PSZCZELNICZE ZESZYTY NAUKOWE
Rok XLIV, Nr 2 2000

SACBROOD VIRUS IN POLISH APIARIES

Grażyna Topolska, Aleksandra Hartwig, Katarzyna Krzyżańska

Laboratory of Diseases of Usable Insects, Department of Infectious Diseases Microbiology and Parasitology, Faculty of Veterinary Medicine, Warsaw Agricultural University, Grochowska 272, 03-849 Warsaw, Poland

Summary

The antiserum produced to sacbrood virus (SBV) was used to identify SBV as a cause of high brood mortality in two colonies from two different apiaries. The investigation of dead bee samples from nine Polish apiaries for SBV presence was also conducted. Samples were collected in 10 colonies of each apiary during five-month period: from May till September. Single cases of SBV presence were detected.

Keywords: sacbrood, honey bee, Poland.

INTRODUCTION

Sacbrood - a disease of bee larvae was thoroughly described by White in 1913 and shown by him to be caused by a filterable agent. Sacbrood virus (SBV) was isolated in the early 1960s (Bailey et al. 1964). Larvae become infected while ingesting contaminated food. The infected larvae fail to pupate. They are unable to shed their larval skin and fluid containing huge amounts of SBV accumulates between the body and this skin. The body colour of the larva changes from pearly white to a pale yellow. The larva dies and becomes dark brown. It dries and changes to a flattened gondola-shaped scale. Although some signs of the sacbrood are quite distinctive and specific, sometimes the disease is taken for a foulbrood and treated as such. On the other hand the cases of brood mortality due to other honey bee viruses (black queen cell virus - BQCV, acute paralysis virus - APV) infection can be taken as caused by SBV. SBV also multiplies in young adult bees without causing obvious disease (Bailey 1969). The adult bees become infected during removal of infected larvae from cells. SBV is widely distributed throughout the world and is a common infection in colonies, but most colonies show no symptoms. Mostly it was detected in the cases of overt diseases in brood, but in Germany it was detected in large amounts in dead adult bees from colonies infested with *Varroa jacobsoni*. It is known that this parasite can transmit SBV (Ball 1994). SBV was also detected in one sample of dead bees while testing bees from a Warsaw apiary.

The aim of this work was to produce an antiserum to SBV and to test bees originated from Polish apiaries for SBV presence.

MATERIALS AND METHODS

Serum anti-SBV was prepared as described by Bailey and Woods (1974). The dead larvae with signs of sacbrood (at the stage of sac), which failed to react with antisera to black queen cell virus and acute paralysis virus, formed the source of SBV for serum production. The titre and specifity of antiserum was kindly tested by Brenda Ball at IACR - Rothamsed in England. Prepared antiserum was used to test two samples of dead brood with signs of sacbrood and to test collected dead adult bee samples.

Samples of brood originated from two different apiaries. In the first apiary - from the surroundings of Poznań - there were 40 colonies with signs of sacbrood. In the other - from Warsaw - in one colony plenty of larvae died with signs of sacbrood. Six pupae of each sample were triturated in 3ml of PB/DIECA, stained with Gram method and examined under a light microscope for presence of pathogenic bacteria. The samples, after adding 27 ml of PB/DIECA to each, were extracted by the method described by Ball and Allen (1988) for bee samples and tested against possessed antisera to APV and BQCV and against prepared SBV antiserum.

Adult dead bee samples originated from nine apiaries situated in different parts of Poland. The bees were collected from 10 colonies of each apiary during five months: from May till September in dead bee traps (modified after Bailey 1965) at colony entrances. In the case of II apiary there was a lack of samples from May. In I, III and V apiary, samples from April were included. Bees were removed from the traps monthly and kept at the temperature -18°C until the investigation. The samples were extracted by method described by Ball and Allen (1988) and tested by immunodiffusion against anti-SBV serum. They were also tested with the previously produced antisera to APV and BQCV. Some were also tested by immunodiffusion at IACR Rothamsted for CPV presence. The presence of filamentous virus was examined by observation of extract in an electron microscope. The initial clearing pellet of extracts were examined microscopically for protozoa. In total 346 samples were examined.

RESULTS AND DISCUSSION

We prepared an anti-SBV serum which proved to be very specific. The titre of antiserum is 1/64 to Polish sacbrood and 1/32 to German sacbrood.

Both samples of brood proved to be free from pathogenic bacteria and extracts of them gave a strong reaction in immunodiffusion test with anti-SBV

serum. They failed to react with antisera against BQCV and APV. Brood mortality was caused by SBV infection.

Dead bee samples in which SBV was detected and the presence of other pathogens in them are shown in tab. 1. SBV was detected in 8 colonies from 5 apiaries. In one apiary SBV was detected in four colonies. Only in one case SBV was present in September, in the others - in spring time: in one in April in three in May and in three in April and May. In four cases SBV was accompanied by another virus (APV, BQCV, FV or CPV) in one by two viruses (APV and FV, FV and CPV).

Table 1

Dead bee samples containing sacbrood virus and the presence (+) or absence (-) of the other tested pathogens in them - Próbki martwych pszczół z wirusem choroby woreczkowej oraz z obecnością (+) bądź brakiem innych badanych patogenów

Apiary Pasieka	Colony No in apiary Nr rodziny w pasiece	Month Miesiąc	*Nosema apis*	Virus - Wirus			
				Acute Paralysis Ostrego paraliż	Black Queen cell Choroby czarnych mateczników	Filamentous Włókienkowy	Chronic Paralysis Paraliżu chronicznego
I	1	May	+	+	-	+	
II	10	Sept.	-	-	-	-	
V	3	April	-	-	-	-	-
		May	+	-	-	-	-
	4	April	-	-	-	-	-
		May	+	-	-	+	+
	6	April	-	-	-	-	+
	7	April	-	-	-	-	-
		May	+	-	-	-	-
VI	8	May	+	-	-	+	
VII	9	May	+	-	+	-	

Although SBV is a common infection in colonies in many countries (Allen and Ball 1996) it rarely causes high percentage of brood mortality as infected bees - which can transmit infection - stop rearing brood and gathering pollen (Bailey and Fernando 1972). Moreover most infected larvae are quickly removed by bees. Outbreak of disease can occur when the division of labour in colonies is poorly developed (at the beginning of brood

rearing season or when forage is limited) (Allen and Ball 1996). In the case of high brood mortality in colony from Warsaw apiary the former was the case. In the case of the apiary from Poznań both circumstances were probably involved, but we can only suppose, that most cases of brood mortality with signs of sacbrood were caused by SBV infection because we were not sent samples to test from the other colonies.

In adult dead bees SBV usually occurs in amounts too small to be detected by immunodiffusion test. By Bailey and Ball (1981) „were all 30 individual of sample to be infected by SBV, the virus could barely be detected by immunodiffusion". It can take place only in colonies where the level of virus infection is high. In previous studies of bees from Warsaw (Topolska et al. 1994) and Germany (Ball and Allen 1988) SBV was detected in three samples. In this study SBV was detected in dead bee samples originated from 8 colonies. We can expect that in these colonies also brood mortality due to sacbrood occurred, although beekeepers did not notice any. Most samples with SBV presence were collected in April and May (10/11). By Bailey and Ball (1994) sacbrood is most evident in spring and early summer. The detection of SBV in as many as 3 colonies from apiary No. V is striking. In the same apiary chronic paralysis virus (CPV) was detected. Maybe, in this region forage was limited - in such circumstances both viruses multiply easily. It is highly possible as the apiary was placed in the easternmost part of Poland where in May bees are often forced to stay in hives because of temperature falls or rainy days. We think, that it is worth adding that this apiary was the cleanest of the investigated ones and that the level of Nosema apis invasion in May was the lowest there - 37% of investigated colonies. In the other colonies the level of Nosema invasion in May was almost 100%.

In one case SBV was accompanied with BQCV. Both viruses frequently multiply even in the same individuals. In one sample presence of SBV and APV was detected. In this case both viruses probably occurred in different individuals, as APV do not multiply in association with SBV. In three samples SBV was accompanied with filamentous virus (FV). It was not surprising because FV as well as BQCV often go together with *N. apis*.

The fact that high level of SBV infection was detected in samples originated from 5/9 tested apiaries indicates that in Poland SBV infection must be as common as in other previously investigated countries.

ACKNOWLEDGEMENTS

We thank Brenda Ball for kindly testing our serum and for help in our struggles on the way of studying the world of honey bee viruses.

REFERENCES

Allen M. F., Ball B. V. (1996)- The incidence and world distribution of honey bee viruses. *Bee World.* 77(3):141-162

Bailey L. (1965)- Paralysis of the honey bee, *Apis mellifera* Linaeus. *Journal of Invertebrate Pathology.* 7;132-140.

Bailey L. (1969)- The multiplication and spread of sacbrood virus of bees. *Annals of Applied Biology,* 63:483-491.

Bailey L., Ball B. V., Perry J. N. (1981)- The prevalence of viruses of honey bees in Britain. *Annals of Applied Biology.* 97:109-118.

Bailey L., Ball B. V., (1994)- Honey bee viruses. In Encyclopedia of virology. New York and London: *Academic Press Ltd.* pp 654-660.

Bailey L. Fernando E. F. W. (1972)- Effects of sacbrood on adult honey-bees. *Annals of Applied Biology.* 72:27-35.

Bailey L., Gibbs A. J., Woods R.D. (1964)- Sacbrood virus of the larval honey bee (*Apis mellifera* Linnaeus). *Virology,* 23:425-429.

Bailey L., Woods R. D. (1974)- Three previously undescribed viruses from honey bee. *Journal of General Virology,* 25:175-176.

Ball B.V., Allen M. F. (1988)- The prevalence of pathogens in honey bee (*Apis mellifera*) colonies infested with the parasitic mite *Varroa jacobsoni. Annals of Applied Biology.* 113:237-244.

Topolska G., Ball B.V., Allen M. F. (1995)- Identyfikacja wirusów u pszczół z dwóch warszawskich pasiek. [Identification of viruses in bees from two Warsaw apiaries] *Medycyna Weterynaryjna.* 51 (3):145-147. In Polish.

WIRUS CHOROBY WORECZKOWEJ W POLSKICH PASIEKACH

Topolska G., Hartwig A., Krzyżańska K.

Streszczenie

Wirus choroby woreczkowej bardzo powszechnie występuje w rodzinach pszczelich na całym świecie, lecz większość zakażonych rodzin nie wykazuje żadnych objawów. Stwierdzany był przeważnie w przypadkach wystąpienia zmian chorobowych u czerwiu. Na ogół ilość wirusa w pszczołach dorosłych jest niewystarczająca do wykrycia metodą immunodyfuzji lecz w Niemczech w znacznych ilościach został stwierdzony w dorosłych pszczołach w rodzinach zaatakowanych przez roztocze *Varroa jacobsoni.* W dużej ilości stwierdzony był także w jednej próbce dorosłych pszczół zebranych w maju w warszawskiej pasiece i przebadanych w IACR- Rothamsted w Anglii. Celem przeprowadzonych badań było przebadanie w kierunku obecności wirusa choroby woreczkowej próbek pszczół zebranych od kwietnia do września w 9 polskich pasiekach. Pszczoły zbierano w 10 rodzinach każdej pasieki i badano w kierunku obecności wirusa choroby woreczkowej metodą immunodyfuzji przy użyciu wyprodukowanej surowicy.

Przebadano też w kierunku obecności wirusa choroby woreczkowej dwie próbki czerwiu wykazującego objawy tej choroby.

Wirus choroby woreczkowej stwierdzono w 9 rodzinach pochodzących z pięciu pasiek. Próbki pszczół zawierające ten wirus były zebrane w kwietniu i maju i, w jednym przypadku, we wrześniu. W próbkach czerwiu stwierdzono obecność wirusa SBV.

Słowa kluczowe: choroba woreczkowa czerwiu, pszczoła miodna, Polska.