EuroReference
journal of Reference
anses
French agency for food, environmental and occupational health & safety

Journal No. 8
Winter 2012

Research

Towards the development of robust protocols for the establishment of MRLs for veterinary drugs in honey

Richard J. Fussell [Richard.Fussell@fera.gsi.gov.uk] (1), Jack F. Kay (2), Eric Verdon [eric.verdon@anses.fr] (3), et Matthew Sharman (1)
(1) Food and Environment Research Agency, Sand Hutton, York, North Yorkshire, United Kingdom
(2) Veterinary Medicines Directorate, Woodham Lane, New Haw, Addlestone, United Kingdom
(3) ANSES - Fougères Laboratory, La Haute Marche, Javené, Fougères, France

Despite widespread concern about the decline in the health of bee colonies in Europe and the rest of the world, beekeepers have access to relatively few veterinary medicines to treat bee diseases. This situation is unlikely to improve in the absence of an internationally agreed protocol for the establishment of Maximum Residue Limits (MRLs) for these medicines in honey. Therefore, a research project has been initiated to develop a statistically valid and harmonised sampling protocol to provide the robust scientific data needed to assist regulators in proposing MRLs for veterinary drugs in honey.

Introduction

Whilst rigorous guidelines exist for calculating the withdrawal time for veterinary medicines in most food producing species, these are not well defined for bees/honey. There is currently no robust protocol for conducting studies which would provide the data necessary to support an application for approval of a treatment and to establish a Maximum Residue Limit. The situation is not helped by the fact that bees are considered a minor species so there is little financial incentive for pharmaceutical companies to invest in the development of new treatments.

The European Commission "*Notice to applicants and Guideline - Veterinary medicinal products - Establishment of maximum residue limits (MRLs) for residues of veterinary medicinal products in foodstuffs of animal origin (Volume 8)*" states that for honey, a depletion study (to determine a withdrawal period) should comprise 5 samples from each of 5 hives, at time points defined according to the period of treatment and the production of the honey. Although helpful, these guidelines could be subject to interpretation.

Outside of the European Union there are no international agreements on setting MRLs in honey. This was highlighted by the fact that the Joint FAO/WHO Expert Committee on Food Additives (JECFA) recently informed the Codex Committee on Residues of Veterinary Drugs in Food (CCRVDF) that there were no recommendations and/or procedures that JECFA could follow for setting MRLs in honey (FAO 2011). In response to this the Codex Committee on Residues of Veterinary Drugs in Food (CCRVDF) set up a working group to:

- collate data on treatments that have been authorised throughout the world ;
- identify common or related parameters used when authorising treatments ;
- propose a risk assessment policy for setting appropriate limits in honey (Codex 2011).

The working group reported that substances to be considered as possible bee treatments should be categorised on the basis of known toxicity, fate and behaviour. For substances considered to be safe it was proposed that a residue study could be waived (e.g. thymol). For substances (e.g. tetracycline) with an established Acceptable Daily Intake (ADI) and/or MRL in a food producing animal or food commodity extrapolation to bees may be possible subject to a depletion study which would also determine an appropriate marker residue. Substances

which are not approved for use in food animals, or which are new drug entities, would require a full residue study, which may not be financially viable for a minor species such as bees.

Such residue studies will set a challenging task because of the need to take into account many variables. Previous studies have identified a large number of factors, including the variability of residue concentrations within and between hives, the effects of timing and application of the treatment, the properties of the substance used for treatment as well as seasonal and climatic factors. A recent study (Fussell et al 2012) concluded that the size of the sample collected and the number of hives in the study should be sufficiently large to minimise the variability in the

Research

measurement of residue concentrations, including sampling, to acceptable values.
The CCRVDF working group considered the evidence available from a recent study (Fussell 2012), together with previous studies, and has proposed a draft protocol for conducting residue studies for honey. The CCRVDF recommendation is to collect all of the honey in supers available from a minimum of five treated hives at each of a minimum of eight different time points. The intervals between the time points should be set to characterise the typical depletion profiles observed in previous studies (see Figure 1). A minimum of five 'control hives' should not be treated while being maintained at a separate location at a distance sufficient to avoid cross contamination by drifting of bees from treated hives.
The Veterinary Medicines Directorate, an agency of the UK Government, is funding a new study to evaluate this CCRVDF draft protocol. The project, being conducted by the Food and Environment Research Agency in the UK, will focus on the development of statistically based methods for collecting samples of honey. It will take place over a minimum of three years in order to take into account seasonal and climatic variations. This is important because the ways in which bees store and move honey within and between hives could influence the distribution of the medicines. The first phase of the project, a field study involving five control hives and 45 treated hives started in May 2012. The hives were treated in late Spring with a 'model compound' (ciprofloxacin), a fluoroquinolone antimicrobial, which was dissolved in syrup solution and applied onto the bees between the frames. Ciprofloxacin was selected because it is stable and yields residues that are measurable by Liquid Chromatography tandem quadrupole Mass Spectrometry.
The honey from all frames in the individual supers (1) from each hive will be extracted, combined and thoroughly mixed to produce a bulk sample to represent that particular super. The results of replicate analysis will be averaged to give the mean residue concentration in each of the individual supers. The results for the individual supers will then be averaged to give the mean residue concentration for each hive. Therefore, the smallest unit for the purposes of statistical analysis will be an individual super. If the results from 2012 demonstrate that satisfactory data can be obtained using fewer hives, then this will be reflected in the experimental design for second phase of the project due to start in 2013. It is envisaged that a reduction in hive numbers in subsequent phases in Years 2 and 3 will encourage collaborators from around the world to contribute. This would enable an assessment of the effects of a greater number of factors including the different genetic strains of bees, different bee husbandry practices, different treatment practices and geographic factors, leading to the generation of a much more comprehensive data set.
Anses-Fougères, as a Reference Laboratory for veterinary drug residues in food, expressed a specific interest in this study. Following the recent signing of a Memorandum of Understanding between Fera-York and Anses-Fougères for potential future scientific collaboration on veterinary drug residues, Anses-Fougères will contribute analytical expertise for the determination of antimicrobial residues in honey and be involved in the statistical assessment of the emerging data.
The objective of this research project is not to support the authorization of any specific product but to develop a protocol that can be used to generate robust residue depletion data.
Data from these experiments will provide statistical information that could be used to better define the experimental requirements for a suitable residue depletion study and hence assist with the calculation of fit-for-purpose withdrawal periods.

Conclusion

It is anticipated that this study will provide a statistically valid protocol that can be used by the UK and others to assist with the establishment of MRLs in honey.
The study will directly support international initiatives within the EU, the Codex Committee on Residues of Veterinary Drugs in Food and JECFA.

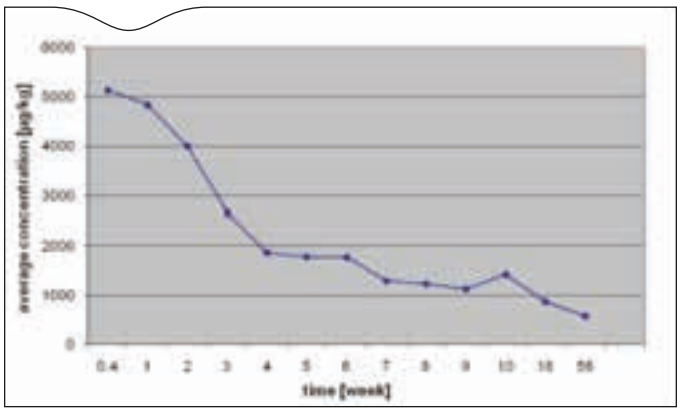

Figure 1. Depletion of ciprofloxacin in super honey collected up to 56 weeks after treatment (based on results averaged from nine individual hives

Spray application of medicine in syrup solution

(1) Supers are the individual boxes that are placed above the brood box, and in which honey is stored prior to collection for human consumption.

Journal No. 8 Winter 2012

Research

References

Codex (2011) Discussion Paper on the Policy for the Establishment of MRLS or other limits in honey, *Codex Alimentarius* Commission, CX/RVDF 12/20/14, December 2011

European Commission (2005) http://ec.europa.eu/health/files/eudralex/vol-8/pdf/vol8_10-2005_en.pdf

FAO (2011). ftp://ftp.fao.org/docrep/fao/011/i0659e/i0659e01.pdf

Fussell RJ, Heinrich K, Dickinson M, Wilkins S, Roelofs V, Murray A, Kay JF, Sharman M. (2012) Investigation into the Experimental Protocols Required to Determine Maximum Residue Limits (MRLs) in Honey. Drug Testing and Analysis, *submitted*

Acknowledgements

The financial support of the Veterinary Medicines Directorate of the UK Department for Environment, Food and Rural Affairs is gratefully acknowledged.

Other publications

Adams S, Heinrich K, Hetmanski M , Fussell RJ, Wilkins S, Thompson HM, Sharman M. (2007) Study of the depletion of tylosin residues in honey extracted from treated honeybee (*Apis mellifera*) colonies and the effect of the shook swarm procedure. Apidologie, 38, 315-322

Adams S, Heinrich K, Fussell RJ, Wilkins S, Thompson HM, Ashwin H, Sharman M. (2008) Study of the distribution and depletion of chloramphenicol residues in bee products extracted from treated honeybee (*Apis mellifera*) colonies. Apidologie, 39, 537-546.

Adams S, Fussell RJ, Dickinson M, Wilkins S, Sharman M. (2009) Study of the depletion of lincomycin residues in honey extracted from treated honeybee (*Apis mellifera*) colonies and the effect of the shook swarm procedure. Analytical Chimica Acta, 637, 315-320