Contamination of honey by oxytetracycline from pig manure

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Keywords
Bees,
Honey,
Oxytetracycline,
Manure,
Water harvesting.

Summary
Although the use of antimicrobial is not allowed in bee industry according to current EU legislation, antimicrobial residues are often detected in honey doomed to human consumption. This study aims to investigate if bees living in hives located nearby tanks filled with pig manure containing residues of oxytetracycline, would naturally harvest water from it, thus contaminating their honey. Data from this experiment were compared with those originating from direct contamination with oxytetracycline through the beehive feeders. Bees did not harvest water from manure, even during the warmest days of summer. Instead, antimicrobial residues were evidenced and quantified in honey from hives directly contaminated with oxytetracycline. Interestingly, antimicrobial residues were also observed in honey from untreated hives thus suggesting that illegal treatments can cause contamination, albeit at low levels, of honey produced in legally-untreated neighboring hives.

Possibile contaminazione di ossitetraciclina da liquami suini nel miele

Parole chiave
Api,
Liquami,
Ossitetraciclina,
Miele,
Raccolta di acqua.

Riassunto
Sebbene la vigente legislazione dell'UE non consenta l'uso di antimicrobici, i loro residui sono spesso rilevati nel miele destinato al consumo umano. Questo studio si propone di indagare se le api che vivono in alveari situati vicino a serbatoi pieni di letame di maiale contenenti residui di ossitetraciclina, raccogliendone l'acqua contenuta, contaminano così il loro miele. I dati di questo esperimento sono stati confrontati con quelli derivanti dalla contaminazione diretta con ossitetraciclina attraverso gli alimentatori dell'alveare. Le api non raccoglievano acqua dai residui, anche durante i giorni più caldi dell'estate. Invece, i residui antimicrobici sono stati evidenziati e quantificati nel miele da alveari direttamente contaminati con ossitetraciclina. Inoltre, nel miele sono stati osservati residui antimicrobici da alveari non trattati, il che suggerisce che i trattamenti illegali possono causare contaminazione, seppur a bassi livelli, del miele da essi prodotto. Invece, i residui antimicrobici sono stati evidenziati e quantificati nel miele da alveari direttamente contaminati con ossitetraciclina.

Introduction

European Union (EU) Regulation 37/2010 established the Maximum Residual Levels (MRLs) of pharmacologically active substances in foodstuffs of animal origin. However, there are no authorised antimicrobial veterinary medicinal products for the control of bee diseases. For this reason, the detection of drug residues in marketed honey results in serious penalties for producers.

Beehives require water to survive. Water is used to keep the osmotic balance of adult bees, to rehydrate honey for larvae nutrition, and to decrease internal temperatures during warm days (Winston 1987). Several authors have hypothesised that bee products might be contaminated by water harvested from manure, in which the presence of drug residues is likely (Mantovi *et al.* 2009). In this regard, previous studies have attempted to clarify this controversial issue, but defined results have not yet been obtained.

Tetracyclines are a class of antibiotics widely used for the treatment of livestock diseases. They are administered to animals through feed or drinking water, and additionally used as a preventive measure. A significant portion of the administered tetracycline dose is excreted through urine and faeces (Mantovi *et al.* 2009). During manure distribution for agricultural fertilisation (150-180 days in outdoor ponds in proximity of farms), the concentration of antimicrobial residues in manure is significantly reduced, but still detectable by means of routine analytical techniques (Mantovi *et al.* 2008). This study aims to ascertain whether bees would harvest water from pig manure thus contaminating with oxytetracycline their honey.

Materials and methods

Field studies

A field study was carried out at the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale' (IZSAM), in the courtyard of the District laboratory of Isernia, Molise region, Southern Italy (41.58417° N, 14.20019° E) from August 1st to October 3rd 2013. The experiment involved 6 hives (Dadant-Blatt composed of 10 honeycombs) of *Apis mellifera ligustica*, placed in a single row, 1 metre away from each other. Hives were labelled as follows: A1, B1, C1, A2, B2, and C2. Colonies were homogeneous in terms of strength according to the method described by Accorti (Accorti 1985) and Marchetti (Marchetti 1985); bees covered each of the 10 combs and had an efficient queen, born in June 2013. The bees were not suffering from any overt diseases, and the parental apiary had never been treated with antibiotics. On August 1st, 2 tanks filled with 200 L each of pig manure were located 6 metres away from the hives (Figure 1). Three foamy plates were placed on the free surface in contact with air in order to help bees (Figure 2). The manure originated from a farm located in Isernia. The manure was combined with 30 g of oxytetracycline 50% (ASCOR Chimici®), resulting in a final concentration of 75 mg/l (Mantovi *et al.* 2008). Oxytetracycline levels were checked 30 and 120 days after the beginning of the experiment.

On August 3rd, supers composed of 9 combs were placed on each hive, together with a queen excluder, in order to avoid eggs being laid in the honeycombs. Feeders were positioned on top of hives B and C, so that only related bees had access to them (Figure 3).

Once a week during a 3-week period, hives belonging to groups B (B1, B2) and C (C1, C2) were fed with 1 L of an aqueous solution of sucrose (sugar syrup) 50% w/v. Once a week for 3 weeks, the sugar syrup of group C was added with 200 mg of oxytetracycline, following guidelines given by

Figure 1. *Position of beehives and tanks filled with manure.*

Figure 2. *Foamy plates placed on the free surface of tanks in order to assist bees for water supply.*

Figure 3. *Feeders placed on hives of group B.*

the U.S. Food and Drug Administration regarding the use of Terramycin® Soluble Powder against the European Foul Brood (EFB) and the American Foul Brood (AFB) (USFDA, 2001).

Observation of bee behaviour

Twice a day (in the morning and in the afternoon for 20 minutes), for 5 days in a week, throughout the experimental period, bees were observed. Bee behaviour has been evaluated also in association to more distantly located puddles of clean water. The evaluation of flying activity was qualitative and consisted of an estimation of the average number of bees leaving the hive for harvesting per minute. An observation of 0 to 30 bees per minute was considered low, 30 to 100 was considered moderate, and over 100 was considered intense. The 6 colonies were homogeneous and were observed all at the same time; the flying activity was similar among hives. The flying activity of bees is strictly related to the external temperature and to the strength of the colony (Danka and Beaman 2007). Based on the data previously reported (Lindauer 1954) we expected to observe at least 2 trips to the manure tanks during 10 minutes of monitoring activity with an outside temperature of nearly 20 °C, and up to 60 trips as temperature rises to 30 °C.

Meteorological data record

Temperature and rainfall data were collected using a Davis VP2 weather forecast station, property of Meteo Molise, an association of amateur meteorologists located 1 kilometre away from our apiary.

Honey harvest and extraction

On October 3rd, all supers were removed and honey was extracted using a radial extractor capable of housing 12 combs. Coarse impurities were removed using a metallic mesh filter. In order to avoid cross-contamination, the honey from each group was extracted and stored separately in sealed plastic containers. Accordingly, the equipment was rinsed with tap water and dried with paper towel. One honey sample from each group (A, B, and C) was analysed for oxytetracycline concentration, physicochemical parametres, and melissopalynological properties.

Oxytetracycline determination in the manure

Oxytetracycline levels in the manure were assessed 30 and 120 days after contamination from a pool of each tank.

Laboratory tests

Determination of Oxytetracycline in honey samples

The method developed by Cristofani and colleagues (Cristofani *et al.* 2009) in order to determine oxytetracycline levels was applied with minor modifications.

Three grams of honey were weighted in a 50 ml centrifuge tube and extracted with 20 ml of succinic acid 0.1M (pH 4) and 20 ml of methanol. The mixture was shaked at 200 rpm for 30 minutes.

After an additional centrifugation (4,000 rpm) for 15 minutes at 4 °C, the extract was filtered through a 0.45 µm PVDF syringe filter, and loaded on a Metal Chelate Affinity Chromatography (MCAC) column containing 1.5 ml of sepharose fast flow suspended in ethanol (GE Healthcare, Uppsala, Sweden) that had previously been activated with 6 ml of ultrapure water, 3 ml of a 10 mM copper (II) sulphate solution, and 4 ml of ultrapure water. The column was then washed in sequence of 2 ml of succinic acid 0.1 M, 2 ml of methanol, and 2 ml of ultrapure water. Oxytetracycline was eluted with 8 ml of McIlvaine-EDTA-NaCl buffer (containing an aqueous solution of 1% EDTA disodium salt hydrate and 0.78% NaCl) into an OASIS HLB cartridge (60 mg/3 ml; Waters, Milford, MA, USA), previously conditioned with 3 ml of methanol, 3 ml of HCl 1N, and 3 ml of ultrapure water. The OASIS cartridge was rinsed with 3 ml of ultrapure water, and oxytetracycline was eluted with 6 ml of methanol.

The eluate was evaporated under nitrogen flow at 50 °C until it was dry, and then re-dissolved in 300 µl of oxalic acid 0.01 M (pH 3), containing 1.5% of tetrahydrofuran. The residue was finally filtered through a 0.45 µm PVDF syringe filter (Millipore, Bedford, MA, USA), and declared to be fit for quantitative analysis using High Performance Liquid Chromatography coupled with Diode Array Detection (HPLC-DAD). Instrumental analyses were performed by injecting 50 µl of the final extract in a RP-18e monolithic column Chromolith SpeedROD (50 x 4.6 mm i.d., Merck, Darmstadt, Germany) equipped with an RP-18e guard column (5 x 4.6 mm, i.d.).

The chromatographic device was the Waters 600 MS (Waters, Milford, MA, USA), equipped with a 717 plus autosampler and a 996 diode array detector.

Oxytetracycline was detected at a wavelength of 360 nm. Spectra in the range 220-400 nm were also acquired and matched with standards in order to confirm the presence of analyte at detectable levels in samples. Chromatographic analyses were performed by a gradient elution, as reported

Table I. *Gradient timetable for HPLC-DAD analyses.*

Time (min)	Flow (ml/min)	Mobile phase A (%)	Mobile phase B (%)
0.0	4.0	0	100
2.2	4.0	0	100
3.0	4.5	10	90
6.5	4.5	15	85
10.0	4.5	15	85
12.0	4.0	0	100
15.0	4.0	0	100

in Table I, using methanol (phase A), and oxalic acid 0.01 M (pH 3) with 1.5% of tetrahydrofuran (phase B). The retention time of oxytetracycline was 3.3 minutes.

The presence of oxytetracycline was further confirmed by Liquid Chromatography coupled with tandem mass spectrometry (LC-MS/MS). Honey samples were extracted and purified as previously described, but the final extract was re-dissolved with 300 µl of oxalic acid 0.1 mM with 0.2% formic acid. For confirmation, chromatographic separation was performed by injecting 10 µl in a XTerra MS C18 column (100 x 2.1 mm, 3.5 µm, Waters, Milford, MA, USA) equipped with a XTerra MS C18 (10 x 2.1 mm, 3.5 µm) guard column.

The chromatographic system was an LC-MS/MS API 3000 PE SCIEX system (Applied Byiosystems, Bedforf, MA, USA) equipped with a Turboionspray® ion source, a Series 200 micro-binary pump, and a Series 200 autosampler (Perkin Elmer, Branford, CA, USA). Chromatographic separation was performed with a flow of 200 µl/min, using as constituents of mobile phase acetonitrile (phase A) and oxalic acid 0.1 mM with 0.2% formic acid (phase B), according to the gradient reported in Table II.

Positive ionization mode was used with the following settings: source temperature: 400 °C; capillary voltage: 5,500 V; curtain gas pressure (N2): 6 psi; collision gas pressure (N2): 6 psi; nebulising gas pressure (air): 15 psi. *Ad hoc* settings were established for mass spectrometric detection: declustering potential (DP), focusing potential (FP), entrance potential (EP), collision energy (CE), and collision cell exit potential (CXP). Selected transitions, retention time (RT), and optimised settings are reported in Table III.

Table II. *Gradient timetable for LC-MS/MS analyses.*

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	5	95
0.5	5	95
10.0	75	25
12.0	100	0
15.0	100	0
16.0	5	95
25.0	5	95

Determination of residues in manure

Samples were filtered through a 0.45 µm PVDF syringe filter. Five ml of the filtrate was purified according to the same procedure used for honey; HPLC analyses were performed using the same conditions used for honey.

Melissopalynological analyses

Melissopalynological analyses were performed using the method described by Von Der Ohe and colleagues (Von Der Ohe *et al.* 2004). For the identification of pollen elements, 10 g of sample were dissolved in 20 ml of distilled water and, after centrifugation at 1,000 rpm for 10 minutes, the supernatant was removed. In order to completely eliminate residual sugar, the remaining material was rinsed with 10 ml of distilled water, and centrifuged for 5 minutes at the same speed. Supernatant was discarded, and the sediment was placed on a microscope slide, covering an area of 22 × 22 mm. The preparation was stained with glycerine jelly added with a 0.1% fuchsine solution. Microscopic examination was carried out using a Nikon Eclipse 100 microscope at a 100x magnification. Qualitative pollen analyses were based on the identification of pollen grains, figurative elements and, finally, on the estimation of the relative frequencies of the types of pollen. In order to define the botanical origin of honey pollen, methods reported by Loveaux and colleagues (Louveaux *et al.* 1978) and Von der Ohe and colleagues (Von Der Ohe *et al.* 2004) were used. For each sample, 4 classes of frequency were considered:

1. Over-represented pollen (frequency > 45%);
2. Accompanying pollen (16-45%);

Table III. *LC-MS/MS parameters (Oxytetracycline).*

Analyte	RT (min)	Selected transition (m/z)	DP (eV)	FP (eV)	EP (eV)	CE (eV)	CXP (eV)
Oxitetracycline	8.7	461.2 → 426.3	51	350	9	27	6
		461.2 → 443.2	51	350	9	19	6

3. Important pollen (4-15%);
4. Present pollen (frequency < 4%).

For each honey sample, at least 300 pollen grains were counted and every different pollen type was accurately identified. Indicators of honeydew were also evaluated.

3. Results

Field studies

Observation of bee habits

An intense flight activity of bees was observed throughout the study, especially from sunshine until 9:00 a.m., when nectar resources were more accessible. The flight activity was similar among the hives. However, during the observation period, bees did not harvest water from manure. Bees harvested water from the puddles of clean water as showed in Figure 4. In contrast, the 'drone fly' *Eristalis tenax* (Diptera, Syrphidae) harvested water from manure.

Honey harvest and extraction

At the end of the trial, group A produced nearly 3 kg of honey, group B 7 kg, group C 10 kg.

Meteorological data

The mean minimum temperature recorded in August was 17.8 °C, the average maximum temperature was 32.4 °C, the average medium temperature was 24.3 °C. Six rainy days with 32.0 mm of rain fell were recorded. In September, the mean minimum temperature recorded was 14.2 °C, the average maximum temperature was 28.3 °C, and the average medium temperature was 20.0 °C. Nine rainy days were recorded, with 77.4 mm of rain fell (Figure 5).

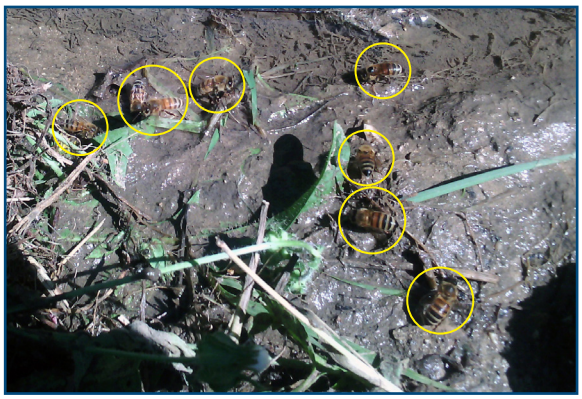

Figure 4. *Foraging bees (evidenced by yellow circles) on puddles of clean water.*

Laboratory tests

Oxytetracycline concentration in honey samples

Oxytetracycline was detected in all honey samples. The results are reported in Table IV.

Oxytetracycline concentration in the manure

The content of oxytetracycline in manure was 75 mg/kg at the beginning of the experiment, 16 mg/kg after 30 days, and 3.2 mg/kg after 120 days (Figure 6).

Melissopalynological analyses

In all samples, the main pollen components were from *Hedera helix* L. (Ivy), with a frequency higher than 45%, followed by *Rubus fruticosus* L. (thorn-free blackberry), and *Ramnaceae*. A frequency higher than 45% of ivy grains was counted in all samples: Sample A showed a frequency of 73%, sample B 90%, and C 94%. Finally, in samples B and C, the presence of honeydew elements was observed in moderate quantities.

Discussion and conclusions

Honey samples of this experiment were classified as 'ivy unifloral honeys'. The pollen spectra were similar

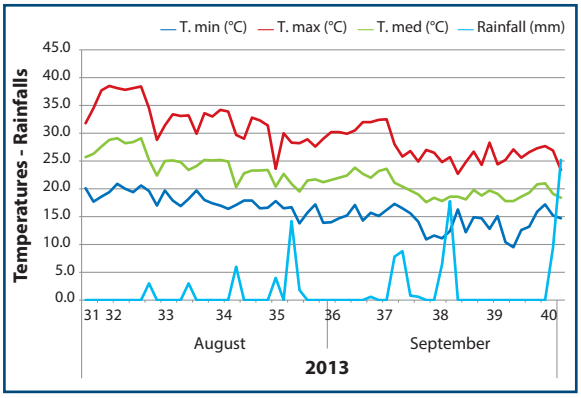

Figure 5. *Temperatures and rainfall recorded during the experimental period.*

Table IV. *Oxytetracycline concentration in honey samples from groups A, B and C.*

Honey sample	Sugar nutrition	Treatment with oxytetracycline	Oxytetracycline (mg/kg)*
A	No	No	0.007
B	Yes	No	0.009
C	Yes	Yes	73.0

*Residual content of Oxytetracycline.

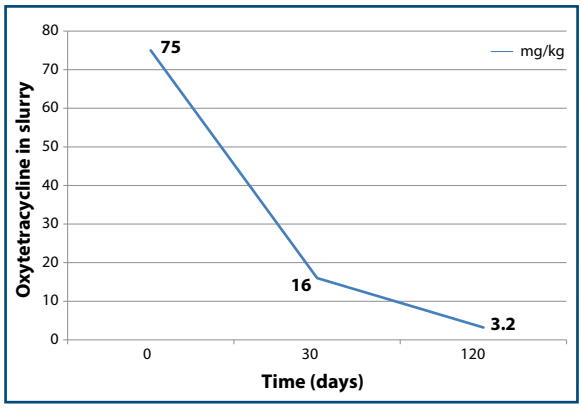

Figure 6. *Residual content of oxytetracycline in the manure.*

and consistent with the geographical origin and the collection period. Indeed, ivy plant blooms in late summer and early autumn are one of the few sources of nectar and pollen in this period of the year.

In summer 2007, a study conducted in Croatia in similar conditions reported that water consumption per beehive was 120 ml per day (Hegic and Bubalo 2007). Since a bee can hold 50 microlitres in its stomach (Balfour *et al.* 2013), in order to meet this demand, in our apiary each hive needed 2,400 water supply trips per day, 14,400 for 6 hives. Harvest of water from manure might occur when bees need a larger supply of water, driving them to collect aqueous waste from livestock holdings, potentially treated with antimicrobials. (Richter *et al.* 2005, Ilari 2011).

Water demand for internal temperature regulation becomes urgent when the outside temperature exceeds 30 °C (Lindauer 1954, Hegic and Bubalo 2007). In our experiment, despite the fact that during 28 days the maximum temperature exceeded 30 °C, bees did not harvest water from manure tanks. We believe that, although more distantly located, bees deliberately preferred a source of clean water. In turn, individuals of 'drone fly' were observed on the manure's surface for several times during the trial, probably to lay eggs. Adult *E. tenax* is able to eat nectar and pollen; it lays eggs in waste waters because its larval stage has saprophagous and coprophagous activities (Altincicek and Vilcinskas 2007). The 'drone fly' can be easily mistaken for a bee (Figure 7). In our opinion, the assumption that bees harvest water from livestock manure could depend by the fact that the two insects may get easily confused for each other. Based on our findings, we cannot conclude that bees harvest water from livestock manure when given at free disposal and in an accessible setting.

Figure 7. *Drone fly (*Eristalis tenax*) evidenced by a yellow circle.*

Honey from group C, produced by bees fed with the aqueous solution of sucrose containing oxytetracycline, showed a contamination level of 73.0 mg/kg, while honey from groups A and B showed very low concentrations, 0.009 mg/kg and 0.007 mg/kg, respectively, despite the lack of therapeutic treatment in these hives. Although these latter results are close to the limit of detection of the method, honey from groups A and B are not in compliance with Regulation 37/2010/EU.

We hypothesise that the presence of residues is caused by the bee drift phenomenon. Bees are, indeed, characterised by specific chemical-olfactory mechanisms, allowing them to identify individuals from their own colony (Hamilton 1964 a, b). However, there is a variable percentage of bees missing from the original beehive. In this study, the experimental field in which hives were lined up, 1 metre apart, was characterised by the same shapes, colours, and orientation. This might have enhanced the possibility of bee drift. As shown by Pfeiffer and Crailsheim (Pfeiffer and Crailsheim 1998), white beehives placed at a mutual distance of 26 cm apart showed a 22% mean percentage of foreign bees. We can therefore speculate that the antibiotic was

Table V. *Chemical composition and physical characteristics of honey in relation to the limits established by Directive 110/2001/EC.*

	Honey A	Honey B	Honey C	Limits*
Glucose (G)	32.3	41.8	39.0	Not provided
Fructose (F)	28.0	31.7	30.9	Not provided
G + F	60.3	73.5	69.9	> 60
Sucrose	0.25	1.14	2.84	< 5
Diastase activity	21.8	15.9	18.4	> 8
HMF	< 0.6	< 0.6	< 0.6	< 40
Acidity	19.5	14.7	13.5	< 50
Insoluble substances	0.06	0.04	0.07	< 0,1
pH	4.01	4.14	4.10	Not provided
Conductibility	0.32	0.27	0.24	< 0,8
Humidity (Moisture content)	19.5	18.9	17.5	< 20

transferred to beehives A and B by bees of group C missing their own beehive.

This scenario may have important consequences for the honey industry. Indeed, illegal antibiotic treatment of a single beehive may result in contamination of untreated beehives. Reasonably, more experiments are warranted in order to disentangle this important phenomenon.

References

Accorti M. 1985. Valutazione numerica degli adulti di *Apis mellifera* L.: variazioni e modifiche al metodo dei sesti. *Apicoltura*, **1**, 63-73.

Altincicek B. & Vilcinskas. A. 2007. Analysis of the immune-inducible transcriptome from microbial stress resistant, rat-tailed maggots of the drone fly *Eristalis tenax*. *BMC Genomics*, **8**, 326.

Balfour N.J., Garbuzov M. & Ratnieks F.L.W. 2013. Longer tongues and swifter handling: why do more bumble bees (*Bombus* spp.) than honey bees (*Apis mellifera*) forage on lavender (*Lavandula* spp.)? *Ecol Entomol*, **38**, 323-329.

Bogdanov S. 2009. Harmonised methods of the International Honey Commission. International Honey Commission. http://www.ihc-platform.net/ihcmethods2009.pdf.

Corradini C., Cavazza A. & Bignardi C. 2012. High-performance anion-exchange chromatography coupled with pulsed electrochemical detection as a powerful tool to evaluate carbohydrates of food interest: principles and applications. *Int J Carbohydrate Chem*, ID: 487564, 1-13.

Cristofani E., Antonini C., Tovo G., Fioroni L., Piersanti A. & Galarini R. 2009. A confirmatory method for the determination of tetracyclines in muscle using high-performance liquid chromatography with diode-array detection. *Anal Chimica Acta*, **637**, 40-46.

Danka R.G. & Beaman L.D. 2007. Flight activity of USDA-ARS Russian honey bees (Hymenoptera: Apidae) during pollination of lowbush blueberries in Maine. *J Econ Entomol*, **100**, 267-272.

European Commission (EC). 2001. Council Directive 2001/110/EC of 20 December 2001 relating to honey. *Off J*, **L 010**, 12/1/2002, 47.

European Commission (EC). 2010. Commision Regulation 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. *Off J*, **L 015**, 20/01/2010, 1-72.

Hamilton W.D. 1964a. The genetical evolution of social behaviour I. *J Theoretical Biol*, **7**, 1-16.

Hamilton W.D. 1964b. The genetical evolution of social behaviour II. *J Theoretical Biol*, **7**, 17-52.

Hegić G. & Bubalo D. 2006. Hygienic water supply for the bees. *J Central Eur Agr*, **7**, 743-751.

Ilari M. 2011. Allevamenti zootecnici e inquinamento da antibiotici per le api. *Apitalia*, **5**, 8-11.

Kiihnholz S. & Seeley T.D. 1997. The control of water collection in honey bee colonies. *Behavioural Ecol Sociobiol*, **41**, 407-422.

Lindauer M. 1954. Temperaturregulierung und Wasserhaushalt im Bienenstaat. *Zeitschrift für vergleichende Physiologie*, **36**, 391-432.

Louveaux J., Maurizio A. & Vorwohl G. 1978. Methods of Melissopalynology. *Bee World*, **59**, 139-157.

Mantovi P., Bonazzi G., Brambilla G., Migliore L. & Barchi D. 2008. Tetracicline nei liquami, il prosciutto non rischia. *Suinicoltura*, **4**, 52-55.

Mantovi P., Soldano M. & Fabbri C. 2009. Tetracicline nei liquami. Nessun effetto sul biogas. *Suinicoltura*, **8**, 73-77.

Marchetti S. 1985. Il "metodo dei sesti" per la valutazione numerica degli adulti in famiglie di *Apis mellifera* L. *Apicoltura*, **1**, 41-61.

Ouchemoukh S., Schweitzer P., Bachir Bey M., Djoudad-Kadji H. & Louaileche H. 2010. HPLC sugar profiles of Algerian honeys. *Food Chem*, **121** (2), 561-568.

Persano Oddo L., Piana M.L. & Sabatini A.G. 1995. Conoscere il miele. Guida all'analisi sensoriale. Bologna, Avenue Media.

Pfeiffer K.J. & Crailsheim. K. 1988. Drifting of honeybees. *Insectes Sociaux*, **45**, 151-167.

Richter D., Bogdanov S. & Edder P. 2005. Résidus d'antibiotiques - de la fosse à lisier au miel? *Rev Suisse Apiculture*, **3**, 20-24.

Seeley T.D. 1995. The wisdom of the hive. The social physiology of honey bee colony. Harvard University Press, Cambridge, MA, 65-66.

Von Der Ohe W., Persano Oddo L., Piana M.L. & Martin P. 2004. Harmonized methods of Melissopalynology. *Apidologie*, **35**, 18-25.

Winston M.L. 1991. The biology of honey bee. Harvard University Press Academic Press Cambridge, MA. 120-121.