samples (A) and calibration samples spiked at concentrations of 0.010 μg/mL of CIPRO and 0.025 μg/mL ENRO (B).

ENRO and CIPRO were detected in 67.2% and 27.9%, respectively, of vultures in CAT ($n = 61$) and in 64.4% and 40% in NAV ($n = 45$) (Table 2). We found significant differences between zones, with the presence of individuals with quantifiable levels of ENRO significantly higher in NAV ($\chi^2 = 6.426$, df = 2, $p = 0.040$). No differences were found between zones for CIPRO ($\chi^2 = 1.729$, df = 2, $p = 0.421$, Table 2). The differences in ENRO concentrations in plasma differed between zones, with higher levels in NAV (Mann-Whitney *U* test, $z = 1.926$, $p = 0.027$, Table 2).

4. Discussion

There is growing concern about the presence of pharmaceuticals in the environment, above all because they are known to affect endangered species of wildlife (Margalida et al., 2014a). Of the non-natural factors threatening vulture populations, the ingestion of veterinary pharmaceuticals such as diclofenac – present in the carcasses of livestock treated with this drug shortly before death – has been identified as the most significant toxicological factor in the past decade. It has led to the near extinction of several vulture species in Asia (Prakash et al., 2003; Green et al., 2004; Oaks et al., 2004; Shultz et al., 2004) and there is alarm about the detrimental effects that it may have on African (Naidoo et al., 2009) and, more recently, on European vulture populations (Margalida et al., 2014b, 2014c; Green et al., in press). The contamination of just 0.3–0.7% of ungulate carcasses with a lethal level of diclofenac was shown in India and Pakistan to be sufficient to cause an annual decline in the oriental white-backed vulture *Gyps bengalensis* population of about 50% (Green et al., 2004). The fall in avian scavenger numbers in Southern Asia due to diclofenac (Green et al., 2004; Oaks et al., 2004) and the recent case in Spain of a dead griffon vulture found to have been intoxicated by flunixin (Zorrilla et al., 2014) demonstrate that certain pharmaceuticals can be passed between domestic animals and wildlife.

Table 1
Concentration in plasma of enrofloxacin and ciprofloxacin in the griffon vultures studied in Catalonia and Navarra. Only positive results are shown.

Catalonia		Navarra	
Enrofloxacin (μg/mL)	Ciprofloxacin (μg/mL)	Enrofloxacin (μg/mL)	Ciprofloxacin (μg/mL)
0.0060	nd	0.0076	nd
0.0063	nd	0.0095	nq
0.0070	nd	0.0109	nd
0.0089	nd	0.0113	nq
0.0410	nq	0.0130	0.0237
nd	0.0053	0.0133	nq
nd	0.0054	0.0139	nq
nq	0.0055	0.0169	nq
		0.0214	0.0062
		0.2136	0.0074
		0.3831	nq

nd: not detected, nq: not quantifiable.

The carrion supplied to scavengers is not subject to withdrawal periods or drug residue controls like those conducted in slaughterhouses on food for human consumption. Despite not demonstrating that antibiotics have a direct effect on vulture populations, our study does provide evidence that medicated livestock is accessible to vultures and that there are quantifiable antibiotic levels in vulture plasma. Quinolone residues were detected in about 65% of individuals ($n = 106$), of which 16 (15.1%) presented quantifiable amounts of ENRO and 6 (5.7%) of CIPRO, the latter a drug that is not registered for use in animals in Spain. Nevertheless, its presence in the samples is not due to its use in the treatment of animals since it is an ENRO metabolite: the vultures that feed on dead animals treated with ENRO ingest the parent drug along with its metabolites. In bovines, large amounts of CIPRO can be found in tissues (up to 50% of residues) after treatment with ENRO. Blanco et al. (2016) detected fluoroquinolones in griffon vulture nestlings, a finding that suggests that vultures do ingest these antibiotics when they feed on the carcasses of medicated livestock. In their development stage, 20% of vultures had quantifiable concentrations of ENRO and CIPRO was detected in 32%, albeit not in quantifiable amounts.

An important proportion of the vultures analyzed contained quinolone residues, which indicates that these antibiotics are ingested and metabolised – to a greater or lesser extent – by most individuals in the sampled populations. Aside from the direct effects on the health of the vulture population, these findings suggest that antibiotic resistance in bacterial flora is likely to develop. Given the long-distance movements of vultures and the frequency with which they come into contact with livestock in the wild and in feeding stations supplied with the carcasses of stabled livestock, the transmission of quinolone-resistant bacteria could become a health problem for human populations. Resistance develops when antibiotic concentrations are high enough to kill all bacteria except resistant mutants. Low concentrations that kill no bacteria are not dangerous to vultures or to other scavengers, nor are they likely to pose a threat to livestock or humans.

Fluoroquinolones have impressive safety records and ENRO, an antibiotic belonging to this group, is known for its minimal adverse side effects (Papich and Riviere, 2009). ENRO is partially metabolised (in some species) to CIPRO by deacetylation in the liver and excreted via the kidney; nevertheless, neither nephrotoxicity nor hepatotoxicity have ever been reported after the use of approved dosages in the studied bird species (Harrenstien et al., 2000). Very high concentrations of fluoroquinolones can produce adverse effects in the central nervous system. Other side effects include anorexia and polyuria in birds, blindness in cats due to retinal degeneration, arthropathy in young animals (dogs

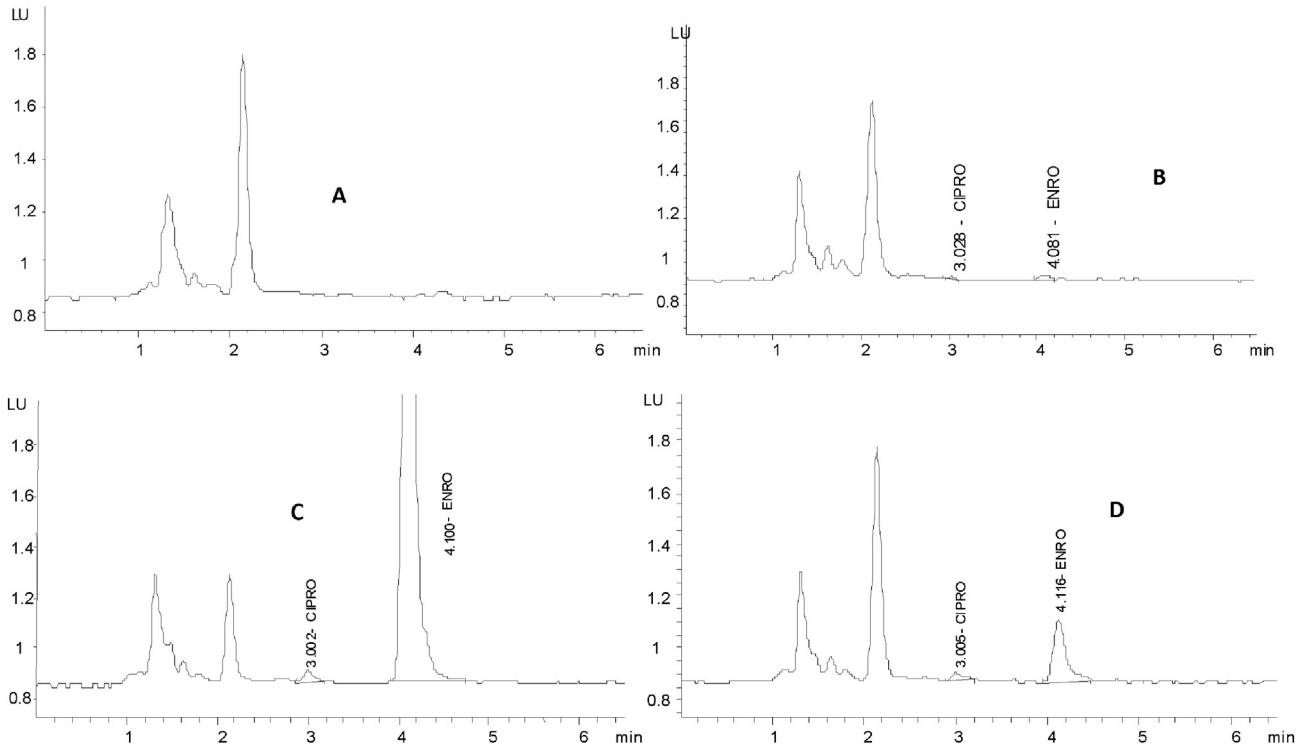

Fig. 4. Chromatograms corresponding to samples GF/15/11 (A), GF/25/11 (B), GF/41/11 (C), and GF/38/11 (D).

and horses), chondrotoxicity in nestling pigeons and chickens, and tendinitis and tendon rupture in humans (but not reported in animals) (Frazier et al., 1995; Papich and Riviere, 2009; Waxman et al., 2013).

ENRO has a half-life of 2.3–14.2 h after oral administration in certain avian species (Frazier et al., 1995; Harrenstien et al., 2000; Knoll et al., 1999), with plasma concentrations of 0.52–2.44 μg/mL depending on the dose administered (5–30 mg/kg) and on the species (Knoll et al., 1999; Papich and Riviere, 2009; Waxman et al., 2013). CIPRO has been administered in dosages of 5–50 mg/kg and gives plasma concentrations of 3.64–4.67 μg/mL (Frazier et al., 1995; Papich and Riviere, 2009). These concentrations are required to attain the desired therapeutic antimicrobial effect but are too small to produce negative side effects. The vultures analyzed in this study had concentrations up to 0.4 μg/mL, which are much lower than those used in treatments. Thus, we can rule out the possibility that these antibiotics have toxic effects on griffon vultures; nevertheless, wildlife should not have access to food contaminated with pharmaceuticals. Our results suggest that griffon vultures access carrion from medicated animals in the field (probably at feeding stations and dumps) and quickly ingest the veterinary antibiotics with which the dead animals had been treated. The exposure to antibiotics may also occur at rubbish dumps because, since the imposition of sanitary restrictions, such sites are now more frequently visited by vultures (Donázar et al., 2010; Margalida et al., 2010) and are also used to illegally dump pharmaceutical products.

Differences in concentrations between regions provide evidence that exposure varies according to location, and differences in carrion management could explain the variability in exposure. In NAV, dead livestock is taken directly to feeding stations and so vultures have more immediate access to medicated carrion than in areas such as CAT, where the availability of food is more unpredictable. A further question – the induced resistance to pathogens – has been raised by the work of Porrero et al. (2013), who isolated a type of MRSA resistant to tetracycline and ciprofloxacin in the NAV population. Other authors have suggested that griffon vultures are a potential vector of *Salmonella* infection and a zoonotic risk in general for the human population (Marin et al., 2014).

Table 2
Results of the detection of enrofloxacin and ciprofloxacin in Eurasian griffon vulture plasma obtained in the two study areas. The percentage appears in brackets and * denotes significant differences ($p < 0.05$) between regions (see Methods for more details).

	Enrofloxacin		Ciprofloxacin	
	Catalonia	Navarra	Catalonia	Navarra
Quantifiable	5 (8.2)*	11 (24.4)*	3 (4.9)	3 (6.7)
Not quantifiable	36 (59.0)	18 (40.0)	14 (23.0)	15 (33.3)
Not detected	20 (32.8)	16 (35.6)	44 (72.1)	27 (60.0)
Total of vultures	61	45	61	45

5. Conclusions

The plasma of vultures analyzed in this study had fluoroquinolone antibiotic residues, evidence that medicated livestock carcasses are available to vultures. However, the detected concentrations were much lower than those used in clinical treatment of scavengers, thereby suggesting that these levels of antibiotics are probably not sufficient to produce intoxication.

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