



Australian Government

Rural Industries Research and
Development Corporation



RURAL INDUSTRIES
Research & Development Corporation

Use of a Sniffer Dog in the Detection of American Foulbrood in Beehives



AUGUST 2013

RIRDC Publication No. 13/O80



Australian Government

**Rural Industries Research and
Development Corporation**

Use of a Sniffer Dog in the Detection of American Foulbrood in Beehives

by Sharon C. de Wet

August 2013

RIRDC Publication No. 13/080
RIRDC Project No. PRJ-004482

© 2013 Rural Industries Research and Development Corporation.
All rights reserved.

ISBN 978-1-74254-573-8
ISSN 1440-6845

Use of a Sniffer Dog in the Detection of American Foulbrood in Beehives
Publication No. 13/080
Project No. PRJ-004482

The information contained in this publication is intended for general use to assist public knowledge and discussion and to help improve the development of sustainable regions. You must not rely on any information contained in this publication without taking specialist advice relevant to your particular circumstances.

While reasonable care has been taken in preparing this publication to ensure that information is true and correct, the Commonwealth of Australia gives no assurance as to the accuracy of any information in this publication.

The Commonwealth of Australia, the Rural Industries Research and Development Corporation (RIRDC), the authors or contributors expressly disclaim, to the maximum extent permitted by law, all responsibility and liability to any person, arising directly or indirectly from any act or omission, or for any consequences of any such act or omission, made in reliance on the contents of this publication, whether or not caused by any negligence on the part of the Commonwealth of Australia, RIRDC, the authors or contributors.

The Commonwealth of Australia does not necessarily endorse the views in this publication.

This publication is copyright. Apart from any use as permitted under the *Copyright Act 1968*, all other rights are reserved. However, wide dissemination is encouraged. Requests and inquiries concerning reproduction and rights should be addressed to RIRDC Communications on phone 02 6271 4100.

Researcher Contact Details

Dr Sharon de Wet
Department of Agriculture, Fisheries and Forestry,
Health and Food Science Precinct
39 Kessels Road
Coopers Plains 4108 QLD

Email: sharon.dewet@daff.qld.gov.au

In submitting this report, the researcher has agreed to RIRDC publishing this material in its edited form.

RIRDC Contact Details

Rural Industries Research and Development Corporation
Level 2, 15 National Circuit
BARTON ACT 2600

PO Box 4776
KINGSTON ACT 2604

Phone: 02 6271 4100
Fax: 02 6271 4199
Email: rirdc@rirdc.gov.au.
Web: <http://www.rirdc.gov.au>

Electronically published by RIRDC in August 2013
Print-on-demand by Union Offset Printing, Canberra at www.rirdc.gov.au
or phone 1300 634 313

Foreword

American Foulbrood (AFB) is the primary disease of honeybee brood in the Australian apiary industry. Both commercial and hobbyist's apiaries are at risk. State departments have been changing policies to put the onus of controlling endemic diseases back to industry. Since AFB is endemic, industry is under considerable pressure to control the disease without government support. The extent of AFB, which is the major brood disease in Australia, has prompted the Australian Honey Bee Industry Council (AHBIC) to formulate a national AFB control strategy.

A detection method that is reliable and less labour-intensive than methodically scrutinising individual brood frames manually is desirable. The objective of this project was to develop a novel, quick and reliable way of detecting AFB that is user friendly and affordable. Apiarists with a large amount of hives will benefit from a sniffer dog to reduce screening times. The pollination industry can benefit as well by giving Apiarists confidence that only AFB free colonies are being used at pollinator destinations, minimising apprehension about the condition of returning colonies. Both commercial beekeepers and major pollinator providers will benefit from this research.

The sniffer dog (Baz) was trained successfully and met a number of expectations. The dog was able to detect as little as one infected cell in a hive. The bee suit for the dog did not meet expectations and is currently under review. In order to overcome this obstacle, alternative sampling methods were developed. These sampling procedures reduced the time for AFB detection considerably when compared to the conventional method. Sensitivity and specificity when compared to human and laboratory detection was excellent. When calculated on the number of hives inspected (n=51), Baz could detect AFB with a sensitivity of 100% and could also correctly identify the 76% of hives that did not have AFB.

The project recommends that more animals such as this one be trained and used. The owner of the dog is open to offers to utilise Baz on a fee-for-service basis. The trainer is considering training another dog to be available for use by the Queensland community, depending on demand.

The project was a joint venture with combined funding by the owner of the dog, Josh Kennett, RIRDC Honey Bee R & D Program and the Department of Agriculture Forestry and Fisheries, Queensland.

This report is an addition to RIRDC's diverse range of over 2000 research publications and it forms part of our Honeybee R&D program, which aims for a productive, sustainable and more profitable Australian beekeeping industry

Most of RIRDC's publications are available for viewing, free downloading or purchasing online at www.rirdc.gov.au. Purchases can also be made by phoning 1300 634 313.

Craig Burns
Managing Director
Rural Industries Research and Development Corporation

Acknowledgments

The author would like to thank John Covey for making available a number of his hives for the trial, as well as assisting with the sampling. I would also like to thank the Apiary officers of DAFF, Queensland (John Zigterman, Hamish Lamb and Patricia Swift) and Howard Prior from the Biosecurity Sciences Laboratory who did the organisation and sampling of the hives with minimum input from me while I was nursing a very sick child in hospital. The trainer, Martin Dominik, also went above and beyond to accommodate difficult timelines. The owner of the dog, Josh Kennett was a delight to work with and a great help with the sampling of the hives for the trial. Thanks to Elroy, the dog that did not make it due to hip problems and finally: Thanks to Baz, Australia's first and only AFB Sniffer dog!

Abbreviations

AFB	American Foulbrood
DAFF	Department of Agriculture, Forestry and Fisheries, Queensland
RIRDC	Rural Industries Research and Development Corporation

Contents

Foreword iii

Acknowledgments..... iv

Abbreviations..... iv

Executive Summary vii

Introduction 1

Objectives..... 2

Methodology 3

Results 10

Discussion 13

Implications..... 15

Recommendations 16

References 17

Tables

Table 1.	Comparison between human and laboratory detection of AFB in hives with the detection by the sniffer dog, Baz.....	12
Table 2.	Sensitivity of AFB detection by Baz when compared to human and laboratory procedures.....	12
Table 3.	Specificity of AFB detection by Baz when compared to human and laboratory procedures.....	12

Figures

Figure 1.	Baz, the black Labrador chosen to be the AFB detection dog.....	3
Figure 2.	Fresh, healthy larvae were infected with 50 µl of an AFB spore suspension in 5 mL polypropylene tubes.....	5
Figure 3.	AFB infected bee larvae incubated at 37°C in CO ₂ atmosphere at different stages to demonstrate the changes from liquefaction up to dried matter simulating scale.....	5
Figure 4.	The contents from affected cells were scooped out of cells and placed into 5 mL polypropylene tubes with a screw lid.....	6
Figure 5.	Samples positive for AFB, EFB and <i>P. alvei</i> , as well as disease free comb was supplied to test the dog's ability to select AFB positives and ignore the non-AFB samples.....	6
Figure 6.	Odour-infused muslin was provided to the trainer in 5mL polypropylene tubes for detection of AFB.....	7
Figure 7.	Polypropylene tubes containing muslin were inserted between the frames the day prior to sampling the hives by an Apiary officer and had to be removed on sampling day to present it to the dog away from the bees.....	7
Figure 8.	Histology cassettes used to enclose muslin cloth. The cassettes were inserted at the bottom of the hives with the aid of a long cable tie.....	8
Figure 9.	A team of 3 Apiary officers and the owner of the hives took 3 days to examine 51 hives.....	9
Figure 10.	Baz indicates an AFB positive sample hidden in the mock hive by sitting down next to the box....	10
Figure 11.	Muslin cloth inside polypropylene tubes between the frames was pulled from the tubes by the bees and in some cases, even found outside the hives.....	11

Executive Summary

What the report is about

This report describes the training and validation of a sniffer dog for detection of American foulbrood (AFB) in bee hives.

Who is the report targeted at?

This report is targeted at AHBIC, Plant Health Australia (PHA), the RIRDC, Honeybee Advisory Committee (HAC), industry, State Departments of Primary Industries and scientists interested in control of honeybee pests and diseases.

Where are the relevant industries located in Australia?

AFB is the major endemic brood disease in Australia. Both commercial and amateur apiaries are at risk. Another relevant party is the agricultural industry reliant on commercial pollination. The sniffer dog currently resides in South Australia (Tintinara). The owner is willing to make the dog available to industry and private beekeepers for screening of hives. The trainer of the dog resides in Queensland (Burpengary) and is considering training another dog if there is enough demand for this screening.

Background

Aims/objectives

The objective of this project was to develop a novel, quick and reliable way of detecting AFB that is user friendly and affordable. A detection method that is reliable and less labour-intensive than methodically scrutinising individual brood frames is desirable. Commercial apiarists with a large number of hives will benefit from a sniffer dog. Screening hives at pollinator destinations will give Apiarists confidence that only AFB free colonies are being used, minimising apprehension about the condition of returning colonies. This may be powerful technology to aid the proposed National AFB control strategy.

Methods used

The sniffer dog: A 19 month old Springer Spaniel (Elroy) was initially chosen and trained. A problem with its hip did not resolve after several physiotherapy treatments. A second dog, four year old black Labrador (Baz) was trained simultaneously and was finally chosen to be the animal for the project.

Training of the sniffer dog: The animal was trained according to internationally recognised standards and protocols for detection dogs. Initially a suit for protection of the dog against bee stings was trialled, but found to interfere with detection. It was found that the dog could not effectively judge distance with the helmet of the protective suit which kept knocking against the hive boxes and broke the dog's concentration. The initial plan for the dog to run through the apiary, sniffing individual boxes, had to be modified. Active and passive sampling techniques were developed to enable the dog to detect the odour away from the hives.

Sample preparation:

A range of samples were prepared to supply the correct odour for recognition and indication by the sniffer dog. Of essence was the need to provide the odour in a pure and concentrated form for training. A number of control odours the dog will encounter in a hive but need to ignore such as wax foundation, frames, fresh, healthy brood as well as European Foulbrood (EFB) with and without *Paenibacillus alvei* was provided.

Results/key findings

The training of the dog was successful and Baz has been handed over to the owner. The owner and the dog were compatible and the subsequent conditioning and management of the animal was transferred to the owner. The initial proposal for the dog to wear a bee suit was unsuccessful and alternative sampling techniques were developed. In the controlled study the sniffer dog detected AFB in hives with a sensitivity of 100% and a specificity (the measure of correctly identifying true negatives) of 76% (n=51 hives; n=408 frames) when compared to human detection and laboratory results. Under field conditions, Baz is able to detect one single infected cell in a hive.

This research will benefit the apiary industry by providing a very sensitive user friendly detection system for AFB. This technology can be a powerful tool to benefit the proposed national AFB control strategy. Early detection of AFB in singular hives as well as in a whole apiary is paramount in the control of the disease.

Implications for relevant stakeholders

Industry can benefit widely by adopting this technology by minimising labour and time to detect AFB. Early detection is the single most important factor in controlling AFB. Providing this service for the commercial pollination industry can potentially expand this service by removing apprehension about contracting the disease when colonies from diverse destinations are brought in close proximity.

Recommendations

These recommendations are targeted at AHBIC, Plant Health Australia (PHA), the RIRDC Honeybee Advisory Committee (HAC), industry, State Departments of Primary Industries and scientists interested in control of honeybee pests and diseases.

It is recommended that adoption of this technology be seriously considered as a tool for the proposed national AFB control strategy. The owner (Josh Kennett, South Australia) is willing to make the dog available to industry and private beekeepers for screening of hives. The trainer of the dog (Martin Dominick) resides in Queensland (Burpengary) and is considering training another dog if there is enough demand for this screening. The methodology for providing the concentrated correct odour have been developed by the Biosecurity Sciences Laboratory and can be utilised for training more animals.

Introduction

The use of sniffer dogs to aid in searching has been well documented over the years. Apart from the use at airports searching for foodstuffs and illegal drugs, their use by military forces in Afghanistan and Iraq to detect explosives has saved many lives. Sniffer dogs are said to be the most reliable way to detect explosives such as roadside bombs. Sniffer dogs are now entering new fields such as bedbug, termite and mould detection in houses and forms of cancers in patients. Their sense of smell is incredible - they can smell as little as one buried truffle in the area of a football field, or a single termite under the floor of a house. The State of Maryland has trained sniffer dogs to detect American Foulbrood (AFB) since the 1970's and are still using them.

For this project, a dog (Baz) was trained to successfully detect the presence of American Foulbrood (AFB) in bee hives. AFB is a lethal bacterial disease that affects the brood of honeybees in the larval stage of development. The infected larvae die and decay in their cells, then form a scale that the bees cannot or will not remove, rendering the cell uninhabitable. This scale releases infectious spores that can then be transferred by bees, honey, hive material or beekeeping equipment. These spores are invisible to the naked eye. The decaying brood however has a unique odour. The dog was trained to detect this odour. Samples were provided to ensure that the odour for the disease in all stages, from freshly infected brood through to scale formation was imprinted on the dog. The dog was trained to search samples from an apiary and indicate hives that had the odour of AFB. The hive can then be inspected and samples taken for testing. This process could be far more economical because it can be done in a fraction of the time of visual inspection which involves looking for what could be just a single cell in an apiary. The average human inspector can inspect 45 hives per day, compared with the sniffer dog that can screen samples from several hives in minutes. AFB is usually not found until the infectious scale has formed because it is then that it looks the most different from European Foul Brood (EFB) and *Paenibacillus alvei*. Not identifying AFB until this stage means that cross infection may have already occurred. With the dog detecting AFB in its early stages it would greatly reduce the risk of cross-infection and destruction of far less hives. The loss of profit from destroying hives is substantial, as it involves not only loss of the boxes and the frames but also loss of production. The only other alternative to burning hives is radiation treatment, but it is very expensive when the treatment, labour and interstate freight costs has been calculated.

As AFB is endemic, State departments have been changing policies to put the onus of controlling endemic diseases back to industry. The extent of AFB, which is the major brood disease in Australia, has prompted the organising of a workshop by AHBIC, Plant Health Australia and the RIRDC Honeybee Advisory Committee (HAC) to formulate a national AFB control strategy.

There has been no real success in combating this disease. The use of antibiotics to control AFB is a non-viable option. Antibiotics only treat the symptoms and do not kill the spores that transfer the disease. The only successful way to control AFB is good management by the apiarist. This involves regular visual inspections of the brood nests, regular honey testing and the use of a good "barrier" system where hive material, including nucleus hives, is not transferred to other apiaries, and all equipment is cleaned thoroughly before use. Unfortunately not all apiarists are entirely diligent, and this can cause major problems because apiaries become neglected. The disease spreads quickly through the apiary from hives dying out and honey being robbed by other hives. Other apiaries in the vicinity are also likely to contract AFB. Management techniques may improve with a quick and simple detection of AFB. Apiary inspectors could use the dog to quickly indicate affected hives.

Objectives

The objective of this project was to develop a novel, quick and reliable way of detecting AFB that is user friendly and affordable. Techniques to supply the dog trainer with a pure odour of AFB in all stages of decay were developed. A suit for the dog to wear during inspection of hives was tested and sampling techniques developed to enable the dog to scrutinise hives for detection of AFB in the field.

Methodology

The sniffer dog: A 19 month old Springer Spaniel (Elroy) was initially chosen and trained. A problem with its hip did not resolve after several physiotherapy treatments. A second dog, four year old black Labrador (Baz) was trained simultaneously and was finally chosen to be the animal for the project (Figure 1). A veterinary examination to ensure the animal is healthy was done. An allergy test for bee stings was conducted during this time as well.



Figure 1. Baz, the black Labrador chosen to be the AFB detection dog.

Training of the sniffer dog: The animal was trained according to internationally recognised standards and protocols for detection dogs. Obedience training was followed by conditioning of the dog to recognise and indicate the correct AFB odour. The dog was conditioned to ignore odours of foundation, frames, fresh, healthy brood as well as European Foulbrood (EFB) with and without *Paenibacillus alvei*. The dog was also conditioned to ignore the odour from all vessels the target odour was provided in. This was done by offering the sterile, 5mL polypropylene tubes for instance, and the dog conditioned to ignore the odour thereof.

The first stage of training involved obedience and searching techniques. A mock apiary was constructed. Thirty painted bee boxes and lids, complete with one or two frames and wax foundation was supplied and used to construct a mock apiary. Once the dog has learnt the searching techniques, the trainer began teaching the dog to detect the odour of AFB. This stage was followed by conditioning the dog to ignore other odours associated with the hive. The dog was also trained to ignore odours emitted by EFB and brood infected with *P. alvei*.

Suit: A suit was made for the dog. The aim of this was to allow the dog to comfortably walk through the apiary without fear of being stung and without impairing its sense of smell. Materials were sourced by Joshua Kennett, the owner, using a local canvas business to sew the suit.

The dog's ability to successfully detect samples hidden in the mock apiary was evaluated with and without the suit.

Sample preparation:

A range of samples were prepared in the laboratory to supply the correct odour for recognition and indication by the sniffer dog. Of essence was the need to provide the odour in a pure and concentrated form for training. A number of control odours the dog will encounter in a hive but need to ignore such as foundation, frames, fresh, healthy brood as well as European Foulbrood (EFB) with and without *Paenibacillus alvei* was provided.

Samples with AFB infected larvae were prepared in the laboratory. A frame with fresh, healthy brood was obtained. Larvae was removed from cells, dipped into 5% Sodium hypochlorite solution to kill surface bacteria and yeasts and dried on sterile filter paper. The larvae were placed into 5 ml polypropylene tubes with a screw lid. The larvae were inoculated with 50 µl of an AFB spore suspension (Figure 2) prepared from a *P. larvae* culture on a sheep blood agar plate. The tubes were incubated for a total of 14 days in a CO₂ incubator. From day 2 onwards a tube containing the liquefied larvae was removed daily, the lids screwed on securely and used as control odour. By day 14 the larvae was dried out and resembled "scale"(Figure 3).



Figure 2. Fresh, healthy larvae were infected with 50 μ l of an AFB spore suspension in 5 mL polypropylene tubes.

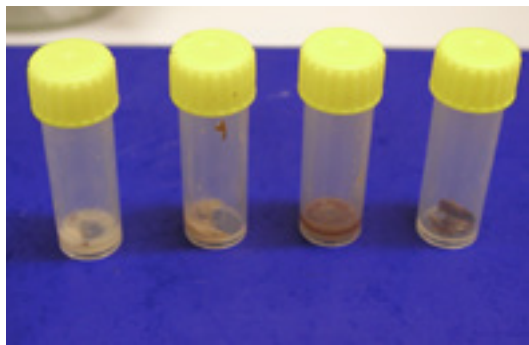


Figure 3. AFB infected bee larvae incubated at 37°C in CO₂ atmosphere at different stages to demonstrate the changes from liquefaction up to dried matter simulating scale. From left to right: larvae incubated for 2, 5, 7 and 14 days.

Brood samples were collected from hives affected by AFB, as diagnosed and confirmed in the BSL Bacteriology laboratory. The contents from affected cells were scooped out of cells and placed into 5 mL polypropylene tubes with a screw lid (Figure 4). The samples were transported to the trainer overnight and used in the conditioning process. Packaging for the samples was uniform, as the dog was trained to ignore the package, and only indicate the odour. As controls for the conditioning, samples from brood affected by European Foulbrood (EFB), fresh brood, foundation as well as *Paenibacillus alvei*, was also supplied. The dog was to be conditioned to ignore these odours. A selection of brood samples submitted to BSL with AFB, EFB and *P. alvei*, as well as healthy and disease free comb was finally supplied to test the dog's ability to distinguish AFB from the other samples (Figure 5).

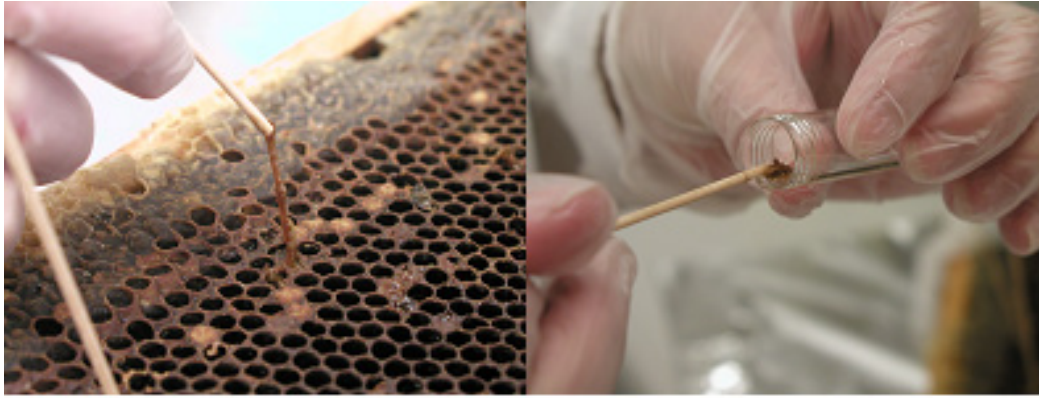


Figure 4. The contents from affected cells were scooped out of cells and placed into 5 mL polypropylene tubes with a screw lid

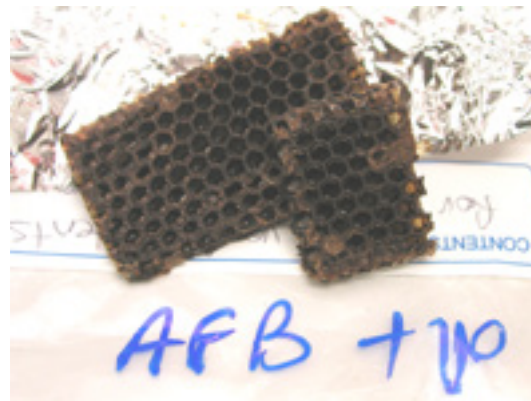


Figure 5. Samples positive for AFB, EFB and *P. alvei*, as well as disease free comb was supplied to test the dog's ability to select AFB positives and ignore the non-AFB samples.

A passive in-hive sampling technique was developed to compensate for the fact that the suit was not entirely successful. A range of samples (AFB and EFB positive brood, *P. alvei* infected brood, foundation and healthy, disease free brood) were incubated at 37° C for 1, 6 and 24 hours in a chamber containing a piece of 3 X 3 cm muslin cloth. Care was taken that the samples do not touch the muslin, by preparing a little “stage” for the muslin to rest on. After the muslin was infused with the odours, it was provided to the trainer in 5mL polypropylene tubes (Figure 6). A negative control of fresh, unexposed muslin cloth was also provided. (See, et al 2001).



Figure 6. Odour-infused muslin was provided to the trainer in 5mL polypropylene tubes for detection of AFB.

For passive in-hive sampling, pieces of 3 X 3 cm muslin cloth in open 5mL polypropylene tubes were inserted between the frames the day prior to sampling the hives by the Apiary officer (Figure 7). On sampling day, the tubes with tubes with muslin were retrieved and offered to the dog.



Figure 7. Polypropylene tubes containing muslin were inserted between the frames the day prior to sampling the hives by an Apiary officer and had to be removed on sampling day to present it to the dog away from the bees.

An improvement for the passive sampling was histology cassettes that were subsequently used to enclose the muslin cloth (Figure 8). The cassettes were inserted at the bottom of the hives with the aid of a long cable tie and prevented the bees from discarding the muslin cloth.

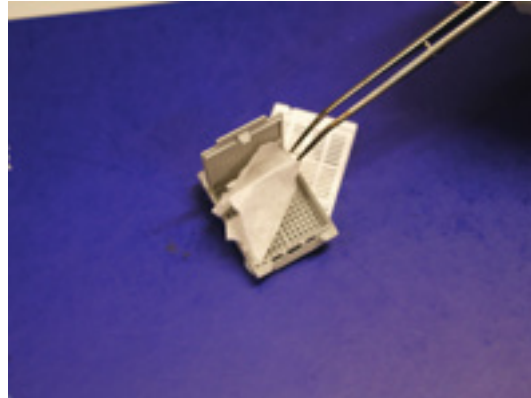


Figure 8. Histology cassettes used to enclose muslin cloth. The cassettes were inserted at the bottom of the hives with the aid of a long cable tie.

Validation of the procedure:

Once training has been completed, the dog was tested under field conditions. 51 hives with 8 frames each (n=408 frames) was screened by both the dog and a team of Apiary officers (Figure 9). Some hives were known to be AFB positive frames. No EFB positive hives were available at this stage in the Apiary. The hives were then manually inspected and samples taken and sent to the laboratory for testing. Sensitivity and specificity of the dog was calculated against the human detection.



Figure 9. A team of 3 Apiary officers and the owner of the hives took 3 days to examine 51 hives.

Further field conditioning and validation of the procedure was conducted in South Australia (Tintinara) by the owner. After handover and training of the owner by the trainer, ongoing trials were conducted in the owners' apiary. Again these trials involved placing AFB and EFB positive hives in the apiary and observing whether or not the dog detects them successfully.

Results

Training: Once Baz was conditioned to the correct odour, brood samples with different disease status were hidden in the mock apiary. The way Baz indicated a positive was to sit down next to a box (Figure 10). If the animal hesitated, but did not sit down to a box or a sample, the behaviour was noted as suspect. When Baz ignored the sample or box completely, the results were recorded as negative.



Figure 10. Baz indicates an AFB positive sample hidden in the mock hive by sitting down next to the box.

Sample preparation: Initially decaying larvae from infected brood frames were scooped into 5mL polypropylene tubes and used as training tool to condition the dog to the odour. Once the dog was tested with disease free and EFB positive brood samples, it became clear that the dog was confused. The odour had to be provided in a purer form that excluded the normal hive odours.

Laboratory infected larvae: Preparation of larvae infected in the laboratory provided a pure and concentrated form of the AFB odour. The samples were incubated until “scale” has formed. In this way, the whole cycle of AFB odours was mimicked. The odours from freshly affected larvae through to the decaying ropey stage as well as the dried out, scaled form of the disease were captured in this manner. These samples were successful in conditioning the animal. When the dog was presented with a range of brood comb samples affected by AFB, European Foulbrood (EFB), fresh brood, foundation as well as *Paenibacillus alvei*, he was competent in indicating the correct samples.

Bee suit : During tests with samples in the mock apiary, the dog could successfully detect the AFB samples at a level of 75% accuracy with the suit on and when the suit was removed, the level of accuracy was 100%.

Passive sampling techniques: When samples from muslin cloth incubated in the presence of brood with different disease status for 1, 6, and 24 hours was presented to Baz, he could indicate the correct odour on all of the different samples, irrespective of the exposure time (data not shown). As a practical measure, it was decided for the trial to insert the tubes into the hive boxes the day before the sampling was to be taking place. On the trial day, it was found that the cleaning behaviour of the bees proved to be a problem, as the muslin cloth was pulled from the tubes and in some cases, even found outside the hives (Figure 11).



Figure 11. Muslin cloth inside polypropylene tubes between the frames was pulled from the tubes by the bees and in some cases, even found outside the hives.

Results from the trial: With 51 hive boxes containing a total of 408 frames, the correlation between the human/laboratory and the sniffer dog results appear in Table 1. Sensitivity of AFB detection by Baz when compared to human and laboratory procedures was 100% (Table 2). Of the 4 positive hives, Baz indicated one positive and three hives suspect. Specificity of AFB detection by Baz in comparison with humans was 76% (Table 3). Twelve of the samples found to be negative were indicated by Baz as suspect and 2 negative hives were indicated as positives. Samples (10 X 10 cm pieces) from the suspect brood were cultured in the laboratory and no AFB isolated (data not shown). Once the dog was transferred to the owner, he was used in a working apiary and was able to indicate a hive being positive which, upon inspection, had one infected cell that was visible to the owner at that stage.

Table 1. Comparison between human and laboratory detection of AFB in hives with the detection by the sniffer dog, Baz.

	Baz	Human
Pos	3	4
Neg	36	47
Suspect	12	0
Total	51	51

Table 2. Sensitivity of AFB detection by Baz when compared to human and laboratory procedures.

	Baz	Human
Pos	1	4
Suspect	3	0
Total	4	4
Sensitivity:		100%

Table 3. Specificity of AFB detection by Baz when compared to human and laboratory procedures.

	Baz	Human
Neg	36	47
Suspect	9	0
Total	45	47
Specificity		76%

Discussion

Initially samples from infected brood frames were scooped into 5mL polypropylene tubes and used as training tool. Competing odours from the hive and brood masked the AFB odour. This confused the dog in the sense that it mixed signals between healthy and diseased AFB and EFB brood when tested. Preparation of laboratory infected larvae provided a pure and concentrated form of the AFB odour with no additional odours from the hive being present to confuse the dog. The samples were incubated until “scale” has formed. In this way, the whole cycle of AFB odours was mimicked. The odours from freshly affected larvae through to the decaying ropey stage as well as the dried out, scaled form of the disease were captured in this manner. The laboratory infected larvae was ideal for training of Baz. When he was tested subsequently by providing brood with a range of diseases, he could indicate the correct samples.

For protection of the dog, it was postulated that a bee suit with a helmet be worn. The plan was for the dog to run through the apiary, sniffing individual boxes at night. Once trialled, however, it interfered with detection. It was found that the dog could not effectively judge distance with the helmet of the protective suit which kept knocking against the hive boxes and broke the dog’s concentration. The initial plan for the dog to run through the apiary, sniffing individual boxes, had to be modified for the purposes of this project. Work on this is ongoing. The owner is still keen to do the search at night with a suit. He is currently trialling a further version. A lot of time is spent currently to condition Baz to feel comfortable with a modified veil.

Due to the inefficiency of the suit, passive sampling techniques were developed. Diseased and healthy brood incubated with muslin cloth was presented to Baz and he could indicate the correct odour on all of the different samples. The practical application of this sampling procedure was tested during the field trial. As a practical measure, it was decided for the trial to insert the tubes into the hive boxes the day before the sampling was to be taking place. The cleaning behaviour of the bees proved to be a problem, as the muslin cloth was pulled from the tubes and in some cases, even found outside the hives. Histology cassettes were subsequently used to enclose the muslin cloth. These cassettes had the added benefit that it could be inserted at the bottom of the hives with the aid of a long cable tie. The benefit of this sampling technique was that passive sampling could be done without the hives being opened by the Apiarist.

Sensitivity for the dog versus human detection was 100%. This means that Baz did not overlook any AFB positive hives. Detection of a hive with one infected cell in the owners’ apiary confirms this magnificent sensitivity. The specificity of his performance was 76%. Baz errs to the side of over sensitivity. This is more desirable than overlooking AFB. During this trial, two negative hives were indicated as being positive and 12 negative hives were indicated as suspect. Upon inspection, the hives were negative. Samples from the brood were cultured in the laboratory and no AFB isolated (data not shown). With some of the muslin swatches being removed by the bees and found on the ground, this could have had an influence on the odour being called suspect by Baz. There were hives with active AFB in the same vicinity, so it is quite possible that odour contamination could have taken place after the muslin was removed from the hives.

In practice, the false positive or suspect hives can be visually inspected, sampled and results confirmed by the laboratory. It is possible that Baz detects a “subclinical” infection that is not yet established. It will be prudent and very interesting to monitor those hives, as it could be that they are at risk of developing AFB. If a small percentage of the hives in an apiary is suspect, the time to check them is still only a fraction of the time needed to do the whole apiary. It took about 2 hours to retrieve the muslin samples from the boxes while Baz inspected the samples in about 45 minutes. It took 5 apiarists 3 days to do the same job due to distance and bad weather.

Further work

The owner is keen to develop a suit with a veil that the dog is more comfortable with. This work is ongoing. Active sampling techniques, where air from a contained space is actively being sucked across filter paper, activated charcoal or other odour-absorbing material, is also being considered by the owner. This technology is already in use for detecting contraband.

Implications

The outcome of this study on industry in Australia has the possibility of having a significant impact, IF this technology can be widely adopted.

Contribution to RIRDC goals

One of the key challenges for the honeybee and pollination-dependent industries are development of methods and strategies for management of existing and threatening pests and diseases in the industry.

Further, two objectives for the Honeybee R&D Program are

- Pest and disease protection and
- Productivity and profitability enhancement to lift beekeeper income.

This project is innovative and an Australian first. This project will contribute to RIRDC's goals for pest and disease management. This successful training of the AFB detection dog can, if widely adopted, revolutionize disease management. We could see a huge decline in cross infections, minimize neglected apiaries and it would help with disease management. The project could be used in quality assurance programs to ensure high bee density areas like Almond and Lucerne pollination are as disease free as possible.

Further, production and profitability would both increase as a result of less hives being destroyed because of AFB infections. It would reduce the number of hours being spent on visual inspection and this would free up time for apiarists to focus on more productive areas of their business.

Recommendations

It is recommended that adoption of this technology be seriously considered as a tool for the proposed national AFB control strategy. The owner (Josh Kennett, South Australia) is willing to make the dog available to industry and private beekeepers for screening of hives. The trainer of the dog (Martin Dominick) resides in Queensland (Burpengary) and is considering training another dog if there is enough demand for this screening. The methodology for providing the concentrated correct odour have been developed by the Biosecurity Sciences Laboratory and can be utilised for training more such animals.

References

See, S. D. A., B.W.; Parker, D.B.; Koziel, J. and Sweeten, J. (2001). Evaluation of fabric swatch types for relative odour intensity at beef cattle feed yards. 2001 ASAE Annual International Meeting. Sacramento, California, The Society for Engineering in agricultural, food and biological systems. **01-2260**.

Use of a Sniffer Dog in the Detection of American Foulbrood in Beehives

By Sharon C. de Wet

Pub. No. 13/O80

This report describes the training and validation of a sniffer dog for detection of American foulbrood (AFB) in bee hives. AFB is the major endemic brood disease in Australia.

A detection method that is reliable and less labour-intensive than methodically scrutinising individual brood frames manually is desirable. The objective of this project was to develop a novel, quick and reliable way of detecting AFB that is user friendly and affordable.

The sniffer dog (Baz) was trained successfully and was able to detect as little as one infected cell in a hive.

RIRDC is a partnership between government and industry to invest in R&D for more productive and sustainable rural industries. We invest in new and emerging rural industries, a suite of established rural industries and national rural issues.

Most of the information we produce can be downloaded for free or purchased from our website <www.rirdc.gov.au>.

RIRDC books can also be purchased by phoning 1300 634 313 for a local call fee.



RURAL INDUSTRIES
Research & Development Corporation

Phone: 02 6271 4100

Fax: 02 6271 4199

Bookshop: 1300 634 313

Email: rirdc@rirdc.gov.au

Postal Address: PO Box 4776,
Kingston ACT 2604

Street Address: Level 2, 15 National Circuit,
Barton ACT 2600

www.rirdc.gov.au

Cover image: Baz indicates an AFB positive sample hidden in the mock hive by sitting down next to the box