

BEENET: ITALIAN BEEKEEPING MONITORING NETWORK – PRELIMINARY DATA

Porrini C¹, Lodesani M², Libertà A³, Bortolotti L², Gallina A⁴, Colombo R², Sgolastra F¹, Medrzycki P², Bozza MA⁴, Mutinelli F⁴

¹DipSA, Università di Bologna, Italy; ²Consiglio nazionale per la Ricerca e la sperimentazione in Agricoltura (CRA) Bologna, Italy; ³Sistema Informativo Nazionale per lo sviluppo dell'agricoltura (SIN) Roma, Italy, ⁴Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (Padova) Italy

Abstract

Introduction The BeeNet monitoring network was activated in September 2011, with an increase of the number of apiaries compared to the previous Apenet network (2009-2010). In 2011 there were 97 apiaries for a total of approximately one thousand beehives. In 2012 the monitoring network progressed up to 303 apiaries located in all the Italian regions with approx. three thousand beehives.

Research methods Each monitoring unit is composed of five apiaries with ten beehives each, managed by a referent person who is in charge to carry out visits in 4 different periods of the year: 1st, end of Winter; 2nd, Spring-Summer; 3rd, end of Summer-beginning of Autumn; 4th, before wintering. At each visit, environmental and beehive data are recorded, while at visit 1 and 3, samples of beehive matrices are collected (beebread and live honey bees) to carry out chemical (pesticides), pathology (*Nosema*, virus and *Varroa*) and nutritional (beebread raw protein) analyses. The data observed are transmitted by referents person of the monitoring unit using software facilities for data-entry, the software facilities are activated via internet connections and the data collected are stored in a georeferred database.

Results and discussion points In 2011 the total mortality was not correlated to any of the investigated parameters. However, the total mortality almost doubled in apiaries infected by *Nosema* compared to the negative ones. Furthermore, *Varroa* infection is directly correlated to ABPV, while Winter mortality is negatively correlated to the percentage of beebread raw protein. According to the data obtained from the first six months of monitoring in 2012, the infection by *N. ceranae* is between low and medium; DWV is present in 95.1% of the samples and in 20% of cases the concentration is above 10 million viral copies per bee. ABPV and CBPV are also present, with a prevalence of 50 and 70% respectively, but the number of samples with a viral load above 10 million viral copies account only for 1 and 3% respectively. Beebread collected from beehives located in southern Italy showed a higher raw protein content; however, the percentage of beebread contaminated by active substances used in *Varroa* control is higher than in beebread collected in northern Italy. Overall 50.4% of beebread samples analyzed was positive to at least one active substance. Moreover, a bee emergency service team has been established who is in charge of field intervention, samples and data collection, and epidemiological investigation in case of mortality report, in collaboration also with Health Authorities.

Introduction

BeeNet is a network for studying bees-environment interactions and monitoring honeybee mortality and colony losses in Italy. The BeeNet monitoring network was activated in September 2011, with an increase of the number of apiaries compared to the previous Apenet network (2009-2010). In 2011 there were 97 apiaries for a total of approximately one thousand beehives. In 2012 the monitoring network progressed up to 303 apiaries located in all the Italian regions with approximately three thousand beehives (Figure 1).

Through BeeNet it has been possible to investigate the presence of *Nosema apis*/*Nosema ceranae* and of three viruses, Deformed Wing Virus (DWV), Acute Bee Paralysis Virus (ABPV) and Chronic Bee Paralysis Virus (CBPV) in Italian apiaries distributed in selected sites to cover the national territory.

The health of honey bee colonies is influenced by numerous factors, among which are the nutritional status of the colony and the intoxication by pesticides. In particular, the nutritional quality of the beebread influences both the longevity and the susceptibility of the bees to several stressors (e.g. pesticides). Within the nation-wide monitoring project, several analysis were carried out on different matrices. Beebread samples were analyzed for pesticide residues and protein content.



Figure 1. Location of the monitoring units involved in the BeeNet project.

Research methods

Each monitoring unit is composed of five apiaries with ten beehives each, managed by a referent person who is in charge to carry out visits in 4 different periods of the year: 1st, end of Winter; 2nd, Spring-Summer; 3rd, end of Summer-beginning of Autumn; 4th, before wintering. At each visit, environmental and beehive data are recorded, while at visit 1 and 3, samples of beehive matrices are collected (beebread and live honey bees) to carry out chemical (pesticides), pathology (*Nosema*, virus and *Varroa*) and nutritional (beebread raw protein) analyses.

The information system has interactive tools of data extraction from the georeferenced data base and software services of data processing to compute geographic statistics of the variables monitored and to compose analytical tables and thematic maps.

Varroa destructor infestation

The level of *Varroa destructor* infestation (% calculated on a sample of 250 honey bees) was estimated in the apiary using the “powder sugar roll method” during the 3rd visit, i.e. end of Summer-beginning of Autumn.

Nosema and viruses

From Autumn 2011 to Autumn 2012 (sampling at the beginning of Autumn 2011; at the end of Winter and at the beginning of Autumn 2012), 620 and 636 samples of adult honey bees from selected apiaries were collected respectively for: a) diagnosis of *N. apis*/*N. ceranae* infection and spores quantification; b) virus detection and quantification. Crushed honey bee were examined by light microscopy for the presence of *Nosema* spp. spores and, after DNA extraction, a specific real time PCR (1, 2) was performed for species identification (*N. apis*/*N. ceranae*) and spores quantification. Virus detection and quantification were performed, after RNA extraction, by using a specific quantitative one step real time RT-PCR for DWV, ABPV and CBPV (3), respectively.

Beebread protein content and pesticides

The protein content was determined using the Kjeldhal method. A portion of sample was digested with a mixture of concentrated H₂SO₄ and K₂SO₄, using CuSO₄ as a catalyst to thereby convert organic nitrogen present to (NH₄)₂SO₄. Excess NaOH was added to the cooled digest to liberate NH₃ that was distilled into excess H₃BO₃ solution and titrated with HCl. The protein content was calculated by multiplying the nitrogen content by 6.25, obtained from NH₃ value.

The determination of pesticides was performed by extraction and purification of a portion of sample using the QuEChERS[®] technique. In particular, we used the kit “Quechers Extract tubes, EN Method” together to “Dispersive SPE 15 ml, Fatty Samples, EN” (Agilent Technologies). The instrumental determination was performed by GC-ECD and HPLC-MS using a quantification by external standards. The number of tested pesticides was higher than 150.

Results

Varroa destructor infestation

In Marche (6.3%) and Lazio (3.9%) (central Italy), Campania (3.3%) and Puglia (3.0%) (southern Italy) regions the highest infestation levels were recorded, the lowest ones in Toscana (0.3%) (central Italy) and Emilia-Romagna (0.5%) (northern Italy) regions at the 3rd visit (end of Summer-beginning of Autumn).

Nosema and viruses

N. ceranae was present in all Italian regions, while *N. apis* or *N. apis/N. ceranae* co-infection were not detected. Of the 620 samples analyzed, 454 were positive for *N. ceranae* with an overall positivity rate of 73%. Only in 3.4% of the samples more than 10 million *N. ceranae* spores per bee were detected.

DWV, ABPV and CBPV were detected in Italian apiaries in different combinations. DWV was present in almost all samples (96.7%) and in 40% of cases exceeded 10 million viral copies per bee. For ABPV and CBPV the percentages were lower, 53.6 and 57.7% respectively, but the samples that exceeded 10 million viral copies per bee were only 5.9 and 5.4%, respectively.

N. ceranae and viruses results for each sampling [at the beginning of Autumn 2011 (a); at the end of Winter (b) and at the beginning of Autumn (c) 2012] are summarized in Figure 2a, b, c and Figure 3a, b, c, respectively.

a	Sampling 2011	<i>Nosema ceranae</i>	b	1 st sampling 2012	<i>Nosema ceranae</i>
	Total number of samples	122		Total number of samples	207
	POSITIVE	46 (37.7%)		POSITIVE	178 (86%)
	of which with more than 10 ⁷ <i>N. ceranae</i> spores per bee	0		of which with more than 10 ⁷ <i>N. ceranae</i> spores per bee	6 (3.4%)
	NEGATIVE	76 (62.3%)		NEGATIVE	29 (14%)
			c	2 nd sampling 2012	<i>Nosema ceranae</i>
				Total number of samples	291
				POSITIVE	230 (79%)
				of which with more than 10 ⁷ <i>N. ceranae</i> spores per bee	15 (6.5%)
				NEGATIVE	61 (21%)

Figure 2a, b, c. Results of the analyses directed to the determination of *Nosema* spp.

a	Sampling 2011	DWV	ABPV	CBPV
	Total number of samples	126	126	126
	POSITIVE	118 (93.6%)	65 (51.6%)	54 (42.9%)
	of which with more than 10 ⁷ viral copies per bee	88 (74.6%)	9 (13.8%)	5 (9.2%)
	NEGATIVE	8 (6.3%)	61 (48.4%)	72 (57.1%)

b	1st sampling 2012	DWV	ABPV	CBPV
	Total number of samples	218	218	218
	POSITIVE	208 (95.4%)	129 (59.2%)	170 (78%)
	of which with more than 10 ⁷ viral copies per bee	43 (20.7%)	4 (3.1%)	11 (6.5%)
	NEGATIVE	10 (4.6%)	89 (40.8%)	48 (22%)

c	2st sampling 2012	DWV	ABPV	CBPV
	Total number of samples	292	292	292
	POSITIVE	289 (99%)	147 (50.3%)	143 (49%)
	of which with more than 10 ⁷ viral copies per bee	116 (40.1%)	7 (4.8%)	4 (2.3%)
	NEGATIVE	3 (1%)	145 (49.7%)	149 (51%)

Figure 3a, b, c. Results of the analyses directed to the determination of viruses.

Beebread protein content and pesticides

In Autumn 2011 the beebread contained a lower percentage of protein and pesticides, compared to Spring 2012. In Spring 2012 the colonies located in the south of Italy contained beebread with the highest protein content and positivity to pesticides of the country.

In Autumn 2011, 22 different active ingredients were found: carbaryl (7.2% of the samples, range: 11-82 ppb), chlorpyrifos (4.0%, 8-47 ppb) and fluvalinate (3.2%, 17-150 ppb) were the most frequently detected. Only one sample contained neonicotinoids (imidacloprid, 16 ppb).

In Spring 2012, 50 different active ingredients were found: fluvalinate (14.5%, 15-134 ppb), chlorfenvinphos (12.8%, 19-126 ppb) and chlorpyrifos-ethyl (8.5%, 8-109 ppb) were the most frequently detected. Among neonicotinoids, imidacloprid (2.6%, 12-62 ppb) and thiamethoxam (1 sample, 18 ppb) were found.

The results of chemical analyses are summarized in Figure 4.

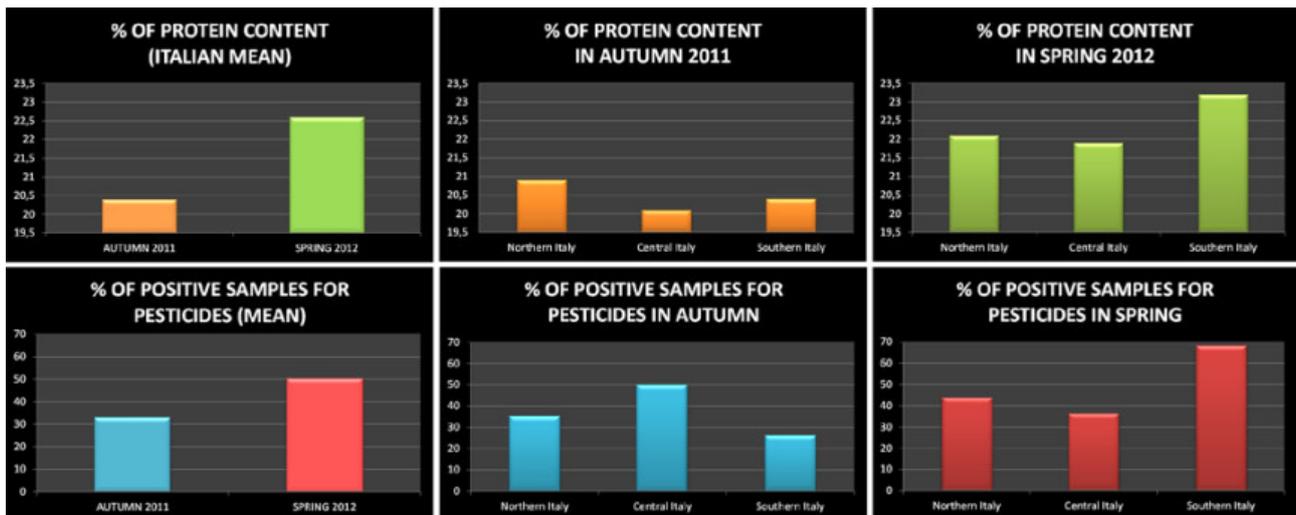


Figure 4. Results of the analyses directed to the determination of protein content and pesticides.

Honey bee colony losses

The average mortality in 2011 amounted to 18.3% (as calculated on 839 colonies); in the year 2011 and Winter 2011/12 amounted to 6.3 and 12.0% respectively.

The average mortality in 2012 amounted to 12.8% (as calculated on 2,501 colonies); in the year 2012 and Winter 2012/13 amounted to 7 and 5.8% respectively.

In northern Italy colony mortality amounted to 4% (1,227 monitored colonies) and Winter mortality to 8.5%; in central Italy colony mortality amounted to 12.9% (584 monitored colonies) and Winter mortality to 4.9%; in southern Italy colony mortality amounted to 7.6% (920 monitored colonies) and Winter mortality to 3%.

Conclusions

The monitoring units are characterized by different landscape i.e. agricultural, urban, forest and humid areas according to the peculiarities of the regional territory. In particular, many monitoring units are surrounded by cereals cultivation in Emilia Romagna region (northern Italy), while in Sicily and Puglia (southern Italy) by citrus and olive groves, respectively.

In 2011 the total mortality was not correlated to any of the investigated parameters. However, the total mortality almost doubled in apiaries infected by *Nosema* compared to the negative ones. Furthermore, *Varroa* infestation is directly correlated to ABPV, while Winter mortality is negatively correlated to the percentage of beebread raw protein.

In 2012, the infection by *N. ceranae* was between low and medium; only in 3.4% of the samples more than 10 million *N. ceranae* spores per bee were detected. *N. ceranae* was present in all Italian regions, neither *N. apis* nor *N. apis/N. ceranae* co-infection were detected. DWV, ABPV and CBPV were detected in Italian apiaries in different combinations. DWV was present in almost all samples (96.7%) and in 40% of cases exceeded 10 million viral copies per bee. Less frequent is the detection of ABPV and CBPV (53-58%) and less than 6% of the samples exceeded 10 million viral copies per bee. Moving from north to south of Italy, an increasing trend of virus infection is evident.

Further investigations are needed to possibly correlate the health status of apiaries with *N. ceranae* and virus quantitative analyses.

In Autumn 2011 beebread contained a lower percentage of protein and pesticides, compared to Spring 2012. In Spring-Summer 2012 beebread collected from beehives located in southern Italy showed a higher raw protein content; however, the percentage of beebread contaminated by active substances used in *Varroa* control is higher than in beebread collected in northern Italy. Overall 50.4% of beebread samples analyzed was positive to at least one active ingredient.

All the parameters considered in the present study seem indicative of a good honey bee health condition.

Mean annual mortality and Winter mortality were remarkably lower than those recorded in Italy during the previous years through Coloss and Apenet monitoring (19-37%).

In addition, the bee emergency service team established with the Apenet project (2009-2010) has been reinforced and is active at national level for field intervention, samples and data collection, and epidemiological investigation in case of mortality report, in collaboration also with Health Authorities. Mortality and depopulation phenomena are usually the result of the interaction among different causes. However, it has often been possible to identify the main cause responsible for these outbreaks, such as the inappropriate use of pesticides in areas characterized by citrus grove and vineyards. Among infectious agents, the main complaints went to *Varroa* and viruses (DWV).

References

1) Chen et al., J. Invertebr. Pathol. 2009; 101: 204-209; 2) Burgeois et al., J. Invertebr. Pathol. 2010; 103: 53-58; 3) Blanchard et al., J. Virol. Methods 2012; 180: 26-31

Funding

The project "BEENET: apicoltura e ambiente in rete" is funded by the Italian Ministry of Agriculture Food and Forestry.