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A Review of Enrofloxacin for Veterinary Use

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Abstract

This review outlines the current knowledge on the use of enrofloxacin in veterinary medicine from biochemical mechanisms to the use in the field conditions and even resistance and ecotoxicity. The basics of biochemistry, the mechanisms of action and resistance and pharmacokinetics are presented. Then an overview of available veterinary products, their efficacy and their toxicity against target species, human and environment is provided.

Keywords

Enrofloxacin, Antibiotic Resistances, Veterinary

1. Introduction

Enrofloxacin (**Figure 1(c)**), or 1-Cyclopropyl-6-fluoro-7-(4-ethyl-1-piperazinyl)-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid, belongs to fluoroquinolone family which is a subfamily of quinolone. The first quinolone is the Nalidixic acid (**Figure 1(a)**) used in animal at the beginning of 1980s, enrofloxacin is the first fluoroquinolone patented in 1984 [1]. The huge evolution in the quinolone family is the addition of a fluor atom on the 6th position which improves quinolones' antibacterial spectrum [2] and creates the fluoroquinolone subfamily. Quinolones have an action on bacterial topoisomerase. The marketing authorization reports a large antimicrobial spectrum for enrofloxacin, which is efficient on most gram-negative and gram-positive bacteria but not efficient on anaerobic bacteria [3]. But with 3.6 tons sold per year in France for animal use [4], fluroroquinolones are an important family in veterinary medicine that increases the probability of selecting resisting bacteria.

2. Action Mechanism of Enrofloxacin

2.1. Important Physicochemical Properties of Enrofloxacin

Enrofloxacin is a zwitterionic molecule with a pKa_1 = [5.88 - 6.06] and a pKa_2 = [7.70 - 7.74] [5]. The lowest

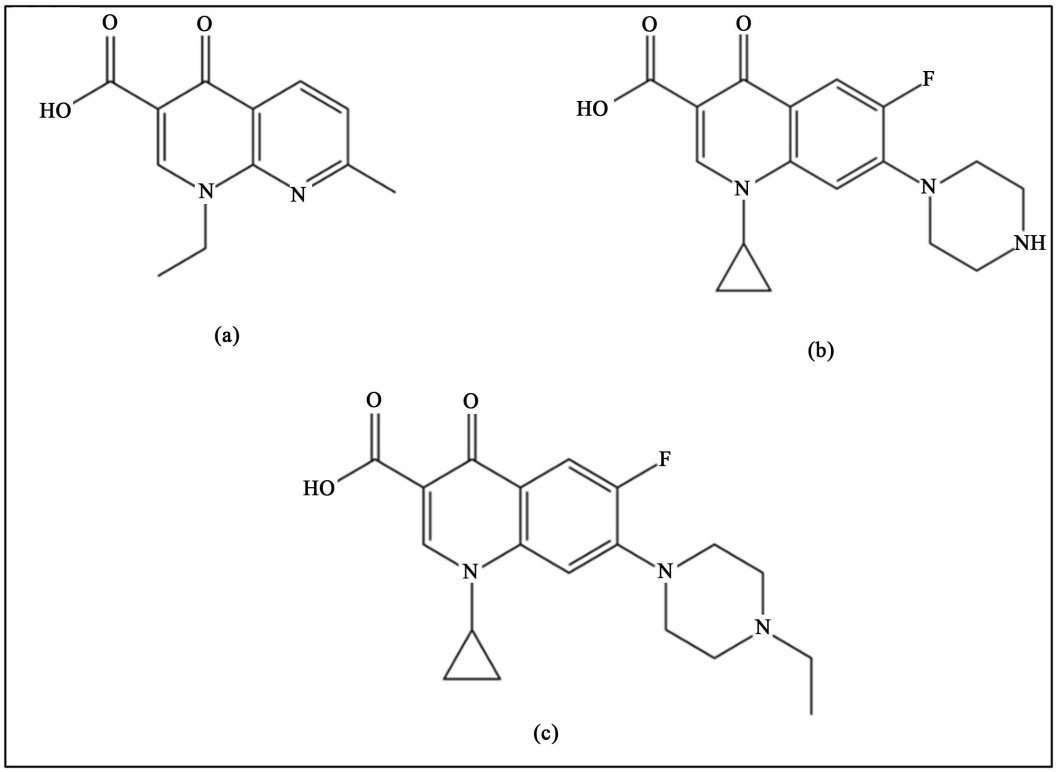

Figure 1. Structure of (a) Nalidixic Acid; (b) Ciprofloxacin and (c) Enrofloxacin.

pKa is due to the carboxyl acid group and the second to the basic tertiary amine. So enrofloxacin doesn't bear charge between this two pH. Moreover it is a lipophilic molecule with a logP of 4.70 at a pH of 7 [6], but 1.88 ± 1.43 with ACDLabs.

The Active metabolite of enrofloxacin, ciprofloxacin (**Figure 1(b)**) or 1-Cyclopropyl-6-fluoro-1, 4-dihydro-7-(1-piperazinyl)-4-oxo-3-quinoline carboxylic acid, which is available as a drug product on veterinary and human market, is a multiple acid with $pKa_1 = 5.15$ and $pKa_2 = 8.25$ [7]. Ciprofloxacin is less lipophilic than enrofloxacin with a logP of −1.11 at a pH of 7.4 [8].

2.2. Targets' Mechanism of Action

Enrofloxacin, like the other quinolones, has two mains targets of the topoisomerase family. Although these proteins exist in eukaryotes cells, quinolones have less affinity for eukaryotes' topoisomerases than for the DNA Topoisomerase II (Gyrase) and the DNA Topoisomerase IV (Topo IV) two major bacterial topoisomerase [9]. The Gyrase and the Topo IV are two tetramers (**Figure 2(a)**) formed respectively of two GyrA and two GyrB and of two ParC and two ParE. Moreover GyrA and GyrB are homologous respectively with ParC and ParE [10].

The Gyrase has an important role in bacteria's life by modifying the topology of the spiral DNA. Indeed, the positive supercoiling stabilizes the DNA and the strands' separation becomes more difficult [11]. Moreover transcription generates a positive supercoiling accumulation that can stop transcription. This positive supercoiling can be released by the Gyrase, which enhances transcription [12]. To do this, the Gyrase binds and wraps around itself a strand of DNA (**Figure 2(b)**) with the help of the C-terminal domain [13] and cleaves it (**Figure 2(c)**) with mediating of the catalytic tyrosine Tyr 122 [14] of each GyrA. Tyrosines form covalent phosphotyrosyls with each 5' phosphoryl terminus of both strands, which remain binding during the reaction [15]. This reaction forms a gap in sequence (called G-DNA). The GyrB part catches the other DNA sequence (called transported DNA or T-DNA). The T-DNA is passed through the opened G-DNA (**Figure 2(d)**) [16]. The G-DNA is closed in an ATP-dependent reaction (**Figure 2(e)**) [17]. Of this reaction results an adding of negative coiling.

The Gyrase is able to do an intermolecular strand passage at the end of the replication like the Topo IV but

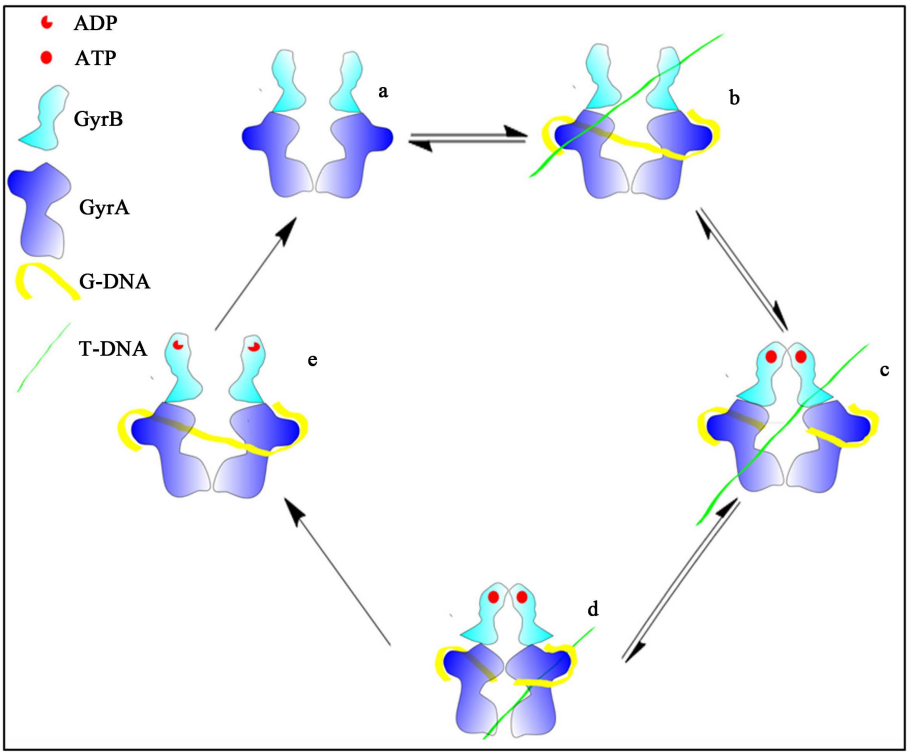

Figure 2. Mechanism of action of Gyrase, with intervention of Gyrase part A (GyrA) and B (GyrB), gap DNA sequence (G-DNA), transported DNA sequence (T-DNA), ATP and GDP. (a) Gyrase with GyrA dimer and GyrB dimer; (b) Interaction of Gyrase with two DNA sequences; (c) Fixation of an ATP molecule and cleavage of the G-DNA strand; (d) Passage of the T-DNA strand through G-DNA; (e) Closing of the G-DNA strand with ATP degradation.

without wrapping the DNA as the Topo IV, this is the major difference between reactions of both enzymes [18]. Topo IV catalyzes the segregation of the two daughter DNA molecules after the replication [19] more efficiently than Gyrase [20].

2.3. Interactions between Fluoroquinolones, DNA and Its Target

The penetration in the bacteria differs between Gram-positives and Gram-negatives bacteria. For Gram-negative bacteria, fluoroquinolones pass the outer membrane mainly through porins [21] [22]. The trimeric OmpF porin is mainly used by fluoroquinolone, this pathway can be modulated by Mg^{2+} [23]. But it seems that some quinolones can promote a pathway by interacting with the Lipopolysaccharide (LPS) and create a lipophilic passage [24]. For Gram-positive bacteria the diffusion process is the main uptake pathway [25].

In the case of mycobacteria the high lipid level of the membrane allows fluoroquinolones to diffuse through membrane [26].

After the entrance in the bacteria, quinolones have two effects on the bacteria: bacteriostatic at low concentration level or bactericide at high concentration level. Rapidly after the Gyrase has formed a complex with the DNA strand, two quinolones bind with this complex, before the DNA's cleavage [27]. This binding is reversible and induces a conformation modification in Gyrase [28]. This modification induces a cleavage of DNA in a particular location and forms a cleaved complex [29] [30]. At this step the action is still reversible, but reduces rapidly the activity of replication and has a bacteriostatic effect [31].

The next two pathways are possible one for bacteriostatic concentration and the other for bactericide concentration. For bacteriostatic concentrations, quinolones induce the SOS regulon controlled by the repressor lexA [32] [33]. Activation of the regulon induces the sfiA gene which codes for an inhibitor of cell division and bacteria form a long filamentous structure. This effect is still reversible [34]. This filamentous structure contributes to the death of bacteria [35].

For bactericide concentration quinolones induce a chromosome fragmentation by creation of a suicide factor or by destabilization of a GyrA dimer [35]. The fragmentation can induce a rapid death or, by reassociation of the GyrA dimer, genomic mutation in bacteria [36].

2.4. Resistance Mechanism

The resistance to fluoroquinolones in general, is today a main subject for the use of antibiotic substances in veterinary medicine and was the subject of an EMA report [4]. Many studies show an augmentation in the development of resistances to enrofloxacin [37] [38]. Moreover, another study shows the increase of resistance of *Escherichia coli* to quinolones and fluoroquinolones after using of enrofloxacin in calves [39].

The mechanism of resistance to fluoroquinolones is based on many pathways: the change of targets, the protection of target, the lower expression of target, the improvement of the efflux pumps, and inactivation of fluoroquinolones. These mechanisms can be chromosomal or plasmide-mediated (**Table 1**) [40].

The most common mechanism is the mutation of Gyrase. In the subpart GyrA the mutation is mainly between Ala67 and Gln 106 for *E.coli* and other bacteria [41] [42]. The mutation is near the GyrA active site which is called Quinolone Resistance-Determining Region (QRDR). For GyrB two mutation are reported at Asp426 and Lys447 [43]. Only one Gyrase mutation is able to increase the Minimum Inhibitory Concentration (MIC) up to 64 times [41], but the GyrB mutation seems to be less effective in resistance against nalidixic acid than the GyrA mutation [44].

The mutation of Topo IV is also described but, until today, always with at least one Gyrase mutation. Therefore, these mutations seem to be developed in a second time to increase the resistance more than only Gyrase's mutations. A QRDR can be also characterized in the parC of topoisomerase IV between Tyr 57 and Glu84 [45].

The protection of target can be attributed to a qnr gene often present on a Plasmide-Mediated Quinolone Resistance (PMQR) or in the chromosomal DNA. The Qnr protein is a dimere which binds with Gyrase and decreases the binding of quinolones with DNA-Gyrase-Qnr complex. But it seems that the Qnr protein reduces the interaction between DNA and Gyrase [46]. Another theory is that the Qnr protein destabilizes the DNA-Gyrase-quinolone complex and promotes the DNA repair after cleavage [47].

An hypothesis of resistance is the slow growth of cell, bacteria growing slowly seem to resist better, but this mechanism is not known today [40] [48].

Although resistances are often attributed to the Gyrase or Topo IV mutations, decrease of influx and increase of efflux mechanisms are important pathways of resistance common to a large number of unrelated antibiotics [49]. This defines a Multiple Antimicrobial Resistance (MAR) phenotype. *E. coli* bearing a MAR operon has higher resistance for the quick death by fluoroquinolones [50]. This operon can induce a decrease of OmpF pumps [51] and an increase of efflux with AcrAB protein [52]. The decrease of porins can also be attributed to PMQR [53].

2.5. Recommendations to Prevent Resistance

Given the rapidity of development of resistance, we have to consider with the MIC, the Mutant Prevention Concentration (MPC). MPC is a recent indicator which is not well standardized. Commonly MPC50 is the concentration where 50% of the colonies doesn't contain resistant mutant after 72 hours of incubation. To count the mutants, a Polymerase Chain Reaction (PCR) is used but only known resistant mutation is tested [54]. Between MIC and MPC there is a window where enrofloxacin is effective but also where mutants are selected.

The data recorded in **Table 2** show that the window between the MIC and the MPC can be very important with a MPC/MIC between 1.6 and 64. This high difference can explain the explosion of resistance against enrofloxacin and might be a base to define new guidelines and dosage for enrofloxacin.

Table 1. Possible origins of resistances to fluoroquinolones.

Chromosomal resistance	Plasmide-mediated resistance
Mutation of target	Lower expression of target
Lower expression of target	Protection of target Improvement of efflux pumps
Protection of target Improvement of efflux pumps	Inactivation of fluoroquinolones

Table 2. MIC and MPC of enrofloxacin for some bacteria.

Bacteria	MIC (µg/mL)	MPC (µg/mL)	(MPC/MIC)	References
Escherichia coli	[0.022 - 0.03]	[0.17 - 0.5]	[7.8 - 16]	[67] [107]
Staphylococcus pseudintermedius	0.13	0.27	2	[108]
Rhodococcus equi	[0.5 - 1]	[8 - 64]	[16 - 64]	[109]
Mannheimia haemolytica	0.16	0.25	1.6	[110]
Salmonella Typhimurium	[0.06 - 0.125]	[1 - 2]	16	[67]
Pseudomonas aeruginosa	2	[16 - 32]	[8 - 16]	[67]

3. Pharmacokinetic of Enrofloxacin

3.1. Absorption

There is high variation of bioavailability after oral administration of enrofloxacin between polygastric and monogastric animals from 10% to 80% [55] [56]. This has a direct consequences on the galenic, oral presentations are reserved for pigs, poultry, calves, and carnivores and only injectable solutions are available for cattle. Moreover, bioavailability depends on whether the animals are fed or fasted [55] [57], and of the presence of ion [58]. These interferences can be explained by the formation of a complex between cation and fluoroquinolone which cannot permeate through the digestive barrier [59]. Another consequence of this complex is the influence of the hardness of water notably in poultry farming where the dilution in water is used [60]. But lipophilic compounds in food can enhance oral bioavailability of fluoroquinolone [61].

In addition to passive diffusion allowed by enrofloxacin lipophilicity, active transporters have also an important role in intestinal absorption [62]. But these transporters are also important to eliminate fluoroquinolones [62].

The intramuscular bioavailability is 96% with a maximum concentration 3 hours after administration [3]. Intramuscular bioavailability can be enhanced by solid lipophilic nanoparticles, this technic enhances the duration of enrofloxacin in plasma [3].

3.2. Metabolism

After administration a high part of enrofloxacin is metabolized into ciprofloxacin in most of species (**Table 3**). Enrofloxacin has an active metabolite, ciprofloxacin [63], obtained by deethylation of the ethyl on the piperazin ring. Other metabolites are obtained but they don't have antimicrobial effects [64].

Only poultry does not have a huge part of enrofloxacin metabolized in ciprofloxacin. The first hepatic pass doesn't have a lot of effect, only 7% of enrofloxacin is metabolized [65]. Moreover ciprofloxacin seems a more potent drug than enrofloxacin [66] [67]. These elements are in favor to grant a more important place to ciprofloxacin in the effects of enrofloxacin-based drugs.

3.3. Distribution

Distribution of enrofloxacin and ciprofloxacin to tissues depends on drug's free concentration, which depends itself on the concentration of protein and the strength of this biding.

The difference between ciprofloxacin and enrofloxacin protein binding (**Table 4**) are in favor of the theory of enrofloxacin's role of prodrug. Indeed, in some species ciprofloxacin is less bound with proteins, so it is able to be more available to be effective. Moreover, enrofloxacin can interact with other protein-binding drugs for instance it increases the clearance of flunixin meglubine [68].

As described in **Table 5**, the volumes of distribution of enrofloxacin and ciprofloxacin in different species are all higher than 1. So enrofloxacin and its metabolite diffuse strongly in tissues, and molecules can be present in cells, so being inactive. Contrary to what we might expect considering its protein binding, ciprofloxacin doesn't diffuse in tissues more than enrofloxacin. Data of **Table 6** suggest a high affinity of drugs for lung and kidney, even if data confirm enrofloxacin's and ciprofloxacin's good diffusion.

Table 3. Percent of ciprofloxacin after enrofloxacin given.

Species	Ciprofloxacin percent of enrofloxacin plasma concentration	References
Dogs	40	[65]
Dairy cows	59	[75]
Steers	64	[75]
Chickens	<10	[111]
Pigs	51	[112]
Goats	34	[71]

Table 4. Protein binding of enrofloxacin and ciprofloxacin.

Species	Percent of bond enrofloxacin (%)	Percent of bond ciprofloxacin (%)	References
Dogs	34	18	[113]
Dairy cows	59.4	33.7	[75]
Steers	60.8	49.6	[75]
Chickens	23	ND	[114]
Pigs	[31.1 - 37.1]	35	[115]

Table 5. Pharmacokinetics parameters of enrofloxacin and its metabolite ciprofloxacin.

Species	$t_{1/2}$ (h) *		Cl (mL/min/kg) **		V_{ss} (L/kg) ***		References
	Enro	Cipro	Enro	Cipro	Enro	Cipro	
Dogs	2.3	2.8	12.16	7.8	2.45	1.92	[65]
Dairy cows	3.69	2.96	24.16	ND	1.56	ND	[75]
Steers	5.5	7.60	11.6	ND	1.59	ND	[75]
Chickens	6.99	3.11	3.30	15.45	1.98	4.04	[111]
Pigs	26.6	2.60	3.0	17.30	6.40	3.80	[69]
Goats	1.39	[1.82-2.72]	22.18	19.59	1.27	3.33	[71] [116]

*elimination half-live, **clearance, ***volume of distribution at the steady state. Enro: enrofloxacine, Cipro: ciprofloxacine.

Table 6. Maximal concentration (C) and area under the curve (AUC) of enrofloxacin (ENR) and ciprofloxacin (CIP) after an intravenous injection of 5 mg/kg of enrofloxacin [117].

	Plasma†		Muscle‡		Liver‡		Spleen‡		Lung‡		Kidney‡	
	ENR	CIP	ENR	CIP	ENR	CIP	ENR	CIP	ENR	CIP	ENR	CIP
C	4.44	0.18	3.48	0.19	3.67	2.95	11.04	0.25	3.82	0.86	8.98	2.78
AUC	3.52	0.61	5.61	0.62	5.76	2.02	10.44	2.08	9.44	4.79	9.03	4.91

†Unit of C is µg/mL and unit of AUC is µg.h/mL; ‡Unit of C is µg/g and unit of AUC is µg.h/g.

3.4. Elimination

Elimination parameters show great difference between species (**Table 5**), especially for pigs where the elimination half-life is high with 26 h [69]. Moreover, for chickens and pigs, clearance of ciprofloxacin is five times higher than enrofloxacin's clearance and their enrofloxacin clearances are lower than other species. This differences might be attributed to a difference of elimination way.

Enrofloxacin's elimination way is mainly renal. This has been proved in rat by nephrecotomizing and comparing with CCl_4 hepatic impairment [70] and in goat with probenecid [71]. Probenecid has been shown to reduce the renal clearance of fluoroquinolones [72]. On the other hand, ciprofloxacin's elimination is both hepatic and renal [73]. For both molecules there is an intestinal recirculation via the bile excretion, moreover, two hour after given, there is no significant difference in the concentration of enrofloxacin in the intestinal content between oral and intramuscular administration of enrofloxacin [74]. Lactation can influence significantly enrofloxacin's and ciprofloxacin's elimination by increasing two folds clearance in dairy cows comparative to steers [75]. It might be explained by an ionic trap.

4. Veterinary Medicinal Products

4.1. Available Veterinary Medicines

The first veterinary product based on enrofloxacin was launched by the laboratory Bayer in 1991 (marketing authorisation in 1991) under the trade name Baytril® and it was an oral form for poultry [3]. Now, many veterinary products on the basis of enrofloxacin are available on the market with at least thirty four veterinary medicines under different forms including oral and injectable forms, tablets or bolus [3]. There are many target species including domestic carnivores (dogs and cats), farm animals (cattle, pigs, poultry) and even exotic pets since 2010 [3].

4.2. Therapeutic Indications and Uses in the Field Conditions

Enrofloxacin is indicated in the treatment of local and systemic diseases caused by a wide range of Gram-negative and Gram-positive bacteria [4] [76]. The most important indication of enrofloxacin in all of the species is the treatment of respiratory infections but it is also indicated in the treatment of digestive, urinary, joint, genital, mammary and dermal infections [3] [77].

However, enrofloxacin is a third generation fluoroquinolone with a very large spectrum of activity so it has to be reserved to second intention. Indeed, in order to avoid fluoroquinolone resistances, it is important to reserve the use of enrofloxacin to infections resistant to over antibacterial agents and if possible under a susceptibility study [4].

In the field conditions, enrofloxacin is often off-label used empirically to prevent uterine infections in susceptible embryo-transfer mares. In fact, a conventional dose (5 mg/kg body weight) given pre-breeding followed by two further doses at 36 - 48 h post breeding are supposed to prevent bacterial adherence and provide effective bactericidal concentrations in utero [78].

Enrofloxacin is often used in aquaculture in Indonesia, Thailand and Vietnam. Indeed, because of the non-hygienic and stressful conditions in aquaculture facilities, the risk of bacterial infections is high and motivates the widely use of antibiotics in fish feed for prophylactic and therapeutic purpose [79].

5. Therapeutic Efficacy

Enrofloxacin is a powerful antimicrobial which have shown efficacy against a lot of bacterial diseases [4] [80]. The effectiveness of enrofloxacin against some bacterial infections in cattle, poultry, domestic carnivores (dogs and cats), rodents, lagomorphs and crustaceans has been assessed in many published studies, as well during natural infections as during experimental infections (**Table 7**). Among these studies, some relate to classical infections contained in the Summary of Product Characteristics (SPC) of veterinary products but some evaluate the efficacy of enrofloxacin in species for which there is no marketing authorisation or against specific bacteria such as *Anaplasma marginale* in cattle, *Ehrlichia canis* and *Brucella canis* in dogs, *Bartonella henselae* or *Bartonella clarridgeiae* and *Chlamydophila felis* in cats, *Toxoplasma gondii* in *Calomys callosus* or even *Vibrio harveyi* in Artemia franciscana (**Table 7**).

6. Adverse Effects

Overall, the fluoroquinolones are well tolerated with fewer adverse effects that are not very serious, especially when compared to their benefits [80] [81]. The most common side effects of enrofloxacin are digestive disorders including nausea, abdominal discomfort, vomiting and diarrhoea [81] [82] and inflammatory reaction at the site

Table 7. Twenty published studies examining the efficacy of enrofloxacin.

Target specie(s)	Disease(s) and/or bacterial agent(s)	Type of infection	Regimen of enrofloxacin	Purpose of the study	Number of animals	Result(s)	References
CATTLE							
Dairy cows	Acute clinical mastitis (*Escherichia coli*)	N	5 mg/kg IV then SC 24 h later	Comparative efficacy of a ENR + KET treatment and a KET treatment	132 (64 ENR + KET, no controls)	Inefficacy of ENR to treat acute clinical E. coli mastitis	[118]
Sahiwal cattle	Anaplasma marginale	N	5 mg/kg IV SID for 5 days	Comparative efficacy of ENR, OXY and IMI	60 (15 per group + 15 controls)	Inefficacy of ENR to clear persistent infection	[119]
Holstein calves	Anaplasma marginale	E	7.5 mg/kg in a single dose or twice every 3 days	Comparative efficacy of ENR (2 regimens) and LA-OXY	24 (6 per group + 6 controls)	Superiority of ENR	[120]
Calves	Pneumonic pasteurellosis (Pasteurella haemolytica A1)	E	2.5 mg/kg SC SID for 3 days	Efficacy of ENR	36 (12 ENR + 12 positive controls + 12 negative controls)	Inefficacy of ENR to treat experimentally induced pneumonic pasteurellosis	[121]
Feeder calves	Bovine respiratory disease (Mh, Pam, Hs, Mb)	N	12.5 mg/kg SC in a single injection	Comparative efficacy of ENR and TUL in two different states of USA	500 (125 per group per site)	Superiority of TUL	[122]
POULTRY							
Chickens	*Escherichia coli* (Ec) and Pasteurella multocida (Pam)	E	10 mg/kg BID in drinking water for 5 days	Efficacy of ENR (given immediately or 6 hours after infection)	Ec: 30 (10 per group + 10 controls) Pam: 20 (7 per group + 6 controls)	Efficacy of ENR against Ec and Pam	[123]
Broilers	Mycoplasma gallisepticum (Mg)	N	5 mg/kg in drinking water at 1 - 10 and 22 - 32 days of age	Efficacy of ENR in a treatment program against Mg in offspring of Mg-infected chicken-broiler breeders	45,000 (22,500 ENR + 22,500 controls)	Efficacy of ENR	[124]
Chicks (White Leghorn)	Salmonella enterica Serovar Typhimurium DT104	E	10 mg/kg for 5 days (1) or 25 mg/kg for 2 days (2) or 50 mg/kg for 1 day (3) (continuously or pulsed in water or gavage)	Efficacy of ENR (comparative efficacy of high dose short duration treatments and conventional treatment)	481 (151 in (1) + 141 in (2) + 89 in (3) + 100 controls)	Efficacy and best compromise for the 2-day 2.5 dosing treatment	[125]
Broilers (male chicks)	Colibacillosis (*Escherichia coli*)	E	50 ppm in drinking water for 7 days	Comparative efficacy of ENR and bacteriophage (individually and in combination)	320 (40 for each one of the 8 treatments)	Superiority of ENR but synergy between ENR and bacteriophage	[126]
Turkeys (poults)	Respiratory infections due to Ornithobacterium rhinotracheale (associated with avian metapneumovirus)	E	10 mg/kg in drinking water during 20 hours for 5 days or 50 mg/kg during 5, 10 or 20 hours in a single day	Efficacy of ENR (comparative efficacy of 4 regimens)	80 (16 per group + 16 controls)	Superiority of the 10 mg/kg 5-day ENR treatment	[127]

Continued

DOMESTIC CARNIVORES							
Dogs	Ehrlichiosis (*Ehrlichia canis*)	E	5 or 10 mg/kg PO BID for 21 days	Comparative efficacy of ENR (2 regimens) and DOX	13	Inefficacy of ENR to clear Ehrlichia canis infection	[128]
Dogs	Recurrent superficial (S) and deep (D) pyoderma	N	5 mg/kg PO SID continued up to 1 (S) or 2 (D) weeks after clinical recovery	Efficacy of ENR	9 (S) + 3 (D)	Efficacy, safety and convenience of ENR	[129]
Dogs	Uncomplicated urinary tract infections	N	18 - 20 mg/kg PO SID for 3 days	Comparative efficacy of ENR (high dose short duration treatment) and AMO-CLA	68 (35 ENR + 33 AMO-CLA)	Non-inferiority of a high dose short duration ENR treatment	[82]
Dogs	Brucellosis (*Brucella canis*)	N	5 mg/kg PO BID for 30 days	Efficacy of ENR for the eradication of Brucella canis in a kennel	12	Incomplete efficacy of ENR but safety use during gestation	[130]
Cats	Chronic bartonellosis (*B. henselae* and *B. clarridgeiae*)	N (25 cats) + E (18 cats)	22.7 mg PO BID for 14 or 28 days	Comparative efficacy of ENR and DOX (each one with 2 treatment durations)	43 (23 ENR + 17 DOX + 3 controls)	Efficacy of high dose long duration ENR treatment (4 to 6 weeks)	[131]
Cats	Conjunctivitis (*Chlamydophila felis*)	N	5 mg/kg SC SID for 3 days then PO SID for 11 days	Comparative efficacy of ENR and DOX	25 (14 ENR + 11 DOX)	Equal improvement of ENR and DOX in clinical signs and infection status	[132]
RODENTS							
Mice	Systemic infections (*Escherichia coli*)	E	5 mg/kg SC or PO	Efficacy of ENR (2 different routes of administration)	NP	Efficacy of ENR and superiority of the injectable form (SC)	[133]
Calomys callosus	Toxoplasmosis (*Toxoplasma gondii*)	E	3 mg/kg SC SID for 3 days	Comparative efficacy of ENR and SUL	15 (5 ENR + 5 SUL + 5 controls)	Efficacy of ENR as a potential alternative drug	[134]
LAGOMORPHS							
Rabbits	Pasteurella multocida (Pam)	N (11 rabbits) + E (12 rabbits)	5 mg/kg SC BID for 10 days	Efficacy of ENR in the elimination of Pam from asymptomatically infected rabbits	11 (N) + 12 (E)	Inefficacy of ENR to eliminate Pasteurella multocida	[135]
CRUSTACEANS							
Artemia franciscana (nauplii)	Vibrio harveyi (strain PN9801)	E	ENR 4h before infection (A) or 24 h after infection (B)	Comparative efficacy of ENR (2 regimens)	600	Efficacy of ENR to stop the course of a bacterial infection in Artemia franciscana	[136]

of injection for injectable forms, particularly in pigs [3]. However, some more serious adverse effects of enrofloxacin could appear targetting the juvenile joints, the reproductive system, the ocular system and the central nervous system.

The best known adverse effects of enrofloxacin concern the joints of young animals and result in arthropathy, articular cartilage degeneration, tendonitis and other forms of tendon injury [80] [81] [83] [84]. In order to try to understand quinolone related-arthropathy, a study was carried out in 1994 on juvenile New Zealand White Rabbits indicating that quinolones stimulate the cellular respiratory burst of immature articular chondrocytes which results in the production of oxygen-derived compounds highly toxic for the cartilage [83]. However the mechanisms underlying fluoroquinolones-induced tendinopathy and cartilage degeneration remained incompletely understood. Thus, further studies were necessary such as the study carried out in 2008 on canine Achilles tendon cells and chondrocytes suggesting that enrofloxacin-induced tendinopathy and cartilage damage could be explained by the inhibition of cell proliferation, induction of apoptosis and DNA fragmentation [84]. In young chickens, a study led in 2009 to determine the chondrotoxic effects of enrofloxacin on avian articular cartilage indicates that only very high dosage of enrofloxacin can induce toxic effects in articular cartilage of growing chickens and that the intensity of chondrotoxicity is dose- and time-dependent. Thus it is suggested that quinolone-induced arthropathy is far less expressed in birds than in mammals [85].

To assess the effects of enrofloxacin on adult joints, a study was carried out in 2000. The effects of long-term administration of an injectable enrofloxacin solution were evaluated by the monitoring of physical and musculoskeletal variables in adult horses. Adverse effects were only detected with high doses and consist of lameness, cellulitis, tendinitis, sheath effusion and even transient neurologic signs [86].

The adverse effects of enrofloxacin on the reproductive system were mainly investigated in males in order to assess the impact of this antibiotic on the fertility parameters. A study carried out in 2008 in male chickens suggested that enrofloxacin at therapeutical dose does not affect the sperm motility, the weight of testes, wattles and combs and the testicular concentration of testosterone, ascorbic acid, total protein and cholesterol [87]. On the opposite, a study led in the same year to evaluate toxic effects of enrofloxacin on sperm quality in male mice indicates that a fixed 150 mg/kg dose of enrofloxacin could lead to structural damages in the testicular tissue resulting in disruption of spermatogenesis in the testes with deterioration of motility, content and morphology of sperms [81]. Although the toxicity of enrofloxacin on the female reproductive tract was less investigated, a recent study on the use of intrauterine enrofloxacin infusion in healthy mares reveals acute effects including endometrial ulceration, necrosis and haemorrhage and chronic effects including fibrosis and inflammation [88].

The ocular toxicity of enrofloxacin has been suggested by the association of enrofloxacin, retinal degeneration and blindness in cats [89] [90]. A retrospective clinical study carried out to assess the possible connection between the administration of parenteral enrofloxacin and the onset of acute retinal degeneration in cats highlights the potential retinotoxicity of parenteral enrofloxacin which can result in acute and diffuse retinal degeneration, particularly with dosages exceeding 5 mg/kg once daily (which is the manufacturer's current dosage recommendation). Mydriasis and blindness were frequently observed but some cats may recover their sight [89].

At slightly higher doses, central nervous system signs of lethargy, anorexia and hypersalivation were observed in dogs as shown in a recent study carried out to assess a high dose short duration enrofloxacin protocol in dogs with uncomplicated urinary tract infections [82].

7. Residues and Toxicity for Consumers

In many animal species, the use of enrofloxacin lead to its de-ethylation to its primary metabolite ciprofloxacin and both enrofloxacin and ciprofloxacin would be found as drug residues in animal muscle and tissue [76] [91].

The consumption of meat containing these residues represent a significant threat against human health because it may result in disruption of the colonization barrier, development of drug-resistant bacterial strains or even allergies [91] [92]. In this regard, a recent study provides information on in vitro testing to determine if concentrations of veterinary antimicrobial agent residues entering the human colon remain microbiologically active [92]. Thus, the authorities have defined Acceptable Daily Intakes (ADI) of antimicrobial veterinary residues by human. The European Medicines Agency (EMA) have set the overall ADI value for enrofloxacin at 6.2 µg/kg body weight [92] [93] corresponding to the microbiological ADI because it is lower than the toxicological ADI of 12 µg/kg body weight which was calculated by applying a safety factor of 100 to the No Observed Effect Level (NOEL) of 1.2 mg/kg body weight per day [94].

In order to protect consumer's health, many countries have defined Maximum Residue Limits (MRL) of enrofloxacin and ciprofloxacin in animal-derived products [76]. In the European Union (EU), the MRLs of enrofloxacin and ciprofloxacin in muscle tissues and milk of all species are 100 µg/L [93] but as there is no MRL for enrofloxacin in eggs, enrofloxacin is forbidden in animals form which eggs are produced for human consumption [93].

To measure the enrofloxacin and ciprofloxacin residues in animal-derived foods including milk, eggs, honey and even mane and tail hair in horses, many analytical methods have been developed. Among these methods, we can mention High Performance Liquid Chromatography (HPLC); HPLC-Diode Array Detection method combined with liquid chromatography-mass spectrometry; HPLC-Ultraviolet Diode Array Detection method; molecularly imprinted solid-phase extraction procedure; liquid chromatography using a metal chelate affinity column; finally, the method of ChemiLuminescence Enzyme ImmunoAssay [76] [77] [91] [95]-[97].

8. Ecotoxicity

The release of enrofloxacin and ciprofloxacin in the environment is mainly due to the direct discharge of aquaculture products and the excretion in urine and feces of livestock animals [98]. It results in the contamination of soil, surface water, sediment, ground water and biota [99]. Once released into the environment the behavior of a chemical substance is determined by its tendency to partition from the aqueous phase to the atmosphere which is expressed by the Henry's Law Constant (HLC) and its affinity to adsorb on solid which is expressed by the Octanol-Water Partition Constant (Kow or log Kow) [99]. Enrofloxacin have a very low HLC at ambient temperature (<10 - 15 $atm \cdot m^3 \cdot mol^{-1}$) resulting in a negligible volatile loss and its Kow is low (0.83) while enrofloxacin have a high affinity for sludge, soils and sediments [99].

In the environment, enrofloxacin and ciprofloxacin can undergo degradations by different processes including photolysis, biodegradation and oxidation by mineral oxides but they are not sensitive to hydrolysis [99]. Despite these degradation mechanisms, environmental half-life time of enrofloxacin and ciprofloxacin are very long (half-life time estimated between 1155 and 3466 days for ciprofloxacin in a mesocosm soil study performed by Walters et al. in 2010, indicating an important persistence in soil matrices [100].

This long environmental persistence of enrofloxacin and ciprofloxacin can affect the growing or the activity of the soil microbial communities [101]. Indeed, although some edaphic organisms may utilize and decompose enrofloxacin as a nutrient, it is suggested that enrofloxacin or ciprofloxacin concentrations exceeding 0.2 mg/kg result in significant toxicity for the edaphon [101] [102]. Thus, edaphic ammonification and nitrification are affected and the edaphon community structure is modified, which can impact the soil fertility [102]. Furthermore, a quite recent study carried out in 2008 indicates that the exposure of whole earthworms and their different tissues to various concentrations of enrofloxacin can lead to changes in catalase activity and to a lesser extent, in growth rate [103].

The toxicity of enrofloxacin and ciprofloxacin for aquatic ecosystems has been assessed in many studies, as well on microorganisms as on algae or even aquatic vertebrates and invertebrates [104] [105]. Among these studies, three were carried out recently in zebrafish embryos [106], in tropical freshwater ecosystems with the monitoring of macro-invertebrates, zooplankton, phytoplankton, periphyton, bacteria, organic matter decomposition and nitrogen cycling [105] and in three tropical aquatic species (the green-algae Chlorella sp., the micro-invertebrate Moina macrocopa and the Nile tilapia Oreochromis niloticus) collected in a stream receiving effluents from a Pangasius catfish farm which uses enrofloxacin [104]. The results of these three studies are similar, suggesting that residual concentrations of enrofloxacin and ciprofloxacin in aquatic environment are not likely to result in direct or indirect severe toxic effects on aquatic ecosystems [104]-[106].

9. Conclusion

The fluoroquinolones are one of the most useful classes of antibiotics, as well in human medicine as in veterinary medicine. Thanks to their broad spectrum of activity against a wide range of bacteria and their physicochemical properties, their use is increasing. Enrofloxacin, which is a third generation fluoroquinolone only available in veterinary medicine, is thus used in many species with few adverse effects. However, there are recent concerns about the emergence of quinolone-resistant bacterial strains and the impact on the environment of the overuse of these drugs. Thus, there is now an important need to use fluoroquinolones with caution to preserve their effectiveness for many years. In veterinary medicine, it is essential to reserve these drugs for cases

requiring a powerful antibiotic and to prescribe and/or administer them only under a good clinical assessment and with appropriate regimens.

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Abbreviations

ADI: Acceptable Daily Intakes;
EMA: European Medicines Agency;
Gyrase: DNA Topoisomerase II;
G-DNA: Clived DNA Sequence;
HLC: Henry's Law Constant;
HPLC: High Performance Liquid Chromatography;
MAR: Multiple Antimicrobial Resistance;
MIC: Minimum Inhibitory Concentration;
MPC: Mutant Prevention Concentration;
MRL: Maximum Residue Limits;
PCR: Polymerase Chain Reaction;
NOEL: No Observed Effect Level;
PMQR: Plasmide-Mediated Quinolone Resistance;
QRDR: Quinolone Resistance-Determining Region;
SPC: Summary of Product Characteristics;
Topo IV: DNA Topoisomerase IV;
T-DNA: Transported DNA Sequence.