RT-PCR analysis of Deformed wing virus in the workers of honeybee subspecies *Apis mellifera carnica*

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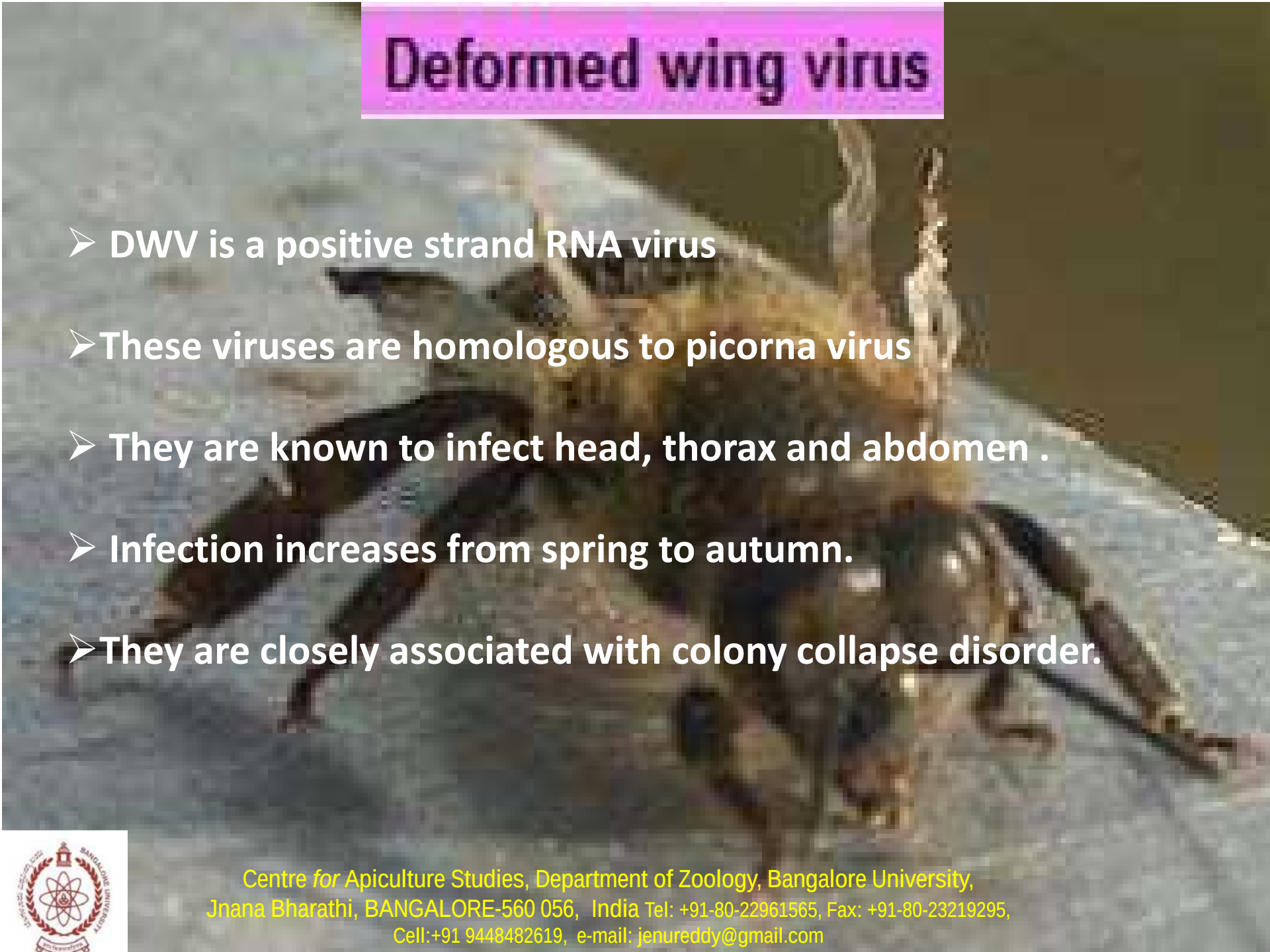
Deformed wing virus



- DWV is a positive strand RNA virus
- These viruses are homologous to picorna virus
- They are known to infect head, thorax and abdomen .
- Infection increases from spring to autumn.
- They are closely associated with colony collapse disorder.

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SYMPTOMS

- Deformity of wings
- Paralysis
- Discoloration of body
- Bloated abdomen
- Weakness
- Sudden collapse

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Effects of DWV on honeybees

- Shortened life span
- Paralysis and mortality of honeybees
- Decreased fecundity rate of queen
- Cognitive impairments
- Colony collapse disorder

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MATERIALS AND METHODS

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RNA EXTRACTION

- Frozen bees were dissected into head using a fresh scalpel for every cut to avoid cross-contamination of viral RNA.
- Total RNA of each body part was extracted using standard methods following the manufacturer's protocol (RNeasy kit; Qiagen).

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RNA EXTRACTION

- RNA extraction from larval food (30 µl each) was performed by standard methods following the manufacturer's protocol (Viral RNA kit; Qiagen).
- Total RNA from individual mites was extracted using the Purescript RNA extraction kit (GentraSystems) following the manufacturer's protocol with minor modifications.

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RNA EXTRACTION

- Briefly, single mites still frozen at –70 °C were crushed with a pestle pre-frozen at –70 °C and homogenized in 100 µl lysis buffer.
- DNA precipitation buffer was added (33 µl) and probes were incubated on ice for 5 min, prior to centrifugation at 16 000 *g* for 3 min to pellet the DNA.

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RNA EXTRACTION

- RNA was precipitated from the supernatant by adding 100 µl 100 % 2-propanol and a subsequent centrifugation step at 16 000 *g* for 3 min.
- The RNA pellet was air-dried for 15 min and washed with 70 % ethanol.
- The air-dried RNA pellet was resuspended in 18µl hydration buffer and stored at −70 °C.

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RT PCR ANALYSIS

One-step RT-PCR (Qiagen) were performed with incubations for 30min at 50 °C, 15 min at 95 °C followed by 35 cycles with 30 s at 94 °C, 30 s at 54.3 °C, and 30 s at 72 °C, followed by a final elongation step for 10min at 72 °C.

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RESULTS

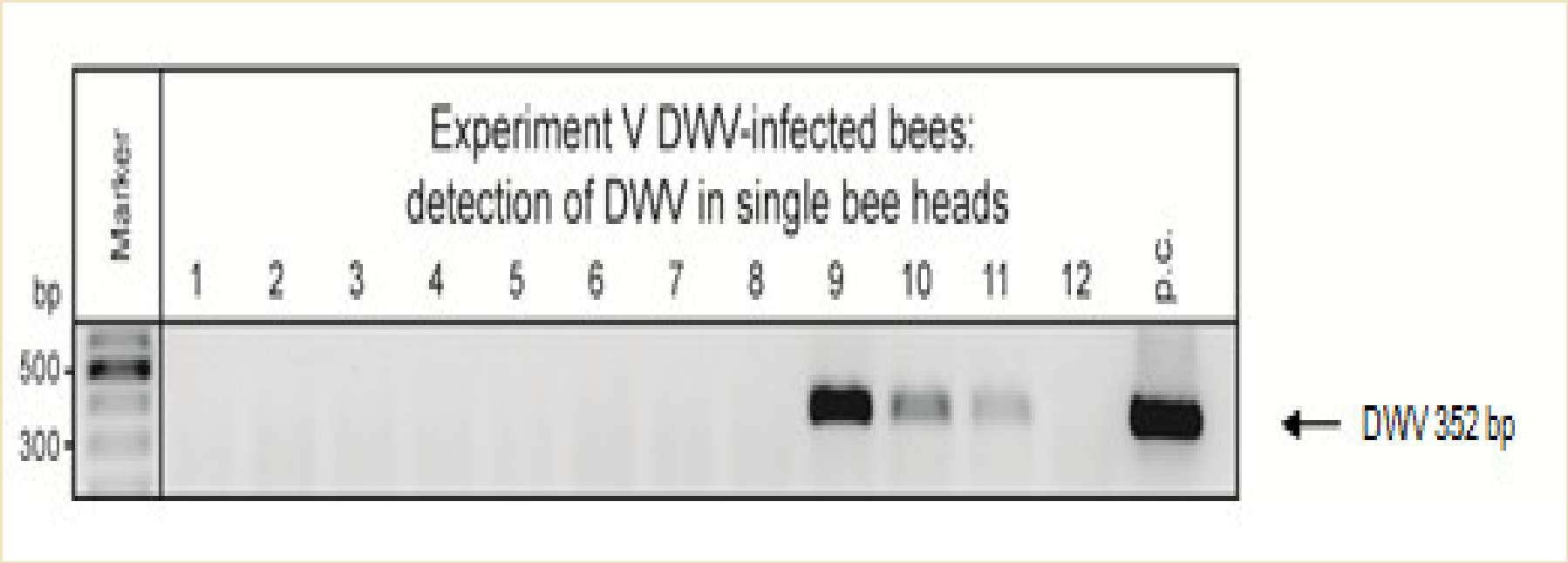

Fig. 1. RT-PCR detection of DWV in infected bees collected from different colonies of Bremen.

P.c. = positive control;

n.c. = negative control;

M = marker.

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RESULTS

RT-PCR analysis of head region of different colonies of *Apis mellifera carnica* revealed the presence of DWV (Fig-1)

Analysis of crippled bees for the presence of DWV demonstrated replicating virus in the head (Fig. 1), while only faint bands could be detected in asymptomatic bees, showing signals for replicating viral RNA from head(Fig. 1).

The absence of DWV could be due to the absence of replicating virus or because of low titer of virus

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CONCLUSION

- DWV infections are a potential threat to honeybee colonies.
- Different strategy for transmission
- World wide distribution
- Potential effect on the bee physiology

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