Perspective
The incidence of honey bee pests and diseases in England and Wales

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Abstract: The Central Science Laboratory (CSL) National Bee Unit (NBU) has been responsible for maintaining the Integrated Bee Health Programme in England and Wales since the early 1990s. The role of the Bee Health Programme is to protect the honey bee, a major pollinator of agricultural and horticultural crops and wild flora, and to provide up-to-date technical support to beekeepers. The Bee Health Programme is funded in England by the Department for Environment, Food and Rural Affairs (Defra) and in Wales by the Welsh Assembly Government (WAG). The work includes inspection of honey bee colonies, disease and pest diagnosis, development of contingency plans for emerging threats, minimising the risk of introduction of potentially serious exotic pests and diseases through importation by import risk analysis and related extension work and consultancy services to both government and industry. There is also an underpinning programme of research and development.

Keywords: honey bee; health; pests; protection; inspection

1 INTRODUCTION

There are an estimated 274 000 colonies of honey bees in the United Kingdom, kept by about 44 000 beekeepers. Some 230 000 colonies are managed by 32 900 beekeepers in England,[1] with the remainder being found in Northern Ireland, Scotland and Wales. Around 200 beekeepers manage bees on a professional basis and are members of the Bee Farmers' Association; collectively they manage around 40 000 colonies. The remainder are small-scale producers, many of whom are members of national and local beekeeping associations. Bees make an essential contribution to agriculture and the environment through pollination. Here, the honey bee (*Apis mellifera* L.) plays a dominant role through the pollination of both wild plants and commercial crops. Honey bees are currently the major managed pollinator available within the UK,[2] although both bumblebees and solitary bees also have an important role. Recent estimates for agricultural and horticultural crops grown commercially in the UK that benefit from bee pollination are in the region of £200 million per annum.[2] In addition to this, the bees also produce honey and wax.[2,3]

Honey bees, as well being affected by contaminants and pesticides, can be affected by a range of diseases, pests and parasites[4] that are of importance for the health of colonies and also from the point of view of regulation and the movement of bees in trade around the world. Pests and diseases can cause high levels of colony losses, creating a reduction in available pollinators for important crops. Recent experience with a lack of bees available for almond pollination in California demonstrates this.[5,6]

The Integrated Bee Health Programme is run by the National Bee Unit (NBU), which is part of the Central Science Laboratory (CSL), a Defra executive science agency, on behalf of core Defra policy customers (Defra Plant Health Division) and the Welsh Assembly Government (Department for Environment, Planning and the Countryside). The NBU has a long track record in bee husbandry and bee disease control (since 1946) and in 1994/1995 took on and modernised the bee inspection services.[7] The programme is funded to safeguard the bee population owing to the major importance of this to the environment and economy, and is underpinned by a programme of research and development to provide up-to-date technical support to beekeepers. The work includes disease and pest diagnosis, research into bee health matters, development of contingency plans for emerging threats, import risk analysis and related extension work and consultancy services to both government and industry.

2 STRUCTURE OF THE NBU

The NBU consists of the home-based inspectorate team and the laboratory diagnostic team based at

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(*Received 8 March 2006; revised version received 26 September 2006; accepted 19 October 2006*)
Published online 19 September 2007; DOI: 10.1002/ps.1461

Published by John Wiley & Sons, Ltd. *Pest Manag Sci* 1526–498X/2007/$30.00

CSL, York. In addition to the NBU staff, members from a range of teams within CSL are heavily involved in cross-group working with supporting research into bee health issues.

2.1 The Bee Health Inspectorate

The inspectorate team consists of approximately 45 home-based members of staff. It is headed by the National Bee Inspector (NBI), whose role it is to run the statutory disease control and training programmes. The NBI has management responsibility for eight home-based regional bee inspectors (RBIs), one heading each of the seven regions in England and one covering Wales. The RBIs in turn manage a number of experienced seasonal bee inspectors (SBIs). The RBIs and SBIs in England organise inspections under the Bee Diseases and Pests Control (England) Order 2006 SI 2006/342 (there is similar legislation for Wales, Scotland and Northern Ireland), submit suspect samples for diagnosis, treat colonies for foul brood and train beekeepers in bee husbandry for better disease control and greater self-sufficiency. In addition, the bee inspectors also collect honey samples for residue analysis under the statutory honey collection agreement with Defra Veterinary Medicines Directorate (VMD). With the small hive beetle (SHB), *Aethina tumida* Murray (Coleoptera, Nitidulidae), and *Tropilaelaps* spp. now being notifiable under UK and EU law, inspectors also undertake surveillance for these exotics in 'at-risk apiaries', for example close to ports of entry.

2.2 Bee disease diagnostic team

The NBU's diagnostic team provides a rapid, modern diagnostic service for both the inspection service and beekeepers. The NBU laboratory is Good Laboratory Practice (GLP) compliant, a quality accreditation scheme administered by the Department of Health. All diagnostic tests are conducted according to the OIE (Office International des Epizooties) Manual of Standard Diagnostic Tests and Vaccines. The OIE is the world organisation for animal health and produce internationally recognised disease diagnosis guidelines. Specialists, including microbiologists, support the diagnostic service. Across CSL, diagnostic support is provided from teams of microbiologists, acarologists, insect virologists and molecular specialists in the CSL Molecular Technology Unit (MTU).

3 BEE LEGISLATION AND STATUTORY DISEASES IN ENGLAND AND WALES

The Bees Act 1980 UK empowers Agriculture Ministers to make Orders to control pests and diseases affecting bees, and provides powers of entry for authorised persons. Under the Bees Act, The Bee Diseases and Pests Control (England) Order 2006, SI 2006/342 designates American foulbrood (AFB), European foulbrood (EFB), *A. tumida* (SHB) and *Tropilaelaps* mites as notifiable pests and defines the action that may be taken in the event of outbreaks.

At the European level, the Directive on animal health requirements for trade in bees is called the Balai Directive 92/65/EEC and lists AFB, SHB and any species of the *Tropilaelaps* mite as notifiable pests and diseases throughout the EU.

3.1 Incidence of bee pests and diseases in England and Wales

Of the four notifiable diseases under UK and EU legislation, EFB and AFB are widespread in parts of England and Wales. Until March 2006, *Varroa destructor* (Anderson & Trueman) was also a statutory notifiable disease, and at the time of writing *A. tumida* and *Tropilaelaps* spp. remain undetected in the UK.[8]

3.2 American and European foulbrood

Honey bees are affected by a range of pests and diseases, but two of the most serious are bacterial diseases that affect the developing brood, i.e. American foulbrood and European foulbrood.

The names American foulbrood and European foulbrood do not relate to their distribution but to where the first scientific investigations were carried out on the diseases. Both diseases have a wide geographical distribution, either one or both being found in most areas where bees are managed.[4,7,9] The causative agent of AFB is the spore-forming bacterium *Paenibacillus larvae* subsp. *larvae* (White), formerly known as *Bacillus larvae*.[10] EFB is caused by the bacterium *Melissococcus plutonius* (Bailey and Collins), formerly known as *Melissococcus pluton*.[10] Both diseases are serious economic threats to beekeeping. AFB, if left unchecked, will always lead to the death of the infected colony. EFB, on the other hand, is sometimes referred to as a stress disease. Colonies displaying signs of EFB, if left untreated, may show signs of complete recovery and the disease signs disappear; however, if the colony is put under stress, the clinical signs of the disease reappear. Inevitably the productivity of these infected hives will be affected. Both diseases are readily transmissible, the primary source of spread being the beekeeper, either through contaminated equipment or by the transfer of frames from infected colonies to healthy colonies. However, infected weak or dead colonies also act as a source of infection when robbed out by other bees.[11] It is therefore important that, in areas where these diseases occur, suitable methods of control are in place to deal with outbreaks.

3.2.1 American foulbrood strategy in England and Wales

With respect to AFB, the UK operates an inspection/eradication policy. There is a well- established system to eradicate AFB in apiaries in which it is detected.[12] There is no use of antibiotics – all infected colonies are destroyed. AFB-infected colonies are killed by the introduction of a small quantity of petrol through the top of the hive when the bees have been

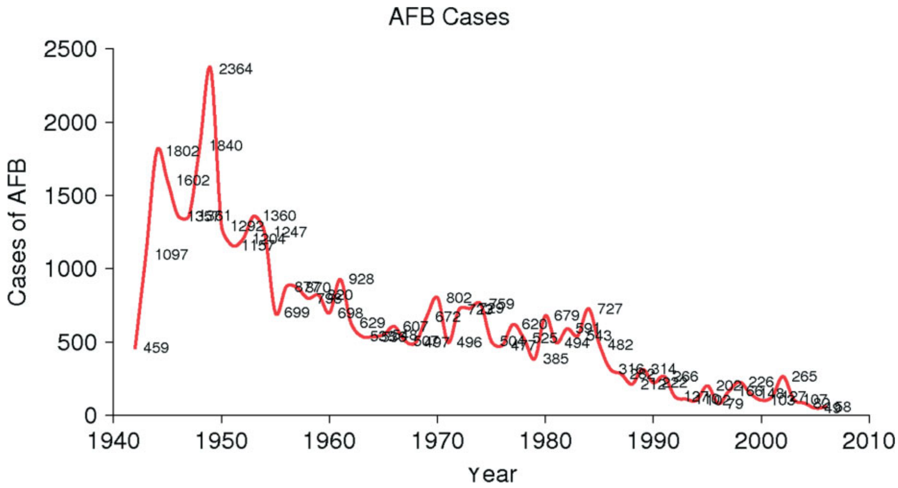

Figure 1. Incidence of American foulbrood in England and Wales, 1942–2006.

sealed in once they have stopped flying (i.e. early in the morning or late at night). The bees, frames and any other moveable frame parts such as queen excluders or quilts are then destroyed by incineration. The hive boxes, floors and other hive parts are sterilized by scorching with a blowtorch. This strategy has resulted in a low incidence of the disease over the last 15 years (Fig. 1). In the season 2005 there were 49 cases of AFB diagnosed from a total of 37 apiaries in England and Wales.[13]

3.2.2 European foulbrood strategy in England and Wales

The system run in England and Wales is more complex for EFB than for AFB; the UK policy is one of inspection/treatment for control. Infected colonies may be treated with either oxytetracycline (OTC; Terramycin®) or the shook swarm method or destroyed on the basis of well-established criteria. As soon as disease is suspected, a sample is sent to the laboratory for diagnosis and the apiary placed under standstill. The decision to treat or destroy is based on the level of infection within the colony; if the colony is heavily infected or too small to respond to treatment, then it will be destroyed. In other cases the colony will be treated either with the shook swarm method (shaking the bees onto clean foundation and destroying the infected comb)[14] or will be treated with OTC. This antibiotic has been used in beekeeping since 1967 by the government veterinary authorities in the UK for the treatment of EFB.[7] It is supplied on prescription by the Veterinary Laboratories Agency (VLA) and applied to the colonies to be treated by an appointed bee inspector. The drug is applied as a single dose of 1 g active ingredient in 200–250 mL aqueous sucrose (64% w/w) poured into empty brood combs in the brood chamber. Only when the apiary is free of clinical signs will the standstill be lifted. If treated with OTC, any honey harvested after the treatment date may not be sold for human consumption for 6 months post-treatment.[15] In the beekeeping season for 2005 there were 675 cases of EFB diagnosed in 243 apiaries. Of these colonies, 222 were destroyed (as for AFB), 263 were treated with OTC and 190 were treated with the shook swarm method.[13]

3.2.3 Development of the shook swarm treatment method

As can be seen in Figs 2 and 3, EFB is well established in England and Wales, with many hundreds of cases

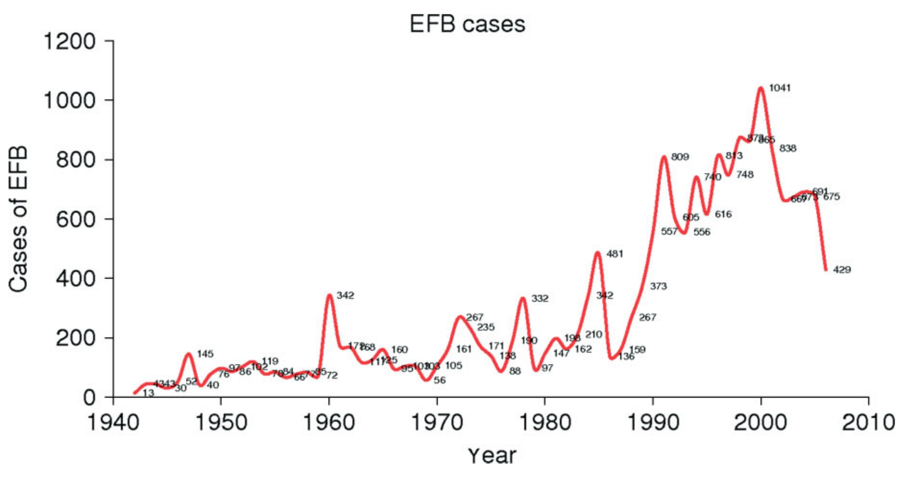

Figure 2. Incidence of European foulbrood in England and Wales, 1942–2006.

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DOI: 10.1002/ps

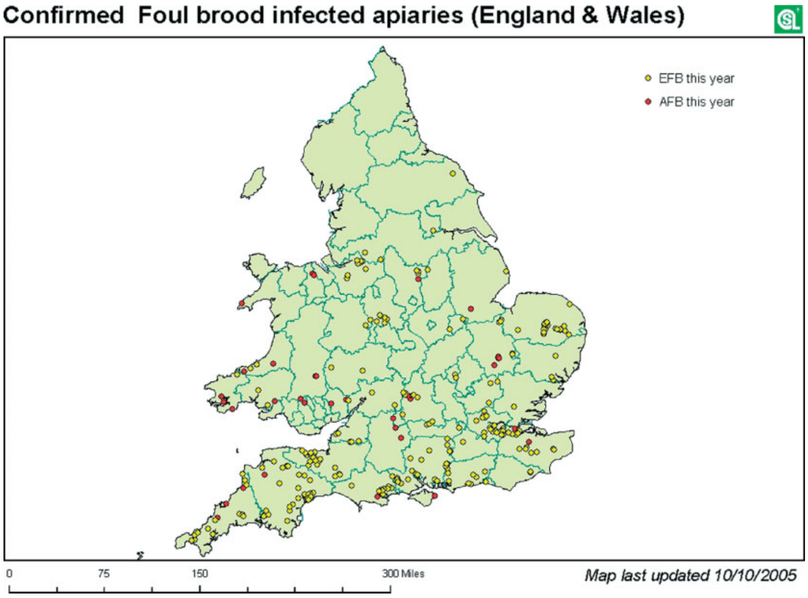

Figure 3. Distribution of apiaries infected by American and European foulbrood in England and Wales, 2005.

of infected colonies found each year. Until recently, if the disease was diagnosed in a colony, there were only two possible options available, either treatment with OTC or destruction.

Evaluation of the use of a husbandry control method for EFB, known as the 'shook swarm', began about 10 years ago at the NBU. One of the major problems with EFB control using antibiotics is the relatively high rate of recurrence from year to year, somewhere in the order of 20–25%. There is also the potential for antibiotic residues to accumulate in hive products.[15] As the shook swarm name suggests, the principle of the technique is that the bees from the infected colony are shaken onto a new foundation and the sources of reinfection (the combs) are removed and destroyed.[14] One of the principal aims behind this investigation has been to determine whether it is possible to develop a method that beekeepers could apply themselves without the need for intervention of an appointed bee inspector.

The potential advantages of using shook swarm include a reduction or possible cessation of the use of the antibiotic OTC, a reduced risk of veterinary medicine residues in the final hive products and an improvement in the control of the disease.[14] Initial experiments investigated whether shook swarm was effective in combination with the use of antibiotic and whether it demonstrated good efficacy and low levels of recurrence. Results obtained supported this theory. In 2004, experiments were initiated to look at control of EFB using shook swarm alone (without the use of OTC). Results to date appear to demonstrate that shook swarm could provide a good level of control of EFB. Work will continue on the technique, including a comprehensive analysis of the data generated, such as parameters on the time of year for applying the method and also if success rates are correlated with levels of disease prior to shaking.

3.2.4 Diagnosis of foulbrood diseases

The laboratory diagnosis of AFB and EFB is a relatively straightforward procedure. Until recently suspected diseased samples were sent to the NBU laboratory, either as whole combs or as diseased larvae in a small vial. The submitted samples were examined for the causative bacterium of the suspected disease using a simple staining technique. This involves examining the samples under light microscopy once they have been stained with an aqueous preparation of the negative stain nigrosine. Although simple, this method is highly effective and is used throughout the world. Owing to the statutory nature of the two diseases in the UK there is a 24h turnaround time on the samples, and the results of the diagnosis must be sent out on the same day as sample receipt. The NBU laboratory still maintains the ability to carry out the microscopic diagnosis of these diseases, but from the 2006 beekeeping season the majority of foulbrood diagnosis will be carried out using rapid field diagnostic test kits.

3.2.5 Development of field testing kits

For some years it has been recognised that there is a need to develop a rapid field diagnostic kit to detect AFB and EFB. The obvious advantages of this would be the reduction in turnaround times, thus allowing any necessary treatments or destructions of infected colonies to be completed much more rapidly, and improved efficiency and targeting of inspection resources. Over the past three years a collaborative project between CSL and Vita (Europe) Ltd has led to the successful development of rapid field diagnostic kits for the diagnosis of both AFB and EFB. These kits are known as lateral flow devices or LFDs.

The kits are based on existing generic technology originally developed by CSL, Pocket Diagnostic™, a team on-site devoted to development of these kits.

Pocket Diagnostic™ has produced similar kits for plant viruses and bacterial pathogens for use by the Defra Plant Health and Seeds Inspectorate (PHSI).[16] The foulbrood LFD kits use monoclonal antibodies to either *P. larvae* subsp. *larvae* (AFB) or *M. plutonius* (EFB), and the technology is fundamentally similar to that used in human pregnancy test kits. Each type of kit is specific for a given disease. They are designed to detect bacteria from symptomatic larvae, so therefore cannot be used as a tentative screening mechanism; they are a confirmatory tool of colonies showing disease signs.

In 2003, the AFB LFD kits were tested for field confirmation of AFB by the inspectorate team. The kits have proven to be completely specific for AFB and highly sensitive. As a result, in the 2004 beekeeping season the AFB LFD kits were used routinely for the first time by the inspectorate. The kits are now used as the routine diagnostic method in both the field and laboratory for any samples submitted for diagnosis.

Following on from the successful development of the AFB LFD kits, work was continued on the development of EFB kits. Laboratory validation has been completed and field validation testing carried out in 2004–2005. Success rates for the kits were 99% in 2004 and 96% in 2005. It is the intention to introduce these kits as the routine diagnostic method for EFB during the 2006 beekeeping season.

3.3 Pyrethroid resistant Varroa mites

Varroa destructor, first discovered in the UK in 1992, is now endemic within the UK and until March 2006 was a statutory notifiable pest. It has now been removed from the statute books. As part of routine field screening carried out by the NBU field inspection team (started in 2000), the first known case of pyrethroid-resistant varroa mites in the UK was discovered in apiaries in Devon in August 2001.[17] Test kits developed in-house enabled detection of pyrethroid resistance in the early stages, prior to widespread colony collapse which had been experienced in other countries. As soon as resistance was identified and confirmed, the NBU undertook a resistance-monitoring programme of neighbouring apiaries around the site affected and stepped up resistance testing in other areas. Resistance to pyrethroids has now been discovered in many areas within England and Wales, and the situation in 2005 is shown in Fig. 4. A training programme has been undertaken to transfer the technology to beekeepers, allowing them to monitor their own stocks of bees.

3.4 Potential exotic threats

With the recent discovery of SHB larvae in a consignment of queens imported into Portugal illegally from Texas, awareness of the potential threat of exotic incursions must be heightened. The potential cost from an accidental exotic incursion can be astronomical; for example, it has been estimated that in New Zealand the additional costs due to the introduction of varroa will be in the region of $NZ 400–900 million over the next 35 years (McMillan D, NZ AgriQuality; private communication).

The NBU field inspection team monitors for *A. tumida* and *Tropilaelaps* spp. as part of a routine surveillance programme for exotic threats. The field inspection team is able to use the NBU IT facilities at its disposal to assist in planning inspections. The NBU has been developing a new online database called BeeBase which currently holds the details of approximately 27 000 beekeepers and 48 000 apiaries (approximately 20 000 active beekeepers and 40 000 active apiary sites), and information from this can be coupled with mapping tools, GIS planning and modelling tools. This allows the NBU to plan inspections by targeting 'at-risk apiaries' to assist with

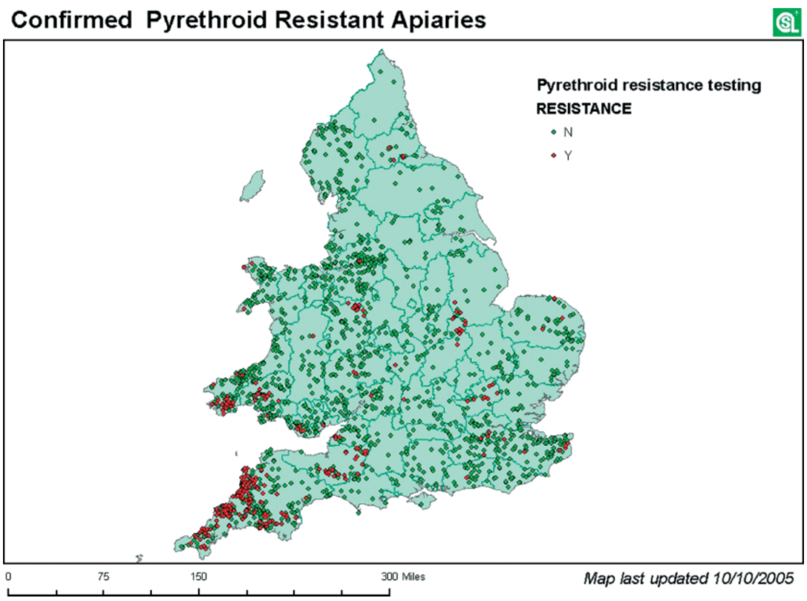

Figure 4. Confirmed pyrethroid-resistant apiaries in England and Wales, 2005.

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DOI: 10.1002/ps

contingency planning in case of exotic incursions, and to model the possible spread and environmental impact if an incursion happens. These resources are not only useful for potential 'at-risk' areas for exotics but for routine disease inspections and management of data. The laboratory team also routinely screens import samples and suspect samples submitted for identification by both beekeepers and the field team.

3.5 Development of a 'one-stop shop' for disease diagnostics

The development of generic techniques enabling testing for a full range of pests and pathogens using single types of assay in a centralised facility will allow for the provision of complete health status checks for bee hives. This approach allows the delivery of very rapid, comprehensive and large-scale testing services both directly to beekeepers and, in the case of survey work, to regulatory bodies. Work at CSL has focused on real-time polymerase chain reaction (PCR; TaqMan®) systems to provide high-throughput diagnostic services in the laboratory.

3.5.1 Taqman® real-time PCR for detection of pests and pathogens

PCR amplifies a specific genetic sequence (DNA or RNA) from a sample. The amplification process means that the target DNA/RNA can be present at very low levels. It can thus be utilised to detect, for example, viruses from mammalian cells. If a sequence of the target organism is known, primers can be designed to attach to the target DNA/RNA. During PCR, the sequence between these primers is replicated by the enzymes DNA polymerase or, for RNA, reverse transcriptase. After several cycles of replication, DNA, whether amplified from DNA itself or from an RNA template, will be amplified to detectable levels. The usual procedure is then to run the product of PCR on an agarose gel. This separates the DNA according to size, after which it can be identified.

The Taqman® (Applied Biosystems, Warrington, UK) process uses real-time PCR. As with 'conventional' PCR, target sections of genetic material are amplified, but with real-time PCR there is no need to run a gel to identify these amplified strands. With real-time PCR the Taqman® process amplifies target nucleotide strands and then, by using a specific probe, is able to identify them immediately. Advantages of this Taqman® system are that it is a very rapid system and also allows large numbers of samples to be processed – as many as 1500 per week.

In 2004, the NBU instigated a survey in England and Wales using this technique. The aim of the project was to collect samples of honey bees throughout both countries and carry out a large-scale survey for viruses. Initially, the research was used to establish whether Kashmir bee virus (KBV) was present in the samples, and other assays have since been developed for a range of other bee viruses.

In this survey, 458 hives were screened and KBV was found at two sites (Fig. 5). Until this survey, it was thought that KBV was exotic to the UK. Over the next 2 years, the NBU would like to be able to put this tool to wider use, such as carrying out further surveys using the TaqMan® assays to determine the health status of bee colonies at a national level.

In addition to the KBV virus survey, further assays using TaqMan® PCR are being developed. As part of the implementation of the national bee health programme for England and Wales, the NBU is currently developing a suite of novel molecular tests to improve the detection and diagnosis of other bee pests and diseases. These tests when developed could be used for large-scale survey work, as well as for routine diagnosis and as a research tool. The NBU aims to develop tests for most of the different pests and diseases of honey bees, a 'one-stop shop' approach. These include honey bee genotypes, foulbrood diseases, pathogenic fungi, tracheal and varroa mites and Nosema, as well as those assays that have already been developed for the viruses. In addition, there is a 2 year programme of funding to develop tests for the full range of pests and diseases of honey bees, including exotics such as tropical mites [beginning with *Tropilaelaps clareae* Delfindo & Baker (Acarina: Laelapidae)] and SHB (all life stages). As well as screening bees, methods are also

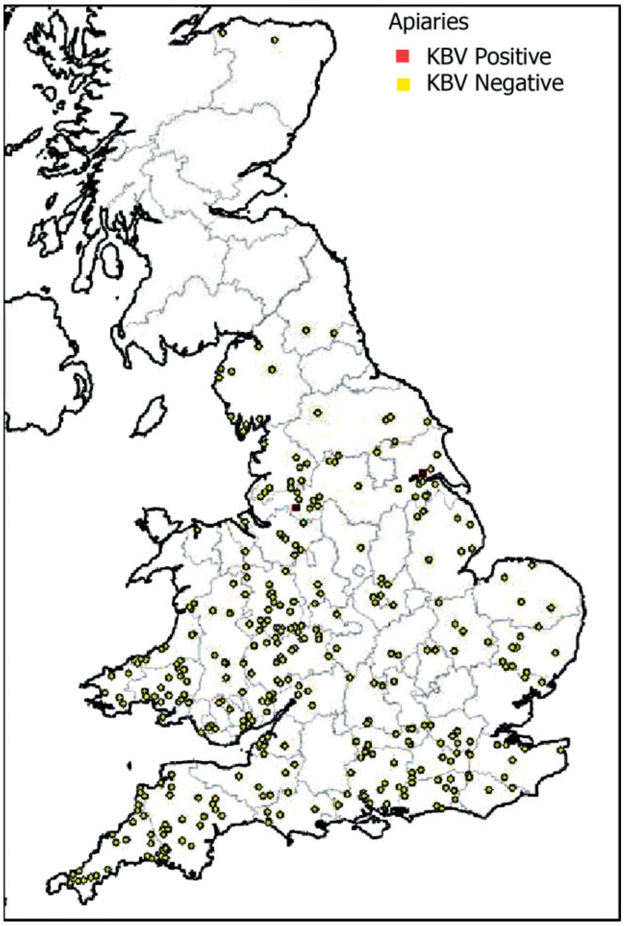

Figure 5. Results of 2004 KBV survey in the UK.

being developed to screen hive debris. Again, it is anticipated that a large number of hive debris samples could be screened rapidly in the event of an incursion of an exotic pest such as the SHB or *Tropilaelaps* mites.

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