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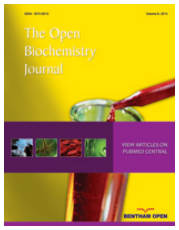

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REVIEW ARTICLE

Tetracyclines: Insights and Updates of their Use in Human and Animal Pathology and their Potential Toxicity

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Abstract:

Tetracycline antibiotics (TCs) have been widely employed to treat bacterial infections and other pathologic conditions in humans and pets. Although most of TCs have been almost ruled out from the human clinical practice they are still used as growth promoters and to treat promiscuity and overcrowding pathologies in the intensive animal farming. As a consequence, TCs are commonly found in all ecological compartments with potential direct or indirect toxicological effects on animals and, generally, on all living organisms. Moreover, clinical and *in vitro* observations raised the hypothesis that the widespread of some adverse food reactions and, to a less extent, antibiotic resistance phenomena could be ascribed to the presence of TCs residues in edible and non-edible tissues of intensive animal farming intended for animal and human consumption. Such residues may pose serious health threat, depending on the type of food and the amount of residue present.

The aim of this review is to provide new insights about the clinical uses of TCs in humans and animals and their potential toxic effects as residues in the environment or as food components.

Keywords: Tetracycline antibiotics, Therapeutic applications, Toxicological effects, Companion animals, Residues, Food components.

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1. INTRODUCTION

TCs, firstly discovered in 1940's, belong to a class of antibiotics known to inhibit protein synthesis in a wide range of gram-positive and gram-negative bacteria, chlamydia, mycoplasma, rickettsia, and protozoan parasites [1].

Based on their nature (natural or synthetic), dosage, elimination time and frequency of administration TCs can be divided into 3 main classes [2, 3]. Class 1 includes Demethylchlortetracycline (DMCT), Oxytetracycline (OTC), Tetracycline (TET) and Chlortetracycline (CTC), have a short duration of action and are used in zootechny; class 2 includes demeclocycline and methacycline and have an intermediate duration of action; class 3 includes newer drugs such as Doxycycline (DOXY), glycylcyclines and Minocycline (MINO) and have a long duration of action. These two latter TCs are mostly employed to treat pet's diseases.

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All TCs derivatives are crystalline and yellowish. They become characteristically fluorescent when exposed to ultraviolet light, which is an interesting property exploited to evaluate their accumulation in tissues. [4]. They are amphoteric, forming salts with strong acids (*e.g.* chloride) and bases (*e.g.* salts of sodium). The most common salt form is the hydrochloride, except for DOXY (hyclate or monohydrate). TCs are stable as dry powders but unstable in aqueous solution, particularly at a 7–8.5 pH range. TCs form poorly soluble chelates with bivalent and trivalent cations, particularly calcium, magnesium, aluminum, and iron. DOXY and MINO exhibit the greatest liposolubility and better penetration of bacteria such as *Staphylococcus aureus* than does the group as a whole. Preparations for parenteral administration must be carefully formulated, often in propylene glycol or polyvinyl pyrrolidone with additional dispersing agents, to provide stable solutions.

TCs can permeate through the bacterial cell wall by passive diffusion and through the cytoplasmic membrane [5, 6], inducing protein synthesis inhibition, blocking attachment of amino acid-bearing tRNAs to the 16S part of the

30S subunit of prokaryote ribosomes [7].

They are more effective at a slightly acidic pH of 6–6.5 against multiplying microorganisms where protein synthesis is essential. They enter microorganisms in part by concentration-dependent diffusion and in part by an active energy-dependent carrier-mediated system. This dual absorption system is essentially responsible for the high concentrations achieved in susceptible bacteria.

TCs can be administered orally, topically, intramuscularly and intravenously [8]. Generally, their absorption ranges from 25 to 60% and mainly occur in the stomach, duodenum and small intestine, with a C_{max} (mg/L) of 1–5 mg/L and a t_{max} of 2-4 h with the exception of demeclocycline whose C_{max} is delayed until 4-6 h [9, 10]. However, absorption can also occur in the uterus and udder, although plasma concentrations remain low [11].

TCs can bind plasma proteins with different degrees (*e.g.* OTC, 30%; TET, 60%; DOXY, 90%) however, once absorbed, they widely diffuse into all tissues (including placenta, ovaries, testes, endocrine glands, skin) and secretions (*e.g.* bile, synovial liquid, and pleural fluids) [12]. However, due to the high affinity for liver, spleen and bone tissues, they are not homogeneously distributed. Moreover, calcium, aluminum, and magnesium can form insoluble chelates with TCs and decrease bioavailability [13].

All TCs are eliminated through the kidney and the liver biliary-GI tract [10]. Generally, 50 to 80% are observed in the urine with variations due to age, route of administration, urine pH, glomerular filtration rate, renal disease, and the TET used. There is a secondary significant enterohepatic elimination pathway with excretion by the bile after some liver metabolization. Biliary path accounts for 10 to 20% of the elimination, even with parenteral administration.

TCs act as ionophores forming lipid-soluble complexes with divalent metal cations such as Ca^{++} and Mg^{++}, which they transport across hydrophobic barriers, such as biological membranes [3, 14]. If TCs help transporting calcium into the cell on one hand, this latter remains fixed in the bones for long periods being gradually released in the blood stream.

Due to their inexpensiveness TCs are widely used in the prophylaxis and therapy of human and animal infections but also as growth promoters in the intensive animal farming [1, 15, 16].

The widespread use of TCs raises the possibility that residues remain in edible tissues of livestock intended for animal and human consumption. Such residues may pose serious health threat, depending on the type of food and the amount of residue present [17].

The aim of this review is to provide new insights about the clinical uses of TCs in humans and animals and their potential toxic effects as residues in the environment or as food components

2. TCS IN HUMAN PRACTICE

TCs have been recognized a safe and cheap class of antibiotics present on the pharmaceutical market, a feature that made them attractive for developing countries. TCs have been placed among the three most used antibiotics worldwide [18 - 20] endowed with antibacterial (respiratory infections [19], malaria [19], *Entamoeba histolytica, Giardia lamblia, Leishmania major, Trichomonas vaginalis and Toxoplasma gondii* infections [1] and filarial nematodes [21]) and nonantibacterial activity:

2.1. Acne

Despite the worldwide rising concerns about antibiotic-resistant acne, TCs still represent the elective treatment of acne [22]. Different clinical trials aimed to establish the better tetracycline (TET, MINO, DOXY and lymecycline) and right dosage (40, 50, 100, 200, 300, 500 and 1000 mg), however, a lack of any significant difference in terms of efficacy between tested TCs was observed [23]. The main activity exerted by TCs in acne treatment is related to a reduction in neutrophil chemotaxis [24], sebum free fatty acids and extracellular lipases [25] as well as to an inhibitory effect on pro-inflammatory cytokines, MMP-9 and *Propionibacterium acnes* [23]. In particular this was observed for TET, MINO and DOXY [26, 27]. Serum samples from patients with acne treated with oral TET were challenged with zymosan and chemotaxis and random migration Polymorphonuclear Leukocytes (PMNLs) were investigated [24]. Both random migration and chemotaxis resulted significantly suppressed with the greatest effect at a daily dosage of 1 g, thus confirming the direct effect on neutrophil movement exerted by TCs.

2.2. Rosacea

Successful results have been also achieved with TCs for the treatment of rosacea, perioral dermatitis, ocular rosacea, and steroid-related rosacea [28, 29]. Along with aforementioned effects on the inflammatory condition, TCs are also supposed to inhibit angiogenesis (through MMP expression decrease) nitric oxide mediated vasodilation, down-regulate inflammatory cytokines and reduce reactive oxygen species levels [30, 31]. Such skin disorder can be treated topically or systemically, while in the first case metronidazole and TCs (TET, MINO and DOXY (0.5% or 2%), remain are the most common treatments, in the second one metronidazole is advisable [32]. Nevertheless, the effectiveness of systemic TET, MINO, DOXY and OTC in controlling papulopustular rosacea and even reducing erythema has been reported [33, 34].

Modified-release DOXY capsules (30 mg/d immediate-release and 10 mg delayed-release beads) were given for up to 12 weeks in 826 patients (235 males and 591 females) with papulopustular (subtype 2) rosacea [33]. Significant improvements in severity rating and erythema were observed in both groups with a treatment success of 73.2% in males and a 75.2% in females, while adverse events, *e.g.* primarily mild or moderate gastrointestinal events, occurred in 9.9% of males and 12.8% of females. Similar results were achieved with same treatment in a study conducted on 826 patients with skin of color (Fitzpatrick Skin Types [FST] IV-VI) [34].

2.3. Bullous Dermatoses

TCs, in particular TET and MINO, have been shown to be effective in bullous dermatoses, Bullous Pemphigoid (BP) [35 - 38], cicatricial pemphigoid [39], linear IgA disease [40 - 42], and lichen planus pemphigoides [43] treatment that are characterized by a splitting or dissolution of the basement membrane accompanied by an inflammatory reaction involving lymphocytes and neutrophils [26]. Despite the lack of studies aimed to warren the effectiveness of TET in combination with Nicotinamide (NAM) with respect to the two single drugs administered as monotherapy, this combination represented a valuable alternative to corticosteroids in the initial treatment of bullous dermatoses. This option might represent an advantage for older patients with concomitant osteoporosis, diabetes mellitus, or hypertension. Indeed, 4 case reports on elderly people with history of hypothyroidism and congestive heart failure, diabetes mellitus, mild pruritic blisters on the arms, legs, thighs, buttocks, and anterior aspect of the chest reported an significant improvements after a course of TET at different dosage, 1500 or 500 mg/d [35].

A combination of TET (500 mg/d) and NAM (500 mg/d) resulted particularly effective in the treatment of BP in 12 patients [36]. On the other hand, 6 patients were treated with Prednisone (PD). 5 out of 12 patients showed a complete response to combined therapy, while 5 had a partial response, 1 was unresponsive and 1 had a disease progression. Only 1 out of 6 patients treated with PD showed a complete response, while the other 5 had a partial response. Similar results were achieved in 7 patients with BP, who received 2 g TET combined with 2 g NAM daily for 7 months achieving a total remission within 6-8 weeks [37]. Nevertheless, BP has been also successfully treated with MINO as adjuvant therapy [38]. 6 out of 22 patients had a major response, 11 a minor response while 5 had no response. TET (2000 g/day) and niacinamide (NIAM) (1500 g/day) were also introduced as an alternative therapy to PD in a patient who showed gastric ulcers [39, 40]. After 3 weeks skin had completely cleared. Further, TET (2000 mg/day) and NAM (1500 g/day) was used to treat 13 patients with pemphigus vulgaris (n = 6), pemphigus foliaceus (n = 3), pemphigus erythematosus (n = 2) and linear IgA bullous dermatosis (n = 2), with or without oral corticosteroids [41]. After a mean follow-up period of 22 months 7 patients had a complete response, 4 a partial response while 2 failed to respond.

Oral TET (1000g/d) and NIAM (900 mg/d) were also successfully introduced in 2 patients with linear IgA bullous dermatosis [42]. After 2 weeks skin lesions resulted improved quickly and cleared and, after healing, milia formation and slight scarring were seen. Over maintenance period of 1 year on TET (500 mg/d) and NIAM (400 mg/d) patients showed no recurrence of pathology along with no adverse side effects. As to cicatricial pemphigoid treatment, Poskitt *et al.* described the effect of MINO (50 mg/d) in alleviating orodynia in 6 out of 7 patients and achieving a complete steroid withdrawal in 2 patients [39].

2.4. Cutaneous Sarcoidosis

Several reports shed light on the use of TCs in the treatment of cutaneous sarcoidosis [44 - 48]. Bachelez *et al*. firstly reported the usefulness of MINO (200 mg daily for 12 months) in a cohort of 12 patients [48]. 8 out of 12 patients showed a complete clearing of their lesions, while only 2 showed a partial response. Although no relapse occurred in the treatment period, the overall length of response ranged from 10 to 41 months. Few years later, a 45-year-old woman with a 7-year history of subcutaneous nodules unsuccessfully treated with corticosteroids underwent a complete remission after a 6-month course of DOXY 200 mg/d [47]. Unfortunately, there is also evidence of TCs, *i.e.* MINO, long-term treatment side effects (papillary thyroid carcinoma) [46]. On the contrary, a study confirmed the effectiveness of MINO in 20 out of 27 patients with moderately extensive lesions (maculopapular, nodular, or plaque lesions on the face, torso, and/or extremities), and 2 with severe ulcerative lesions (scalp) [45]. Further, a 35-year-old man with a history of pulmonary sarcoidosis, who developed raised plaques within tattoos, achieved a complete resolution of pruritus within 4 days and improvement of sarcoidal plaques within one week after a course of MINO 100mg/b.i.d., thus suggesting the antibiotic as a valuable therapeutic option for cutaneous sarcoidosis [44].

2.5. Kaposi's Sarcoma

Chemically modified TCs, *i.e.* COL-3 (6-dimethyl-6-deoxy-4-de(dimethylamino) tetracycline), were instead used in the treatment of Kaposi's sarcoma, a pathology characterized by endothelial cells proliferation and MMP activity increase [26, 49, 50]. A phase I trial involved 18 patients with AIDS-related Kaposi's sarcoma, who received 25, 50, or 70 mg/m2 of COL-3 per day for 25 weeks [50]. One out 18 patients patient showed complete resolution while 7 out 18 had partial improvement of skin lesions. The median therapy response time was 4 weeks and also MMP-2 serum levels resulted significantly reduced. Significant declines in MMP-2, and even in MMP-9, plasma levels were also observed in a phase II trial aimed to confirm and extend previous observations and to define more precisely the optimal drug dose [49]. Seventy-five patients were randomly divided in two groups that differed for the amount of COL-3 administered (group A received 50 mg and group B received 100 mg). The response rate in the group A was 41% while that of group B was 29%, thus placing COL-3 as a promising agent for the treatment of Kaposi's sarcoma.

2.6. Miscellaneous Dermatoses

Although the presence of literature reports concerning the treatment of pyoderma gangrenosum [51], hidradenitis suppurativa [52], Sweet's syndrome [53, 54], a_1-antitrypsin deficiency panniculitis [55], and chronic pityriasis lichenoides with TCs [56], well-controlled studies with a larger number of patients to warren the efficacy of such antibiotics are required. Piamphongsant *et al.* firstly described the usefullness of TC (2 g/d) in the treatment of pityriasis lichenoides [56]. After 6 months 5 out of 13 patients resulted completely cured, while 7 had to take the antibiotic 1 g/day for a further month to prevent relapse. On the other hand, MINO (100 mg b.i.d. for 6-12 months) was successfully treated 7 patients with pyoderma gangrenosum [51]. Further, DOXY was successfully intro-

duced 3 patients with recurrent panniculitis associated with alpha 1-antitrypsin deficiency due to its collagenase activity inhibition [55]. DOXY (100 mg b.i.d.) was also administered to 2 patients with Sweet's syndrome. After 2 weeks of treatment, lesions completely resolved [53]. It is worth noting that a patient Sweet's syndrome experienced an acute neutrophil dermatosis after systemic treatment with MINO [54]. The authors hypoyhesiszed a drug-induced condition after oral administration of TC and DOXY.

2.7. Rheumatoid Arthritis

Despite the unclear etiology of this pathology, a joint destruction and MMP-8 expression have been shown [57]. Many clinical trials described the significant improvements in patients with rheumatoid arthritis treated with MINO [58 - 60]. The efficacy of MINO (200 mg/d) was confirmed by a 48-week trial on 109 patients [58]. An overall improvement in joint swelling, joint tenderness, hematocrit, erythrocyte sedimentation rate, platelet count, and IgM rheumatoid factor levels were observed. MINO was also employed in the treatment of seropositive rheumatoid arthritis in the first year of disease [59]. 40 patients received MINO (100 mg b.i.d.) for 6 months. 18 out of 46 met 50% improvement criteria (modified Paulus composite criteria: morning stiffness, joint tenderness, joint swelling, ESR) at 3 months, and maintained at least a 50% improvement for 6 months with no significant drug toxicity. A similar result was observed in a 2-year study conducted on 60 patients who were also administered low-dose prednisone [60]. Patients were more likely to achieve a 50% improvement response along with a less requirement of prednisone.

2.8. Scleroderma

Literature reports are quite contradictory concerning the use of MINO in the treatment of this pathology [61, 62]. Indeed, in a first open trial 11 patients started receiving MINO (50 mg/b.id.) for 1 month and, after this period, it was increased up to 100 mg/b.i.d. Total skin score (TSS, 0 = normal, 1 = thickened skin, 2 = thickened, unable to move, and 3 = thickened, unable to pinch) and visual analogue scale (VAS, 0 cm = could not be better, 10 cm = could not be worse) were assessed before and at the end of the trial. After 1-year evaluation 4 out of 11 patients had a complete resolution of their disease with a final TSS score of 0 and in 3 of these patients VAS scores improved to 0. In 2 out of 11 patients no improvement in TSS was observed while only 1 of these had a significant improvement in VAS scores.

Conversely, results observed in 31 patients enrolled in an open-label multicenter trial showed no significant difference in TSS and modified Rodnan skin thickness score with respect to subjects treated with D-penicillamine.

2.9. Cancer

Tumor cell adhesion, extracellular matrix proteolysis, and cell migration are the main steps occurring in carcinogenesis, while basement membrane and surrounding connective tissue stroma degradation are key features of cancer invasion and metastasis along with MMP-2 and MMP-9 expression [26, 63, 64]. DOXY, MINO and CMTCs have been shown to inhibit MMPs activity and reduce cell proliferation [65 - 67]. Several clinical trials investigating the use of TCs in cancer treatment have been conducted [68 - 72].

A phase I clinical trial in 2001 aimed to establish the maximum-tolerated dose and dose-limiting toxicity of COL-3 in 30 patients with different cancer types [68]. Besides the recommendation for a dose of 36 mg/m2/d, authors reported disease stabilization for periods of 26+ months, 8 months, and 6 months in hemangioendothelioma, Sertoli-Leydig cell tumor, and fibrosarcoma, respectively.

DOXY (100 mg/b.i.d. orally, for 3 weeks), on the other hand, was also employed in 9 patients with ocular adnexal lymphomas, 4 due to *Chlamydia psittaci* (*Cp*) infection [69, 70]. After 1 month of DOXY assumption, *Cp* was no longer detectable in all 4 patients. Despite 7 out 9 patients responded to therapy, a complete response was observed in 2 out of 9 patients, while 2 other patients had a partial response and 3 a minimal response. Duration of response was 12+, 29+, 31+, 8+, 7+, 2+, and 1+ months, respectively. The same authors reported the usefulness of DOXY also in 27 patients (15 newly diagnosed and 12 having experienced relapse) with ocular adnexal MALT lymphoma and Cp infection [71]. 11 out of 27 patients were *Cp* DNA-positive and 16 negative. All patients received DOXY at a dosage of 100 mg/b.i.d. orally, for 3 weeks. At a 14-month follow-up lymphoma regression was complete in 6 patients, a partial response in 7 patients. Both Cp DNA-positive and negative patients experienced a regression of the lymphoma. Also previously irradiated and nonirradiated patients belonging to relapsed patients group showed a response to therapy. The 2-year failure-free survival rate of patients was 66% and 20 of the 27 patients were progression free.

A case report of a 66-year-old woman with lymphangioleiomyomatosis clearly showed the efficacy of DOXY (initial dose of 20 mg/day and after 1 month up to 100 mg/day for 6 months) as a promising therapy for such disease [72]. A clear improvement in the quality of life as well as increased lung capacity and oxygen saturation were observed along with MMPs urinary levels decrease.

COL-3, a chemically modified TC, was administered at a dosage of 50 mg/m2/d for 8 weeks to 15 patients with advanced and/or metastatic soft tissue sarcoma [69]. Despite de overall tolerability of the drug no objective responses were reported and 5 patients experienced disease progression.

2.10. Cardiovascular Diseases

MMPs production is known to occur before aortic wall elastin degradation, a condition that characterizes abdominal aortic aneurysm [26]. Patients treated with DOXY (100 mg p.o. bid) before abdominal aortic aneurysms surgical intervention showed a 3-fold reduction in aortic wall expression of MMP-2 and a 4-fold reduction in MMP-9 with respect to those untreated [73]. DOXY (150 mg daily) was also used to treat patients infected by *Chlamydia pneumoniae* with aneurysms of 30 mm [74]. Ultrasound investigations accomplished during an 18-month period evidenced a lower expansion rate of the aneurysm. Although DOXY had no clear effect on *Chlamydia*

pneumoniae immunoglobulin G antibody titers, C-reactive protein levels were significantly lower after 6 months.

Further, patients with acute myocardial infarction showed a ≥ 10% reduction in PMNLs activity after 30 and 60 minutes after DOXY administration, thus indicating its effectiveness in reducing myocardial cell damage [75]. A randomized single-blinded open-label study from Srivastava *et al*. recently highlighted an improvement in National Institute of Health Stroke Scale, modified Rankin Scale and modified Barthel Index score in acute ischemic stroke patients receiving MINO (200 mg/day for 5 days) at day 30 and 90 as compared with the controls [76]. These results clearly suggested the use of MINO in reducing acute ischemic stroke-related clinical deficits.

2.11. Periodontal Disease

The condition of periodontal inflammation is characterized by an increased apical proliferation and migration of gingival sulcular epithelial cells, periodontal microbial pathogens, and eventual destruction of collagen in the gingival, periodontal ligament, and alveolar bone causing an irreversible loss of tooth attachment [26, 77]. An *in vivo* study clearly showed the expression of MMP-2, -7, -8, and -13 in gingival sulcular epithelium [77], which resulted inhibited by DOXY and CMTCs [78]. A clinical study showed significant improvement in tooth attachment as well as reduction of pocket depth and bleeding after submicrobial dose of DOXY (20 mg/b.i.d.) [79]. Interestingly, a tetracycline-loaded absorbent membrane along with scaling and root planning resulted in a better prognosis in the reduction of periodontal pockets of 12 patients after only 28 days of evaluation [80].

A further trial aimed to combine a sub-gingival debridement with a three-month regimen of sub-antimicrobial-dose of DOXY (SSD), or with a two-week regimen of DOXY (ADD) or with placebo in a pool of 45 patients with long-standing type 2 diabetes and untreated chronic periodontitis [81]. Clinical measures of periodontitis resulted decreased after 1 and 3 months, moreover, mean HbA1c levels in the SSD resulted reduced of 0.9% units with an overall improvement of 12.5%. No significant change in HbA1c was observed in ADD and placebo group. Such results provided a new point of view for the use of DOXY as parallel treatment of periodontitis with sub-gingival debridement and as hypoglycemic agent.

2.12. Brain Diseases

Besides DOXY, MMP-9, a protein expressed also in Fragile X syndrome, resulted pharmacologically down regulated after treatment with MINO [82]. The treatment also induced the rescue of immature dendritic spine morphology and a significant improvement of abnormal behavior. Due to its excellent penetration into cerebrospinal fluid, established neuroprotective and antiviral properties MINO was also successfully used in the initial management of Acute Encephalitis Syndrome (AES) [83]. 281 patients beyond 3 years of age, who were hospitalized with AES of ≤ 7 days, were randomly assigned to receive either nasogastric/oral MINO or placebo suspension. Despite the lack of significance in 3-month mortality between MINO and placebo group, encouraging trends in patients older than 12 years and in Glasgow Outcome Score were observed. These trends were even accentuated excluding patients who died within one day of reaching hospital.

Due to its beneficial effects observed in various neurologic disorders [84, 85], it has been also proposed the use of MINO in the treatment of schizophrenia [86]. Possible action mechanisms are the inhibition of nitric oxide synthase and blocking of nitric oxide-induced neurotoxicity, although effects on the dopaminergic system and microglial activation inhibition have been hypothesized. 54 out of 70 patients with early-phase schizophrenia were received a MINO dosage of 200 mg/d and were previously treated with risperidone, olanzapine, quetiapine, or clozapine 200-600 mg/d. Few adverse events were reported while beneficial effects on negative symptoms and general outcome as well as on executive functions such as working memory, cognitive shifting, and cognitive planning.

3. TETRACYCLINES IN VETERINARY PRACTICE

As previously mentioned, TCs, in particular OTC and DOXY, are nowadays widely employed to prevent disease, reduce morbidity and mortality and increase overall development in production animals [1, 87, 88]. The improvement in daily growth rates resulting from the use of growth promoters was evaluated between 1 and 10 per cent, with more meat, less fat and increased protein content [89].

On a world scale, the use of antibiotics as animal growth promoters differs dramatically but is still frightening [90]. 25% of total antimicrobials were used in the pre-starter and starter phases, while the rest was used in the grower and finisher phases.

However, some countries like the EU forbid the use of antibiotics for growth promotion purposes, while the USA still uses and allows a wide range of antibiotics. To justify the use of antibiotics, the Animal Health Institute of America (AHI) estimated that, without the use of growth promoting antibiotics, the USA would require an additional 452 million chickens, 12 million pigs and 23 million more cattle [91]. Indeed, mortality rates associated with scouring and proliferative enteritis are 10-15 per cent lower for countries using antibiotics compared with countries that do not use antimicrobial growth promoters. In poultry, antimicrobial growth promoters (*i.e.* TCs) also control infections (*i.e. Clostridium perfringens*), which are potentially fatal, in addition to improve feed conversion efficiency. It was estimated that only this effect translated into an improvement of 1.5 per cent, with added economic benefits from just the reduction of *C. perfringens* infections without even considering other diseases [92]. In addition, it is worth noting that TCs have been employed also as healing agent for digital dermatitis treatment in dairy cattle [93]. For those reasons, pigs, livestock and poultry are still exposed to a huge range of antimicrobials while the long term effect on the environment, besides antibiotic resistance [94, 95], or effects, when consumed by the animal or human species, on the overall individual health status have been partially investigated. As annual production and consumption increase, increasing volumes of antimicrobials will be used each year if nothing is done to reduce their usage with a consequent increase of antimicrobial resistant organisms. Indeed, a 2014 study

conducted on animal feed (fish, poultry, swine and shrimp) feed marketed in Costa Rica revealed worrisome concentrations of TCs along with great abundance of OTC-resistant bacteria from the genera *Staphylococcus* and *Bacillus* [96]. In particular, fish feed contained the highest amounts of TCs (119–8365 mg/kg), poultry (78–438 mg/kg), swine (41–1076 mg/kg) and shrimp (21.5– 50.3 mg/kg) with an OTC MIC_{50} > 256 μg/ml for fish and poultry feed and 192 μg/ml for swine. In this regard clinical studies were conducted to establish the rational dosage for some TCs against resistant bacteria [96, 97].

Conversely, to give an idea of the manifold ways through which TCs are daily delivered by human and pet food, it is noteworthy to mention a 2009 report conducted on 24 commercially available chicken drumsticks [98]. All samples showed important concentrations of OTC and TET ranging from 83.0 to 2049.3 μg/kg and from 197.8 to 2564.3 μg/kg, respectively. A further study conducted by Nonga *et al*. on commercial chicken eggs sold by smallholder farmers in Tanzania confirmed the presence of OTC in all analyzed samples and interestingly revealed the lack of awareness of the farmers concerning the potential toxic effect of such compound on human health [99]. In 2014, Odore *et al*. firstly conducted a pilot trial aimed to clearly establish the amount of OTC present within the bone of broiler chickens reared according appropriate withdrawal times [100]. OTC concentration was far below the established maximum residue levels (100 μg/kg) in the muscle while resulted about 100-fold higher in the bone, posing a serious treat for the health of animals and humans that daily assume amounts of chicken and chicken byproducts, including bone, through pet food and würstel sausages possibly fostering chronic inflammatory phenomena [101, 102].

For instance, such inflammatory phenomena may manifest with clinical symptoms such as drooling, back and neck intense itching, neck eczema, chronic conjunctivitis and stomatitis, which are generally regarded as adverse food reactions [101]. In 2017, a clinical study firstly posed a possible link between the onset of these cutaneous reactions and a chronic intake of a chicken-based diet in a pool of 18 cats [101]. The authors performed an Enzyme-linked immunosorbent assay (ELISA) to evaluate the presence of OTC in the sera of the animals before and after a 60-days nutraceutical diet supplementation (without chicken or chicken byproducts). Interestingly, a significant decrease of serum OTC along with a significant improvement of clinical symptoms was observed at the end of the study. This observation posed a serious concern on an anything but hypothetic pro-inflammatory effect exerted by OTC assumed by a daily intake of contaminated food. This latter consideration was confirmed by Di Cerbo *et al.* who conducted a clinical evaluation on 8 dogs consuming chicken-based commercial diets correlating the appearance of symptoms such as otitis, diarrhea, generalized anxiety and dermatitis with the presence of OTC (19.0 ± 3.7 μg/kg) and calcium, aluminum, silicon, and phosphorous nanoparticles in the kibbles [15]. ELISA revealed also a significant reduction of serum concentration of OTC in all dogs, from 0.22 to 0.02 μg/mL, after 15 days of organic chicken-based diet supplementation, which was associated with an overall significant reduction of the symptoms. Interestingly, OTC serum concentration resulted quite below the cytotoxic concentration observed *in vitro* for its powder and liquid form.

4. POTENTIAL TOXIC EFFECTS OF TCs

Several studies highlighted the effects of the OTC on the immune system [100, 103 - 106], on reactive species and pro-inflammatory molecules (cytokines, myeloperoxidase, malondialdehyde, superoxide dismutase, catalase and glutathione peroxidase) [103, 105, 107, 108], transcription of the cytochrome P-450 [104, 109] and even on DNA [110].

First experiments carried out with TCs revealed a cytostatic effect through the inhibition of mitochondrial protein synthesis [111]. However, since then, further studies showed contradictory results on haemopoietic cells. For instance, Van de Bogert *et al*. reported a T cell proliferation inhibition exerted by OTC while Potts and coworkers observed an anti-proliferative effect on human PBMCs only for DOXY, demeclocycline, methacycline and MINO [112, 113]. Moreover, as with zymosan, DOXY, lymecycline, CTC, MINO and DMCT were able to induce an increase in oxygen consumption in PMNLs once exposed to UV light [114]. Zymosan, but also bacteria, were used to challenge bovine blood mononuclear cells and neutrophils pretreated with OTC [103]. An antibacterial activity inhibition of neutrophils at high concentrations of OTC (500-1,000 μg/ml) and a decreased peroxidase activity at a concentration of 15 μg/ml were observed. Conversely, bovine blood mononuclear cells proliferation resulted inhibited at an OTC concentration of 100 μg/ml, indicating the greater sensitivity of these latter with respect to neutrophils. Besides the countless evaluations aimed to investigate the effect of TCs challenge with immune system, adverse inflammatory effects, due to persistence of these antibiotics in the environment, were also evaluated [109]. Interestingly, zebrafish larvae exposed to OTC for a period of 24 to 96 hours developed a widespread inflammation and showed an improved regeneration capacity in the mechano-sensory system lateral line. Experimental fish were also investigated for OTC-induced oxidative stress evaluation [104]. Yonar *et al*. reported a significant increase in the malondialdehyde level, and a decrease in superoxide dismutase, catalase, and glutathione peroxidase activity of OTC-treated rainbow trout. The authors also observed a decrease in the glutathione level and a significant increase in glutathione-S-transferase in the blood, liver, kidney and spleen of the animals. Further, specific and nonspecific immune system parameters including haematocrit, leucocyte count, oxidative radical production, total plasma protein and immunoglobulin levels and phagocytic activity appeared to be suppressed by the antibiotic.

In 2015, for the first time, a study evaluated the effect of a bone derived by intensive farming chickens treated with OTC on two hematopoietic cell lines, K562 cell and PBMCs [100]. Chicken bone meal is known to be one of the constituents of pet food and, to a less extent, of human food [16, 101, 115]. Results evidenced a significant pro-apoptotic effect of the bone meal on both cell lines at 48h of incubation at different dilutions. However, further experiments on K562 cells challenged with OTC for different incubation times, showed

apoptotic features (*i.e.* blebs formation) already after 12h of incubation by means of Micropipette Aspiration technique [106].

The same bone meal was used to assess its possible pro-inflammatory effects on human PBMCs [105]. A significant interferon-gamma (IFN)-γ secretion from T lymphocytes and non-T cells was observed in the first 10–12 h of cell exposure, paving the way for the hypothesis that OTC-enriched bone might be responsible for a tissue inflammatory spreading or of immune-mediated diseases. These results firstly suggested new potential health risks for humans and pets depending on the entry of TCs in the food supply chain.

Later, Gallo *et al*. shed light on molecular mechanisms related to OTC toxicity on human PBMCs [110]. They observed the expression of DNA damage features including ATM and p53 activation, H2AX histone phosphorylation and modifications of histone H3 methylation of lysine K4 in the chromatin, which were linked to a marked inflammatory response as evidenced by an increased expression of IFN-*γ* and type 1 superoxide dismutase (SOD1).

Recently, cell viability and HPLC-ESI/QqToF assays confirmed the cytotoxic effect of bone derived by intensive farming chickens treated with OTC at 24, 48 and 72h of incubation but ruled out the possible release of OTC and its derivatives from the bone until a concentration of 1 μg/mL [116].

CONCLUSION

Presence of antibiotic residues in carcasses poses a health risk to consumers in terms of antibiotic resistance, teratogenicity, carcinogenicity, endocrine disruptors, hepatic and renal failure. However, embryonic, fetal, chronic or short term and long term immune effects have not been seriously considered. To prevent those problems and safeguard health, the European Union and more recently the USA have fixed Maximum Residue Limits (MRLs) of those compounds in livestock products [117, 118]. On the other hand, very few studies described the fate and toxicity of TCs in the environment, which are omnipresent in most of ecological compartments [119] and are far to be biodegradable once released in the environment. Moreover, since humans and animals do not fully absorb or metabolize those pharmaceuticals, they excrete a significant fraction of such drugs (from 30 to 90%) or of their breakdown products in the environment *via* feces or urine [120, 121], which in turn enter in the environment through the direct discharge of animal wastewater and the discharge of effluents from wastewater treatment plants [122, 123]. Up-to-now, wastewater treatment plants are not capable of removing effectively TCs. They have been detected in USA surface water samples at concentrations of 0.11, 1.34 and 0.15 μg/L, for TET, CTC and OTC, respectively [124] and residual concentrations of TET from 0.15 to 0.97 μg/L were detected in Canada [125] while up to 2.37 μg/L were detected in the final effluent from wastewater treatment plants in the USA [126]. TCs have been found also in sewage sludge, that represents one of the major routes of spread of those compounds in the environ-ment [127]. Further, they can accumulate within organic manure in soil. In most investigations, residual concentrations of TET, CTC and OTC were detected in soil with amounts up to 2.683 mg/Kg, whereas residual concentrations of up to 183.5 mg/kg were detected in manure [128]. In Table **1** human and veterinary clinical application of TCs as well as their potential toxic effects have been summarized.

Table 1. Human and veterinary clinical application and potential toxic effects of TCs.

TETRACYCLINE KIND	HUMAN CLINICAL PURPOSE	VETERINARY CLINICAL PURPOSE	POTENTIAL TOXIC EFFECTS
Doxycycline	*Toxoplasma gondii* infection [1], respiratory infections [19, 129], malaria [131], acne [27], rosacea [29, 33, 34], cutaneous sarcoidosis [47], Sweet's syndrome [53], a_1-antitrypsin deficiency panniculitis [55], leukemia [65, 67], ocular adnexal lymphomas [70], ocular adnexal MALT lymphoma [71], lymphangioleiomyomatosis [72], abdominal aortic aneurysms [73], *Chlamydia pneumonia* infection with aneurysms [74], acute myocardial infarction [75], periodontal disease [78, 79], long-standing type-2 diabetes and untreated chronic periodontitis [81]	filarial nematodes [21, 130], *Leishmania major* infection (Leishmaniasis) [131], disease prevention, morbidity and mortality reduction and growth promotion [87, 88]	DNA synthesis reduction [112], polymorphonuclear leukocyte oxygen consumption reduction [114], environmental, manure, soil, surface waters, groundwater and wastewater pollution [119, 123 - 125]
Achromycin	malaria [19, 132]		
Oxytetracycline	*Entamoeba histolytica* infection (Amebiasis) [133, 134], *Trichomonas vaginalis* infection (Trichomoniasis) and *Leishmania major* infection (Leishmaniasis) [135], *Giardia lamblia* infection (Giardiasis) [133, 135], food intolerances induction [115]	*Giardia lamblia* infection (Giardiasis) [133], disease prevention, morbidity and mortality reduction and growth promotion [87, 88, 127], adverse food reactions induction [15, 101]	pro-apoptotic effect [100, 106], pro-inflammatory effect [105, 107, 109], genotoxic effect [110], neutrophils and mononuclear cells modulation [103], oxidative stress and immunosuppression induction [104], toxicity against catalase [108], villous epithelium damage [111], T lymphoid cells division inhibition [113], cytotoxic [116], environmental, manure, soil, surface waters, groundwater pollution [119, 123, 124, 128]

(Table 1) contd.....

TETRACYCLINE KIND	HUMAN CLINICAL PURPOSE	VETERINARY CLINICAL PURPOSE	POTENTIAL TOXIC EFFECTS
Tetracycline	*Giardia lamblia* infection (Giardiasis) [133, 135], filarial nematodes [136], acne [24], rosacea [28], bullous pemphigoid [35 - 37, 40 - 42], suppurative hidradenitis [52], chronic pityriasis lichenoides [56], metastatic prostate cancer [66]	filarial nematodes [21], *Giardia lamblia* infection (Giardiasis) [133], *Leishmania major* infection (Leishmaniasis) [131], digital dermatitis [93]	environmental, manure, soil, and surface waters, groundwater and wastewater pollution [120, 123-124-125]
Thiatetracycline	*Giardia lamblia* infection (Giardiasis) [134]	*Giardia lamblia* infection (Giardiasis) [134]	
Minocycline	*Toxoplasma gondii* infection [1], acne [26], cicatricial pemphigoid [38, 39], cutaneous sarcoidosis [44 - 46], pyoderma gangrenosum [51], rheumatoid arthritis [58 - 60], scleroderma [61, 62], acute ischemic stroke [76], fragile X syndrome [82], acute encephalitis syndrome [83], schizophrenia [86]	filarial nematodes [137]	groundwater and surface water pollution [124]
COL-3	Kaposi's sarcoma [49, 50], refractory metastatic cancer [68, 69]		
CMT-1, -3, and -5	periodontal disease [78]		
Demethylchlortetracycline			polymorphonuclear leukocyte oxygen consumption reduction [114]
Chlortetracycline		growth promotion [88, 89, 127]	manure, soil, surface waters and groundwater pollution [123, 124, 128]
Meclocycline			manure, soil, surface waters and groundwater pollution [123, 124]

Besides the contradictory results achieved with TCs we retain that their abuse and the consequent introduction into the environment can lead to serious environmental problems including ecological risk, animal and human health damage and even antibiotic resistance.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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