



# BEE NET: ITALIAN BEEKEEPING MONITORING NETWORK

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# BeeNet

- ✓ 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

# Location of the monitoring units involved in the BeeNet project



- ▲ Coordination centre
- Apiary composed of 10 hives



# BeeNet

The network:

- ✓ National monitoring network
- ✓ Dedicated software
- ✓ Data collection on environment
- ✓ Data collection on honey bee colonies health status
- ✓ Analysis on bee hive matrices
- ✓ Data analysis

# BeeNet

The investigation aimed to:

- Colony development and environment data collection
- *Nosema apis/Nosema ceranae*
- Deformed Wing Virus (DWV),
- Acute Bee Paralysis Virus (ABPV)
- Chronic Bee Paralysis Virus (CBPV)
- Beebread protein content
- Beebread pesticide residues

# Materials & 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 at 4 different time points:
  - ✓ 1<sup>st</sup>, end of Winter;
  - ✓ 2<sup>nd</sup>, Spring-Summer;
  - ✓ 3<sup>rd</sup>, end of Summer-beginning of Autumn;
  - ✓ 4<sup>th</sup>, before wintering.
- Colonies are visually inspected
- In each inspection several parameters of each colony are considered:
  - ✓ health and nutritional condition,
  - ✓ number of bees and brood,
  - ✓ queen's age,
  - ✓ climate, land use



# M & M

- At visit 1 and 3, samples of beehive matrices are collected: beebread and live honey bees
- Chemical (beebread pesticides), pathology (*Nosema*, virus and *Varroa*) and nutritional (beebread raw protein) analyses

# M & M

## ***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 3<sup>rd</sup> visit, i.e. end of Summer-beginning of Autumn



# M & M

## **Nosema**

From Autumn 2011 to Autumn 2012

- ✓ 620 samples of asymptomatic live adult honey bees collected for diagnosis of *N. apis*/*N. ceranae* infection and spores quantification
- ✓ Crushed honey bees were examined by light microscopy for the presence of *Nosema* spp. spores
- ✓ After DNA extraction, a specific real time PCR (Chen et al., 2009; Burgeois et al., 2010) performed for species identification (*N. apis*/*N. ceranae*) and spores quantification

## **Viruses**

- ✓ 636 samples of asymptomatic live adult honey bees collected
- ✓ Virus detection and quantification performed, after RNA extraction, using a specific quantitative one step real time RT-PCR for DWV, ABPV and CBPV (Blanchard et al., 2012), respectively

# M & M

## Beebread protein content

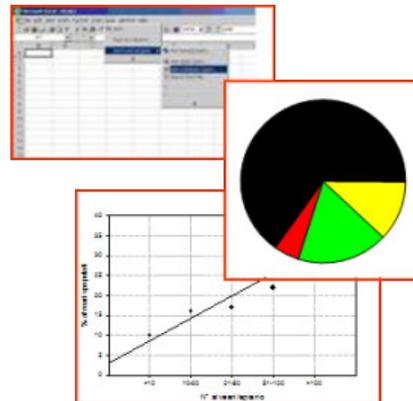
- ✓ The protein content determined using the Kjeldhal method.
- ✓ The protein content calculated by multiplying the nitrogen content (obtained from  $\text{NH}_3$  value) by 6.25

## Pesticides

- ✓ The determination of pesticides performed by extraction and purification using the QuEChERS<sup>®</sup> technique
- ✓ The instrumental determination performed by GC-ECD and HPLC-MS using a quantification by external standards
- ✓ The number of tested pesticides greater than 150

# M & M

- ✓ 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
- ✓ the data collected are stored in a georeferred database



# 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 3<sup>rd</sup> visit (end of Summer-beginning of Autumn)



# Results

- *N. ceranae* was present in all Italian regions
- *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

# *Nosema ceranae*

**a**

| Sampling 2011   | <i>Nosema ceranae</i> |
|---|-----------------------|
| Total number of samples   | 122                   |
| <b>POSITIVE</b>   | <b>46</b> (37.7%)     |
| of which with more than $10^7$ <i>N. ceranae</i> spores per bee | 0                     |
| <b>NEGATIVE</b>   | <b>76</b> (62.3%)     |

**b**

| 1 <sup>st</sup> sampling 2012                                   | <i>Nosema ceranae</i> |
|---|-----------------------|
| Total number of samples   | 207                   |
| <b>POSITIVE</b>   | <b>178</b> (86%)      |
| of which with more than $10^7$ <i>N. ceranae</i> spores per bee | 6 (3.4%)              |
| <b>NEGATIVE</b>   | <b>29</b> (14%)       |

**c**

| 2 <sup>st</sup> sampling 2012                                   | <i>Nosema ceranae</i> |
|---|-----------------------|
| Total number of samples   | 291                   |
| <b>POSITIVE</b>   | <b>230</b> (79%)      |
| of which with more than $10^7$ <i>N. ceranae</i> spores per bee | 15 (6.5%)             |
| <b>NEGATIVE</b>   | <b>61</b> (21%)       |

Results of the analyses directed to the determination of *Nosema* spp.

# Viruses

- DWV, ABPV and CBPV were detected in Italian apiaries in different combinations
- DWV was present in almost all samples (96.7%)
- 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
- The samples that exceeded 10 million viral copies per bee were only 5.9 and 5.4%, respectively

# Viruses

**a**

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

**b**

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

**c**

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

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)

fluralinate (3.2%, 17-150 ppb)

- Only one sample contained neonicotinoids (imidacloprid, 16 ppb)

In Spring 2012, 50 different active ingredients were found:

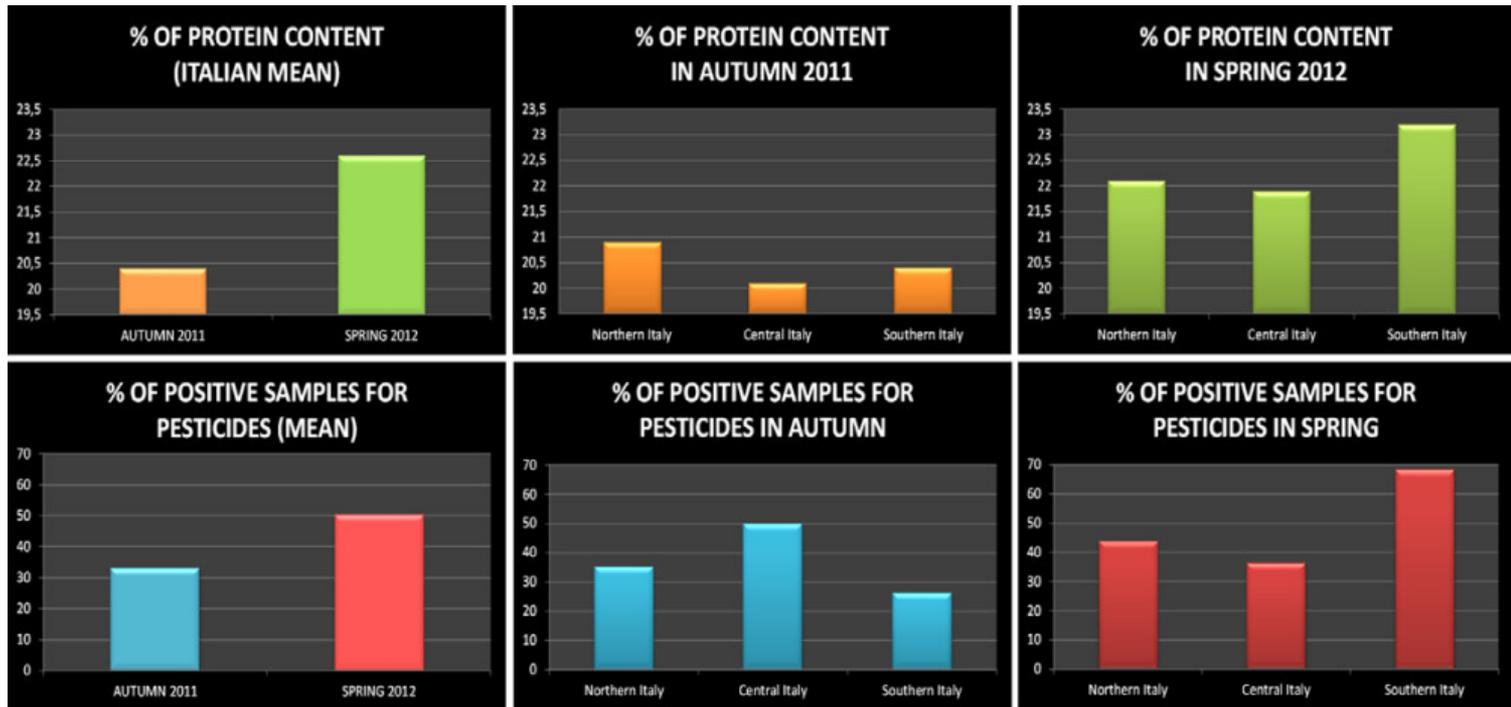
fluralinate (14.5%, 15-134 ppb)

chlorfenvinphos (12.8%, 19-126 ppb)

chlorpyrifos-ethyl (8.5%, 8-109 ppb)

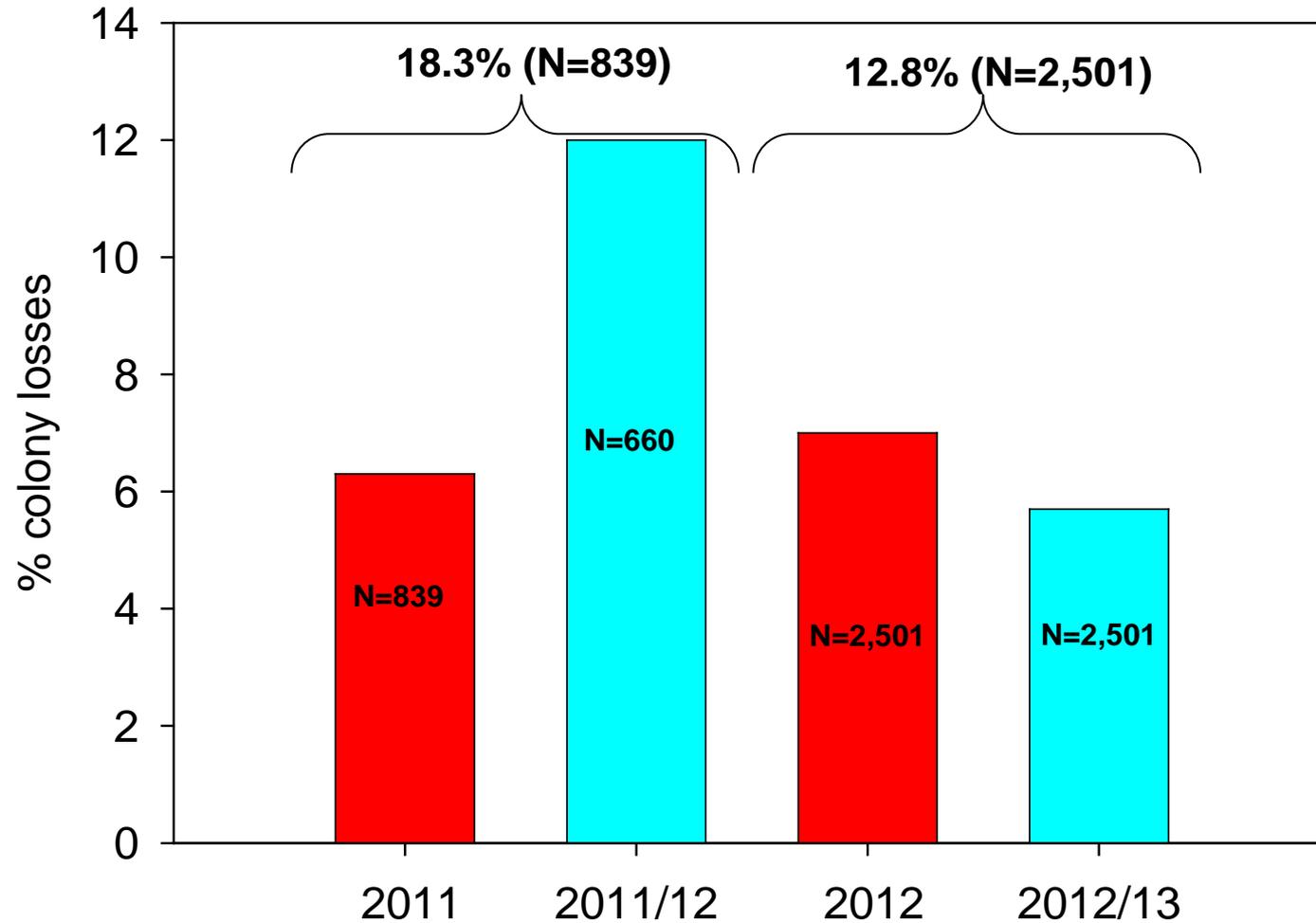
- Among neonicotinoids, imidacloprid (2.6%, 12-62 ppb) and thiamethoxam (1 sample, 18 ppb) were found

# Beebread protein content and pesticides



Results of the analyses directed to the determination of protein content and pesticides

# Honey bee colony losses



# Honey bee colony losses

- 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 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
- ✓ Confirmed the enzootic condition of *N. ceranae* in Italy, with low spore concentration (in 2012, only in 3.4% of the samples with more than 10 million *N. ceranae* spores per bee)
- ✓ Systematically investigated the presence of viruses (DWV, ABPV, CBPV), their quantification and geographic distribution
- ✓ Contributed to the knowledge of beebread nutritional value

# Conclusions

- ✓ Systematically investigated the presence of residues (pesticides, acaricides and neonicotinoids) in beebread
- ✓ Contributed to quantify annual and Winter colony losses
- ✓ 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%).
- ✓ All the parameters considered in the present study seem indicative of a good honey bee health condition
- ✓ Contributed to create a database on honey bee colonies development and health condition in Italy
- ✓ 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.

# Acknowledgements

- People in charge of monitoring units
- Beekeepers
- Ministry of Agriculture Food and Forestry

Thank you for your attention