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Review

Antimicrobials in beekeeping

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ABSTRACT

The bee diseases American and European foulbrood and nosemosis can be treated with anti-infectious agents. However, in the EU and the USA the use of these agents in beekeeping is strictly regulated due to the lack of tolerance (*e.g.* Maximum Residue Limit) for residues of antibiotics and chemotherapeutics in honey.

This article reviews the literature dealing with antimicrobials of interest in apiculture, stability of these antimicrobials in honey, and disposition of the antimicrobials in honeybee hives.

Contents

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1. Introduction

For many centuries, honeybees have been domesticated in artificial hives for the production of honey that has been used as an important carbohydrate source and food sweetener. Like all living organisms, honeybees can be infested with diseases and pests. Some diseases can be treated by antibiotics or chemotherapeutics. This review article provides an overview of antimicrobials of interest in beekeeping, stability of these antimicrobials in honey, disposition in the beehive and the legislation regarding the use of these antimicrobials in treatment of bee diseases.

2. Bee diseases and pests that can be treated with antimicrobials

2.1. American foulbrood

American foulbrood (AFB) is by far the most virulent brood disease known in honey bees. The disease is caused by the sporeforming bacterium, *Paenibacillus larvae*. It attacks older larvae and young pupae, which are digested by enzymes secreted by the bacterium. The comb has a speckled (pepper box) appearance where infected larvae have been removed. Cappings may appear moist, sunken, and perforated. Initially the dead larvae are slimy, and dry to form brown scales that are highly infective (Genersch, 2010a). Because the spores can remain viable for years, many countries require bee colonies with AFB to be burned. Other countries (*e.g.* USA, Canada, Argentina) allow the use of antibiotics to keep the disease in control. Antibiotics can only mitigate, but will not eliminate the disease and therefore infected hives must be treated constantly to prevent a foulbrood outbreak. Left untreated, foulbrood destroys the hive's bee population and can annihilate an apiary. If the infection is moderate without clinical symptoms, a shook swarm method of treatment is recommended (von der Ohe, 2003). Other authors have suggested the use of essential oils to control the disease (Albo et al., 2003).

2.2. European foulbrood

European foulbrood (EFB) is closely related to AFB in symptomatology. However, the causative organism, the bacterium *Melissococcus plutonius*, does not form spores, and therefore the disease is considered less problematic than AFB. The bacterium generally attacks only younger larvae in uncapped cells. Although EFB had previously been successfully controlled by sanitizing measures or requeening with a more resistant stock, these methodologies are now proving to be ineffective (Genersch, 2010b). Some antimicrobials, for example oxytetracycline, have been demonstrated to be an effective treatment of EFB. Additionally, the combination of shook swarm plus oxytetracycline is applied in order to get a lower level of EFB recurrence (Waite et al., 2003).

2.3. Nosemosis

Nosemosis, caused by *Nosema apis* or *N. ceranae*, is by far the most damaging adult bee disease. Infections are acquired by the uptake of spores during feeding or grooming. *Nosema apis* infects the epithelial cells of the hind gut (*ventriculus*) of the digestive tract of the adult bee, giving rise to large numbers of spores in a short period of time and impairing the digestion of pollen which shortens the life of bees. High incidences of *Nosema* are directly related to stress, such as periods of long confinement or nutritional imbalance (Webster, 1993). *N. ceranae* was originally a parasite of the Asian honey bee (*Apis cerana*) but now is widespread in some European regions. Adult bees are affected and this results in depopulation and bee colony losses (Higes et al., 2006). Until recently, *N. apis* was considered to be a spore-forming microsporidian, a single-celled protozoan, but is now classified as fungus or fungi-related (Fischer and Jeffrey, 2005).

2.4. Use of antibiotics and chemotherapeutics in beekeeping

In the EU, honeybees are classified as food producing animals. Therefore, establishment of a Maximum Residue Limit (MRL) for honey is necessary before a marketing authorization for a veterinary medicinal product can be granted. In view of the lack of metabolism in the beehive, an elimination of residues over a certain period of time as defined for other food producing species, does not occur. So, in principle only medicinal products which do not result in residues in honey after use (zero day withdrawal period)

can be authorized for bees as indicated by the Committee for Medicinal Products for Veterinary Use (CVMP). So far, no MRLs have been established for antibiotics and sulfonamides in honey (Commission Regulation (EU) No. 37/2010 and amendments), theoretically meaning that the use of antibiotics in beekeeping is not permitted in the EU. The resulting 'zero tolerance' policy for antibiotic residues causes significant trade problems. Harmonized rules do not exist with regard to acceptable control methods, limits of detection, or sampling methods, resulting in different interpretations by EU Member States. In the absence of either EU MRLs or reference points for action (RPAs), the presence of any detectable (and confirmed) residue in honey imported into the EU would mean that those consignments could not legally be placed on the market in the EU (Regulation (EC) No. 470/2009). As stipulated in Annex II of Council Directive, 2001/110/EC, honey must, as much as possible, be free from organic or inorganic matter foreign to its composition. In view of this, the European Federation of Honey Packers and Distributors (FEEDM) requested the establishment of RPAs in order to allow for control of honey imported from non-EU countries. In the meantime, some EU Member States (Belgium, France, UK) and Switzerland have established action limits, recommended target concentrations, non-conformity, or tolerance levels. The EC Reference Laboratory (Anon., 2007b) proposed recommended concentrations for screening for antibiotics and sulfonamides in honey for national residue control plans established in accordance with Council Directive 96/23/EC. An overview of the limits applied is given in Table 1. At present, in the UK, instead of fixed reporting limits (Anon., 2003) concentrations above the decision limit (CC_α, Commission Decision 2002/657/EC) of the confirmatory method are now reported as non-compliant. Since January 1, 2009 Switzerland stopped the use of tolerances for residues of streptomycin, tetracyclines, and sulfonamides in honey.

The regulatory limit for certain prohibited or unauthorized analytes in food of animal origin is the Minimum Required Performance Limit (MRPL) or the Reference Point for Action (RPA). MRPL is defined as the minimum content of an analyte in a sample, which must be detected and confirmed by the laboratories. MRPLs are foreseen in Article 4 of Commission Decision 2002/657/EC in order to provide harmonized levels for the control of those substances to ensure the same level of consumer protection in the Community. So far MRPLs were set for chloramphenicol (0.3 $\mu g\,kg^{-1}$ in honey), medroxyprogesterone acetate, nitrofuran metabolites (furazolidone, furaltadone, nitrofurantoin, and nitrofurazone) (1 $\mu g\,kg^{-1}$) in poultry meat and aquaculture products, but generally also considered as applicable in honey), and (leuco)malachite green (Commission Decisions 2003/181/EC and 2004/25/EC).

Despite the lack of MRLs for anti-infectious agents in honey, antibiotics and chemotherapeutics could be used in the EU in apiculture based on the 'cascade' system as described in Article 11 of Directive, 2001/82/EC, as amended by Directive 2004/28/EC. The cascade system was introduced to solve the general problem of availability of veterinary medicinal products for minor species. The cascade system is open to all animal species, including honeybees (Anon., 2007a), provided that the active substance concerned has been included in Annex I, II or III of Council Regulation (EEC) No. 2377/90 (recently repealed by Table 1 in the Annex of Commission Regulation (EU) No. 37/2010) and the prescribing veterinarian specifies a withdrawal period. Hence, the use of oxytetracycline is allowed in the UK under the cascade for the treatment of EFB with a withdrawal period of at least 6 months (Anon., 2010a). In France, the treatment of AFB with antibiotics is accepted, provided that the disease is not yet largely developed and the honey and wax are destroyed afterwards (Anon., 2005).

In the USA, antibiotic drugs authorized for treatment of bees include oxytetracycline, tylosin, and bicyclohexylammonium fumagillin. However, the use of these antibiotics

Table 1
Limits (in $\mu g\,kg^{-1}$) for antibiotics and chemotherapeutics in honey in various European countries.

Antibiotic or chemotherapeutic	Limits applied in various countries (in $\mu g\,kg^{-1}$)			
	Belgium		France	EU
	Action limit[a]/MRPL	Proposed recommended target concentration[b]	Non-conformity limit	Recommended concentration for screening[c]
Streptomycin	20	–	10	40
Tetracyclines	20	–	10	20
Sulfonamides	20	–	–	50
Erythromycin	–	20	–	20
Tylosin	–	20	15	20
Lincomycin	–	20	–	–
Enrofloxacin	–	5	–	–
Ciprofloxacin	–	5	–	–
Trimethoprim	–	20	–	–
Metronidazole	–	3	–	–
Chloramphenicol	0.1 (MRPL)	–	–	0.3 (MRPL[d])
Nitrofurans	1 (MRPL[d])	–	–	1[e] (MRPL[d])

[a] Anon. (2001).
[b] Laza et al. (2011).
[c] Anon. (2007b).
[d] MRPL, Minimum Required Performance Limit (Commission Decision 2003/181/EC).
[e] MRPL set for poultry meat and aquaculture products (Commission Decision 2003/181/EC), applicable on honey (Anon., 2007b).

must be discontinued with sufficient time prior to honey flow in order to prevent residues in the honey since there are no authorized residue limits for these antibiotics in honey. Even so, there are no authorizations or tolerances for other drugs like sulfonamides, erythromycin, or streptomycin in treating bees. Fluoroquinolones are prohibited for use in treating honeybees (Anon., 2010c).

In Canada (Anon., 2011a) and India (Johnson et al., 2010) oxytetracycline is the only antibiotic approved for treatment of AFB and EFB. In both countries fumagillin is allowed for use in the treatment of nosemosis. The same antibiotics can be used by beekeepers in Argentina. In addition, also a product containing sulfadimethoxine, trimethoprim, and oxytetracycline as pharmacologically active substances is approved in Argentina to be used against foulbrood and nosemosis (Anon., 2011b).

The problem of availability of veterinary medicines to treat honeybees has been discussed extensively at a workshop held in December 2009 at the European Medicines Agency (EMA) in London, United Kingdom (Anon., 2010a). At the 19th session of Codex Committee on Residues of Veterinary Drugs in Foods that took place in August–September 2010 in Burlington, VT, recommendations were made to consider MRLs in honey and to develop a Codex guideline on Good Veterinary Practice in honey production. Such a guideline could provide harmonized guidance that would ensure the safety of bee products and enable fair trade practices (Anon., 2010e).

3. Antibiotics and chemotherapeutics of interest in apiculture

3.1. Tetracyclines

Tetracyclines are broad-spectrum bacteriostatic antibiotics with a long history in veterinary medicine and are used for the treatment and control of a wide variety of bacterial infections. Oxytetracycline (OTC), usually in its hydrochloride form, has been used in apiculture since the early fifties for the treatment of bacterial brood diseases like AFB (Hopingarner and Nelson, 1987; Spivak, 2000) and EFB (Oldroyd et al., 1989; Waite et al., 2003; Thompson et al., 2005). Four modes of OTC application have been commonly used: antibiotic paper packs (Wilson et al., 1973), a dusting with OTC in powdered sugar, repeated several times at weekly intervals; a solution of OTC in syrup fed to the bees; and now most common, an 'extender patty' consisting of OTC, sugar, and vegetable shortening (Kochansky, 2000). For a considerable length of time, Terramycin® (OTC hydrochloride) (Phibro Animal Health, Ridgefield Park, NJ) has been the only approved drug treatment for the foulbrood diseases in the United States (Anon., 2010c). Terramycin® exists in different application forms. Application of Terramycin® in powder form is likely to result in lower initial residue levels, compared to an application in liquid form (Anon., 2002). Nowadays in the USA, many other animal drug products based on OTC are approved by FDA for use in apiculture. The application should be finished and all product removed at least 6 weeks prior to honey flow to eliminate the risk of residues present in the honey (Anon., 2010c).

Despite the fact that no MRL for tetracyclines has been fixed in honey, oxytetracycline is used in the UK in the statutory treatment of EFB, since this is considered by the authorities as within the cascade system for veterinary medicines under Minor Use, Minor Species (MUMS) (Thompson et al., 2005, 2006). The use is only permitted under certain circumstances, *i.e.* under veterinary supervision and applying long withdrawal periods. The Minor Use and Minor Species law is intended to make more medications legally available to veterinarians and animal owners to treat minor animal species like honey bees and uncommon diseases in the major animal species. In September 2009, approximately 4660 hives were treated with OTC in response to an outbreak of EFB in eastern Scotland (Anon., 2010d).

The product Oxypharm plv. sol.® (Pharmagal s.r.o., Nitra, Slovakia), containing oxytetracycline hydrochloride, is authorized in Slovakia for EFB treatment or preventative application to winter feed (Anon., 2009).

The intensive use of tetracyclines in professional beekeeping resulted in tetracycline-resistant *Paenibacillus* strains in the US (Miyagi et al., 2000), Canada (Colter, 2000), and Argentina (Alippi, 2000). There is now general concern about widespread resistance. The tetracycline resistance involves horizontal transfer *via* a non-genomic (*e.g.* plasmid or conjugal transposon) route (Evans, 2003; Murray and Aronstein, 2006; Alippi et al., 2007).

3.2. Streptomycin

Streptomycin is an aminoglycoside antibiotic used in apiculture to protect bees against a variety of brood diseases. Despite the fact that the drug is not authorized in most countries (EU, USA), the use is often suggested in bee forums and in beekeeping handbooks (Mutinelli, 2003). In China, streptomycin and chloramphenicol were preferred antibiotics to control a large AFB outbreak in 1997, instead of eradication (Ortelli et al., 2004). At the Apimondia meeting in 1997 (Antwerp, Belgium) it was noted that Mexican beekeepers used streptomycin as a reinforcing product in the beehives (Bogdanov and Fluri, 2000).

3.3. Sulfonamides

Sulfonamides play an important role as effective chemotherapeutics for bacterial and protozoal diseases in veterinary medicine. They are frequently administered in combination with dihydrofolate reductase inhibitors of the group of diaminopyrimidines. The use of sulfonamides to protect honey bees against bacterial diseases became a common practice in commercial beekeeping after Haseman and Childers (1944) learned that sulfa drugs, particularly sulfathiazole, could prevent the spread of AFB. The compound sulfathiazole provided a short-term control by suppressing the symptoms of the bee disease caused by *Paenibacillus larvae*. It also prevented the reproductive spores from germinating. The use of sulfa drugs in the bees' food in spring and fall was also encouraged by other authors (Eckert, 1947; Reinhardt, 1947; Johnson, 1948; Katznelson and Gooderham, 1949; Katznelson, 1950). Despite the effectiveness of sulfonamides against AFB, their stability and

consequent residues in honey caused problems, and in the seventies, the registration was allowed to lapse (Shimanuki and Knox, 1994).

Some beekeepers also apply sulfonamides against nosemosis in a prophylactic way by the addition to winter feed sugar solution (Lourdes, 2002). This practice, as suggested in beekeeping manuals (*e.g.* Anon., 2010b) and in publications regarding the treatment of infections due to microsporidia (Didier, 1998), increased after fumagillin became less available in the EU.

3.4. Tylosin

Tylosin, a macrolide antibiotic, has been used globally in beekeeping. Its efficacy was proven by different authors (Hitchcock et al., 1970; Moffett et al., 1970; Peng et al., 1996; Pettis and Feldlaufer, 2005; Alippi et al., 1999, 2005; Reynaldi et al., 2010). Tylosin was found to be more stable in sugar syrup than OTC (Kochansky et al., 1999). In October 2005, Tylan (tylosin tartrate) Soluble® (Elanco Animal Health, Indianapolis, IN) received approval in the US from the Food and Drug Adminstration (FDA) for the treatment of active AFB, but not for preventative use in healthy colonies. The use of tylosin should be discontinued at least 4 weeks prior to honey flow (Anon., 2010c). In countries with infestations of small hive beetles, tylosin application by the dust method is more efficacious than the patty method of delivery (Elzen et al., 2002). As efficacy, safety, and residue studies were available, the UK beekeepers association requested the originator to apply for a 'global marketing authorization' as created by Article 5 of Directive 2004/28/EC. However, such a marketing authorization would prevent data protection for the dossier that would immediately become available for generic competition and this would dramatically impact the return on investment for this product. The use of tylosin against AFB was promoted after *Paenibacillus* was demonstrated to have resistance against tetracyclines.

3.5. Erythromycin

Erythromycin, another macrolide, was first tested in 1955 (Katznelson et al., 1955; Katznelson, 1956). Depending on the literature, erythromycin has been reported to be effective against AFB (Machova, 1970; Okayama et al., 1996) and EFB (Wilson and Moffett, 1957; Wilson, 1962), while other authors found it to be ineffective against AFB (Katznelson et al., 1955; Moffett et al., 1958; Alippi et al., 1999). Despite the doubt about its effectiveness, erythromycin was used by professional beekeepers in the Southern Marmara region of Turkey (Gunes et al., 2008).

3.6. Lincomycin

Lincomycin belongs to the group of lincosamides. Its activity against *Paenibacillus larvae* strains has been reported by some authors (Okayama et al., 1996; Kochansky et al., 2001). Lincomycin was, along with tylosin, tested as potential drug for FDA approval to control tetracycline-resistant AFB disease. Lincomycin was effective in controlling AFB, when applied to honeybee colonies as a dust in confectioners' sugar (Feldlaufer et al., 2001). The FDA approval is still pending for this product.

3.7. Chloramphenicol

Chloramphenicol (CAP) is a potent, broad-spectrum antibiotic and a potential carcinogen and has been banned in the European Union since 1994 for use in food producing animals, including honey bees (Commission Regulation (EC) No. 1430/94). The consumption of CAP contaminated food may pose human health risks associated with the development of a potentially life-threatening blood disorder, called aplastic anaemia. In China in 1997–1998, hundreds of thousands of beehives were infected by AFB and treated by the beekeepers with CAP or streptomycin to save their hives and their industry (Ortelli et al., 2004). In January 2002, concerns regarding 'serious deficiencies of the Chinese residue control system and problems related to the use of banned substances in the veterinary field', lead to the European Union to issue a suspension of imports of all products of animal origin from China. The ban on import of honey from China was lifted in July 2004. Meanwhile, a growing number of rapid alert notifications related to the presence of chloramphenicol in import honey from China have been issued (Anon., 2011c).

3.8. Nitrofurans

There are only few publications noting that nitrofurans are used in the maintenance of bees for honey production. In Europe, nitrofurans are prohibited substances for all food producing animals (Commission (EU) Regulation No. 37/2010). Nitrofurans are rapidly metabolized and covalently bound with proteins or peptides. Results of a simultaneous analysis of the metabolites of four nitrofuran veterinary drugs, furazolidone, furaltadone, nitrofurantoin, and nitrofurazone, in honey have shown that furazolidone is the main nitrofuran antibiotic administered to treat bacterial diseases of bees (Khong et al., 2004).

3.9. Nitroimidazoles

Dimetridazole (DMZ), metronidazole (MNZ), and ronidazole (RNZ) are classified in Europe as prohibited substances for all food producing species (Commission Regulation (EU) No. 37/2010). Zhou et al. (2007) claim that in China in recent years, 5-nitroimidazoles have been commonly used to prevent and control *Nosema apis* in hives. Chinese beekeepers consider it as a cheap alternative to fumagillin. The use of MNZ, DMZ, and RNZ is now prohibited in food animals in China. Tinidazole (TNZ) has never been authorized as a veterinary drug, and is also considered as a banned substance in China. The most found nitroimidazole residue in honey is MNZ.

3.10. Fluoroquinolones

The majority of the clinical use of quinolones is in the form of fluoroquinolones. The base chemical in quinolones is nalidixic acid. Despite the lack of scientific data demonstrating efficacy, the application of fluoroquinolones in

apiculture, especially in Asia, as a prophylaxis for bee diseases has increased during the last few years. This use was confirmed by the detection of residues in honey from that area (Savoy Perroud et al., 2009). Residue testing in honey is demonstrating that enrofloxacin (ciprofloxacin) and norfloxacin are the main fluoroquinolone antibiotics administered.

3.11. Fumagillin

To prevent and control nosemosis, fumagillin was commonly used in beekeeping in several parts of the world. Fumagillin, an antibiotic prepared from *Aspergillus flavus*, was found to be effective by Katznelson and Jamieson (1952). Treatment with the antibiotic fumagillin inhibits the spores reproducing in the *ventriculus* but does not kill the spores (Bailey, 1953; Webster, 1994). Fumidil B® (bicyclohexylammonium fumagillin) (Mid-Continent Agrimarketing Inc., Overland Park, KS) is approved in the US by FDA for use in beekeeping to prevent *Nosema* disease. In order to prevent residues in honey, fumagillin should not be fed immediately before or during the honey flow (Anon., 2010c).

Fumagillin was on the EU market since 1970. However, as MRLs could not be recommended by the CVMP due to the inadequacy of data available to ensure consumer safety, no marketing authorization could be maintained or issued for bees. The CVMP acknowledged that fumagillin would be an essential substance for veterinary medicine for bees (Anon., 2000). Fumagillin can be considered under MUMS, and based on this, data on toxicity is being generated by the animal health company that produces fumagillin (Anon., 2010a). In the meantime, the use of fumagillin in the EU is not permitted. Nevertheless, Fumidil B® (CEVA Animal Health Ltd., Chesham, United Kingdom) with fumagillin bicyclohexylamine salt as active ingredient, is still available in the UK as an authorized product (syrup 20 mg l^{-1}) for the control of *Nosema* in honeybees (Anon., 2009). Studies by Stanimirovic et al. (2007) suggested that fumagillin has genotoxic (clastogenic) potential in mammals *in vivo*. Other studies (Stevanovic et al., 2008) indicated that fumagillin is clastogenic and cytotoxic to cultured human lymphocytes. Since no other antibiotics or chemotherapeutics are registered for the treatment of nosemosis, prevention procedures need to be applied.

3.12. Other antibiotics and chemotherapeutics

Machova (1970) reported good sensitivity of *Paenibacillus larvae* to bacitracin, a polypeptide antibiotic. The isolates of *Paenibacillus larvae* tested by Okayama et al. (1996) were most susceptible to penicillins, macrolides, and lincomycin. Microsamicin among the macrolides, and ampicillin among the penicillins, appeared to be the most effective agents. Ampicillin was also tested in beehives, where it resulted in high residues in honey but only in very low levels in larvae, casting doubt on its utility in disease control (Nakajima et al., 1997, 1998). Kochansky and Pettis (2005) reported later that all β-lactams (penicillins and cephalosporins), while active *in vitro*, are apparently not effective in the field. Kochansky et al. (2001) screened alternative antibiotics against OTC-resistant *Paenibacillus larvae*. Rifampicin, a bactericidal antibiotic drug of the rifamycin group, was by far the most active antibiotic tested; monensin (an ionophore antibiotic), and the earlier described erythromycin, tylosin, and lincomycin showed also to be active *in vitro* to resistant strains of *P. larvae*. In a later study (Kochansky and Pettis, 2005), more antimicrobials were tested. The ionophore antibiotics narasin, lasalocid, salinomycin, laidlomycin, and maduramycin had a high *in vitro* activity but they were inactive in the field. The lincosamide pirlimycin and the pleuromutiline antibiotic tiamulin showed high activity *in vitro* but share the mode of action of tylosin, and therefore offer no benefit. The effectiveness of the macrolide antibiotic tilmicosin against AFB *in vitro* and *in vivo* was reported by Reynaldi et al. (2008).

The list of effective alternatives for oxytetracycline and tylosin to treat AFB is very limited. It is essential that these veterinary drugs, in the countries where their use is authorized, are utilized in a manner that will delay the onset of resistance, and that other methods of dealing with AFB are explored (Kochansky and Pettis, 2005). Despite early data suggesting that alternative drugs might be an alternative treatment for AFB, there are so far no signs of use of bacitracin, microsamicin, rifamycin, monensin, pirlimycin, tiamulin, and tilmicosin in beekeeping.

4. Stability and disposition of antimicrobials in honeybee hives

4.1. Stability and depletion of tetracyclines

In the study of Martel et al. (2006), tetracycline was very stable in honey: the half-life of tetracycline hydrochloride in honey stored at 20 °C and 35 °C in dark was 242 and 121 days, respectively. In other studies tetracyclines were degraded rapidly in honey (Table 2). The half-life of OTC in incurred honey at 34 °C was reported as 12 days stored at 34 °C in the laboratory (Argauer and Moats, 1991), and 2–4 days when undisturbed in the cells of the comb within the active bee colony (Gilliam and Argauer, 1981a). Münstedt (2009) demonstrated that honey fortified with 500 μg kg^{-1} of oxytetracycline, tetracycline, and chlortetracycline and stored in dark at room temperature for 7 months had residues of oxytetracycline below 10 μg kg^{-1} while only epimers of tetracycline and chlortetracycline were found.

There are several studies published regarding the depletion of oxytetracycline in beehives (Gilliam and Argauer, 1981a,b; Matsuka and Nakamura, 1990; Lodesani et al., 1994). Recent publications (Anon., 2002; Thompson et al., 2005, 2006; Martel et al., 2006) demonstrated that, when used in beekeeping, concentrations of tetracyclines up to mg kg^{-1} could be found in the honey of the treated hives. Depletion and degradation of tetracycline is slow with a half-life for oxytetracycline residues of 9–44 days (Thompson et al., 2006) or 11–14 days (Anon., 2002), and 65 days for tetracycline hydrochloride in honey from supers (Martel et al., 2006)). In the study performed at the Central Science Laboratory (presently the Food and

Environment Research Agency or FERA) at Sand Hutton (UK) (Anon., 2002), application of Terramycin® (1 g of oxytetracycline, single dose) in liquid form resulted in very high residue levels in honey with residues of 3.7 mg kg^{-1} 8 weeks after application. Thompson et al. (2006) suggested a withdrawal period of up to 16 weeks was required for colonies treated with oxytetracycline in liquid sucrose, and up to 18 weeks was required for those colonies treated in icing sugar, considering a reporting limit of 50 μg kg^{-1}. Münstedt (2009) treated hives with 200 mg of chlor- and oxytetracycline three times, with an interval of 7 days. Nine weeks after the last dosing, the sum of chlortetracycline and epi-chlortetracycline in the honey ranged from 311 to 512 μg kg^{-1}. Only in one sample of the honey from the five hives treated with oxytetracycline residues were found (13 μg kg^{-1}).

4.2. Stability and depletion of streptomycin

Pang et al. (2004) found streptomycin to be stable in honey stored for a period of more than 4 months at room temperature, without any occurrence of disintegration or metabolic reaction. In a study of the FERA, no significant decline in concentration of streptomycin in honey over 161 days at room temperature was observed (Anon., 2006). In the same study, the stability of lincomycin in honey was shown to be 28 days at room temperature.

The distribution of streptomycin was followed after dosage of 1 g per hive (single dose in sucrose solution) to bee colonies. The highest mean concentration of streptomycin found in honey was 124 mg kg^{-1}, 7 days after dosing. The concentration declined to 8.0 mg kg^{-1} at day 28, with a final concentration of 6.5 mg kg^{-1} at day 332 (Anon., 2006).

4.3. Depletion of sulfonamides

Sulfamethazine residues were found in Flemish honey samples at mg kg^{-1} level the year after sulfa drugs were used by beekeepers in winter feed to prevent nosemosis (Reybroeck et al., 2010).

4.4. Stability and depletion of tylosin

It has been recognized that the parent compound, tylosin A, degrades in acidic media such as honey, to yield the antimicrobially active degradation product, desmycosin (tylosin B) with a half-life of approximately 4 months at 34 °C (Kochansky, 2004). During storage of 16 weeks at ambient temperature, approximately 20% of the tylosin A had degraded to desmycosin (Thompson et al., 2007). In honey sampled from hives approximately 9 months after the last treatment with tylosin, a relatively constant ratio of tylosin A to desmycosin (overall average of 1.2) was observed. In other incurred honey samples this ratio was in the range of 0.9–1.6 (Thompson et al., 2007). Desmycosin seemed to be quite stable in honey, the sum of both tylosin and desmycosin decreased only slightly over 9 months (Kochansky, 2004). It has been demonstrated that honey destined for human consumption should be analysed for both tylosin A and desmycosin, rather than for the parent antibiotic alone (Kochansky, 2004; Thompson et al., 2007).

Tylosin was applied by Feldlaufer et al. (2004) to honey bee colonies in a confectioner's sugar dust (200 mg of active compound) three times with an interval of 7 days. Tylosin concentrations in surplus honey from treated colonies declined from an average of 1.31 mg kg^{-1} during the treatment period, to 160 μg kg^{-1} three weeks after the last treatment. Elzen et al. (2002) demonstrated that the application of a grease patty *versus* dust application of tylosin, resulted in increased residues of tylosin in the hive products wax and honey. Different results were obtained for pollen patties by Thompson et al. (2007). In their experiments, pollen patty treatments contributed to a substantially lower tylosin residue production in honey, in comparison to sugar dusting treatments.

In another study, tylosin was dosed to hives (1 g per hive, single dose) and the distribution of both tylosin A and desmycosin in the honey was followed. The highest mean concentration of tylosin A found in honey was 17 mg kg^{-1}, 3 days after dosing. The concentration declined to 6.1 mg kg^{-1} on day 28, with a final concentration of 930 μg kg^{-1} at day 238. The concentration of desmycosin remained relatively consistent at 2.3 mg kg^{-1} at day 3, 2.6 mg kg^{-1} on day 28, and 1.0 mg kg^{-1} on day 238 (Adams et al., 2007; Anon., 2006).

4.5. Depletion of erythromycin

A degradation time of 35–40 days is required for erythromycin to obtain residue levels below the limit of detection (50 μg kg^{-1}) (Alippi et al., 1999). An erythromycin-fortified cake was fed to bees by Gunes et al. (2008). In this test hive, the erythromycin residue level in honey was approximately 28 μg kg^{-1}, 3 months after dosing.

Table 2
Stability of tetracyclines in honey (different authors).

Pharmacologically active substance	Temperature – storage	Half-life	Reference
Tetracycline hydrochloride	20 °C – lab	242 days	Martel et al. (2006)
	35 °C – lab	121 days	Martel et al. (2006)
	in bee colony	65 days	Martel et al. (2006)
Oxytetracycline	34 °C – lab	12 days	Argauer and Moats (1991)
	in bee colony	2–4 days	Gilliam and Argauer (1981a)
	in bee colony	9–44 days	Thompson et al. (2006)
	in bee colony	11–14 days	Anon. (2002)

4.6. Depletion of lincomycin

Bee colonies treated with 1.2 g lincomycin hydrochloride per hive resulted in a highest mean concentration of lincomycin in honey of 24 mg kg^{-1} 3 days after treatment, a mean of 3.5 mg kg^{-1} after 129 days. Lincomycin was persistent in the hive and detected in all over wintered samples of honey, 290 days after dosing (Adams et al., 2009).

4.7. Stability and depletion of chloramphenicol

CAP and its metabolite chloramphenicol glucuronide (CAP-Glu) are stable in solvent and in fortified matrix (milk powder, shrimp, and kidney) at room temperature tested for at least 20 weeks (Ashwin et al., 2005). CAP did not form significant concentrations of glucosides in honey. Consequently, free CAP is a suitable marker compound for determination and quantification of CAP residues in honey (Adams et al., 2008).

Adams et al. (2008) followed the distribution of CAP after dosage of 1 g per hive (single dose in sucrose solution) to bee colonies. The highest mean concentration of CAP found in honey was 26 mg kg^{-1} at 7 days after dosing. This concentration declined to 2.0 mg kg^{-1} at day 28, with a final concentration of 1.0 mg kg^{-1} on day 332 ('over wintered' sample). Even when the shook swarm procedure was used, in an attempt to 'clean' the bee colonies, CAP was still detected in honey 332 days after dosage (100 μg kg^{-1}). The dosage also resulted in CAP residues in beeswax and royal jelly (highest mean concentration of 6.8 mg kg^{-1} and 3.0 mg kg^{-1} at 7 days, respectively) (Adams et al., 2008).

4.8. Stability and depletion of nitrofurans

Furazolidone in honey, stored at room temperature, showed a rapid decline (>90%) over 14 days, while its metabolite AOZ remained stable. Therefore AOZ is considered to be the most suitable marker compound to detect the use of furazolidone in apiculture (Anon., 2006).

At the Food and Environment Research Agency, the distribution of furazolidone after dosage (1 g of active compound per hive) in beehives was followed (Anon., 2006). On one hand, this was resulting in a dilution of the drug in the honey flow; on the other hand, furazolidone was degrading to AOZ. In super honey, the highest mean residue concentrations were measured seven days after dosing (2.5 mg kg^{-1} of furazolidone and 5.8 mg kg^{-1} T-AOZ (sum of the concentrations of AOZ and parent furazolidone)). The results also confirmed that furazolidone and T-AOZ can still be detected in honey 332 days after dosing with furazolidone (mean concentration: 440 μg kg^{-1} of furazolidone, and 530 μg kg^{-1} of T-AOZ).

4.9. Depletion of fluoroquinolones

Ciprofloxacin was administered to bee colonies. The highest concentration of ciprofloxacin was >10 mg kg^{-1} 3 days after dosing. The average concentrations of ciprofloxacin in honey at 18 weeks were between 622–1370 μg kg^{-1} (Fussell et al., 2010).

4.10. Depletion of other antibiotics

Mirosamicin, mixed in a pollen-substitute paste, was administered to honeybee colonies continuously during one week, at a dosage of 200 mg/hive/week. A relatively low distribution of mirosamicin in honey was observed (Nakajima et al., 1998). Single dosing of mirosamicin in sucrose syrup resulted in a very high and long lasting residue in honey (Nakajima et al., 1998).

Comparable results were obtained for the disposition of ampicillin: a single dose of 30 mg ampicillin per hive administered in syrup resulted in high drug residue levels in honey and residual residues beyond the detection limit for more than 14 days. In the hives where ampicillin (30 mg per hive) was delivered in pollen substitute paste, relatively low honey residues were found (Nakajima et al., 1997).

5. Conclusions and reflections about the use of antimicrobials in hives

Several antimicrobials can be used in beekeeping against AFB, EFB, and nosemosis. However, their use is resulting in high levels of residues in honey. Some active compounds remain very stable in honey while other compounds metabolize. Hence, for residue analysis it is important to look for the suitable marker residue. An overview of the most suitable marker residues for the different antimicrobials of interest in beekeeping is given in Table 3.

In general, the highest residue concentrations in honey are found within one week after dosing. Afterwards, residue levels in honey from supers diminish by a dilution effect of the honey flow and for some compounds (oxytetracycline, tylosin, furazolidone) by a degradation of the parent drug. Residues of the marker compounds for most drugs are still detected in honey, harvested the year after the drug application, even when the shook swarm method was applied after dosing. This is mainly caused by the fact that antimicrobials are not actively metabolized by the honeybees, and consequently, all the food needs to be consumed by the bees in order to eliminate residues in the hive. In view of this, applications in syrup are least desirable, since the syrup is stored directly by the bees. Any sort of application during nectar flow, when supply honey is being stored, also poses a residue risk. In view of the zero-tolerance for residues of antimicrobials in honey in many countries, very long withdrawal periods together with other biotechnical measures need to be considered.

Management of residues in honey is more complex than in mammalian or avian tissues. In the honey matrix, there is no time dependent depletion/elimination of residues as a result of pharmacokinetics. Residues, once present in honey, largely remain intact. Apart from the possible chemical decay of a substance in honey matrix over time, the main variation responsible for the level of residues at harvest time is the honey yield (dilution effect), which in large parts depends on the production site (geographical area) and weather conditions at flowering time. Therefore, the specification of a withdrawal period, the interval between last treatment and harvest of honey, is extremely difficult.

Table 3
Marker residues for antibiotics and chemotherapeutics used in beekeeping.

Pharmacologically active substance	Metabolite	Marker residue[a]
Streptomycin	–	Streptomycin
Tetracyclines	Epimers	Sum of parent drug and its 4-epimers
Sulfonamides	–	Parent drug
Erythromycin	–	Erythromycin A
Tylosin	Desmycosin (tylosin B)	Tylosin A
Lincomycin	–	Lincomycin
Enrofloxacin	–	Sum of enro- and ciprofloxacin
Ciprofloxacin	–	Sum of enro- and ciprofloxacin
Trimethoprim	–	Trimethoprim
Metronidazole	–	Metronidazole
Chloramphenicol	–	Chloramphenicol
Furazolidone	AOZ (3-amino-2-oxazolidone)	AOZ (3-amino-2-oxazolidone)
Nitroimidazoles	Hydroxymetabolites	Hydroxymetabolites

[a] Commission Regulation (EU) No. 37/2010.

According the new MRL regulation, if the metabolism and depletion of the substance cannot be assessed, the scientific risk assessment may take into account monitoring data or exposure data (Anon., 2010a).

6. General conclusions and considerations for the future

The situation regarding the use of antimicrobials in honey production is rather complicated. From one side many antimicrobials have proven their effectiveness against AFB, EFB, or nosemosis. On the other side, in the European Union, no MRLs have been established for antibiotics and sulfonamides in honey, resulting in a 'zero tolerance' for residues of anti-infectious agents in honey. The EU has a honey deficit and is relying for about half of the total honey consumption on import of honey from regions where the use of antimicrobials in apiculture against infectious bacterial brood diseases is allowed or applied. Zero tolerance means in most countries the decision limit (CCα) of the confirmatory method which is resulting in an uneasiness for the honey importers. Every laboratory involved in honey residue analysis has his own CCα-values and hence the same honey sample may be declared compliant or non-compliant depending upon the CCα of the particular testing laboratory.

Residues or their metabolites remain stable for a long period in honey since antimicrobials are not actively metabolized by the honeybees and elimination of the residues in the hive can only happen by consumption by the bees or removal of the contaminated food by the beekeeper. Hence, high numbers of rapid alert notifications for residues of antimicrobials in honey are not surprising.

In beekeeping the setting of a withdrawal time to be respected after the last usage of antimicrobials is not an easy task. In honey, there is no elimination of residues as a result of pharmacokinetics. In practical studies, large variations in residue concentration (*i.e.* high %CVs) were observed between honey sampled from different hives within an apiary and even between honey collected from different frames within a hive. In such respect, more depletion studies of residues in honey are needed.

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