

## POLISH HONEY BEE COLONY-LOSS DURING THE WINTER OF 2007/2008

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Received 21 October 2008; accepted 14 November 2008

### S u m m a r y

During the winter of 2007/2008 beekeepers from most regions of Poland reported extraordinary bee colony losses. The aim of this work was to investigate the extent of this problem and point out possible causes.

We analysed 431 questionnaires completed by beekeepers. Questions concerned the number of colonies that had been prepared for wintering in the autumn of 2006 and 2007 and the number of colonies which survived the winter of 2006/2007 and 2007/2008. The investigations pertained to about 1% of the beekeeper population and to 3% of bee colonies in Poland.

We investigated 448 dead bee samples and 15 capped brood samples (from collapsed or almost collapsed colonies). These were sent by 104 beekeepers from October 2007 to April 2008. The samples were examined mainly for *Varroa destructor*, *Nosema* spp., as well as bees with deformed wings, acute bee paralysis virus (ABPV), black queen cell virus (BQCV), chronic bee paralysis virus (CBPV) and sacbrood virus (SBV).

The collected data indicate that in Poland the colony loss during the winter of 2007/2008 (15.3%) was significantly higher than during the winter of 2006/2007 (9.9%). The mean colony loss for an apiary was 15.9%. The highest percentage of beekeepers (22.9%) who lost 30(+) percent of their colonies was within the group which had 21 to 50 colonies. The problem of colony loss seemed to be more serious in Zachodniopomorskie and Wielkopolskie. These are provinces with fewer (10 – 20) cold winter days ( $T_{max} < 0^{\circ}C$ ) (Lorenc 2005) than in the provinces of Warmińsko-Mazurskie, Kujawsko-Pomorskie, Pomorskie which generally have more cold days.

The investigation of the samples revealed a “Varroa problem” (severe *V. destructor* infestation, bees with deformed wings or ABPV infection detected) in 55% of the apiaries. In 32% of the apiaries a severe *Nosema* spp. infection was detected. We were not able to determine the possible causes of colony losses in about one third of the apiaries.

**Keywords:** honey bee colony-loss, winter 2007/2008, Poland, questionnaire, dead bees.

### INTRODUCTION

The phenomenon of heavy honey bee colony-loss is well known in the history of beekeeping worldwide. Many factors e.g. *Acarapis woodi* and *Varroa destructor* infestations, or different virus infections have been found to be responsible (Bailey

1983, Hung et al. 1996). The new wave of colony collapse started in 2006 in the USA (Johnson 2007) and soon afflicted the apiaries in Europe. In the USA beekeepers mostly blame the mysterious Colony Collapse Disorder (CCD) for the situation. In CCD bees from a colony suddenly

disappear. Only a handful of young bees with their queen and brood, and food stores are found in the hive (Kaplan 2008). In collapsing colonies plenty of different bee pathogens were found, for example, parasites, bacteria and viruses. The direct cause of CCD, however, is not yet known (Watanabe 2008). Israel acute paralysis virus was found in 83% of the colonies with CCD, but it is considered rather an indicator of CCD than the cause (Cox-Foster et al. 2007). According to Pettis (Kaplan 2008), "CCD is likely a combination of factors, as opposed to a single, discrete cause". In many European countries *Varroa destructor* and *Nosema* spp. were supposed to be responsible for the death of most of the colonies (Martin-Hernandez et al. 2007, Neumann 2008). Lack of hard field data on losses, limits a better understanding of the causative factors (Neumann 2008).

During the winter of 2007/2008 beekeepers from most regions of Poland reported extraordinary losses of bee colonies. The aim of this work was to investigate the extent of this problem and point out possible causes.

## MATERIAL AND METHODS

### Questionnaire analysis

Beekeepers who are members of beekeeper associations in Poland, were asked to complete questionnaires. The questions were about the number of colonies which had been prepared for wintering in the autumn of 2006 and 2007 and the number of colonies which survived the winter of 2006/2007 and 2007/2008. 431 questionnaires were returned, of which 394 contained the data from the winter of 2006/2007 and 424 - from the winter of 2007/2008. Statistical analysis was performed using Statistica 8.0 software. Comparison of colony losses in different years was conducted using  $\chi^2$  test, of colony losses in apiaries of different sizes -

Yates corrected  $\chi^2$  test, and in different provinces - V-square test. To compare different provinces from the point of view of colony-loss in individual apiaries, the non-parametric Kruskal-Wallis one way analysis of variance was used. The reason for this is because the arcsin $\sqrt{x}$  transformation of data did not result in a normal distribution.

### Sample processing

We examined 448 dead bee samples and 15 capped brood samples, from collapsed or almost collapsed colonies. The samples were sent by 104 beekeepers. They were sent to our laboratory from October 2007 to April 2008. We treated all the colonies belonging to one beekeeper as one apiary.

Dead bee samples were examined: 1) under a stereoscopic magnifying glass for the presence of bees with deformed wings, *Varroa* mites, sugar crystals, rodent faeces, and wax moth larvae, 2) under a light microscope for the presence of *Nosema* spp. spores, 3) virologically for the presence of black queen cell virus (BQCV), acute bee paralysis virus (ABPV), chronic bee paralysis virus (CBPV) and sacbrood virus (SBV). Brood was examined under a stereoscopic magnifying glass for the presence of *Varroa destructor* mites and offspring, and virologically for BQCV, ABPV, CBPV and SBV.

The level of *Varroa* infestation in a colony was evaluated as high if one or more mites were found per ten bees (on average), or if one, or more female mites were found in 5 brood cells.

Microscopic examination was performed as described by Hartwig and Topolska (1995), with the use of a different extracting fluid. We used buffer pH 6.7 with DIECA (45 ml of the buffer plus 5 ml of DIECA per 100 bees). The level of *Nosema* sp. infection was evaluated according to the following pattern: (-) no spores; (+) single or few spores not in

every field of vision; (++) single or few spores in every field of vision; (+++) numerous spores in every field of vision. In this study (+++) was used to describe heavy infection.

After being examined under a microscope we then used the sample for further virological examination (Bailey et al. 1981, Ball 1999). We performed an AGID test with the use of rabbit polyclonal diagnostic antisera raised against black queen cell virus (BQCV), acute bee paralysis virus (ABPV), chronic bee paralysis virus (CBPV) and sacbrood virus (SBV) (Topolska et al. 2000, 2001, Topolska and Hartwig 2003, Topolska 2008).

## RESULTS

The data from 431 questionnaires pertained to about 1% of the beekeeper population and 3% of the bee colonies in Poland.

The analysis of data revealed that during the winter of 2007/2008 beekeepers in Poland lost about 15.3% of bee colonies, which was significantly more ( $\chi^2$  test,  $p < 0.001$ ) than during the winter of 2006/2007 (Table 1).

Mean colony loss for an apiary was 15.9% and it was almost the same in each group of apiaries. The apiaries were grouped in the following manner: up to 20 colonies, from 21 to 50 colonies, from 51 to 100 colonies, and above 100 colonies. The highest percentage of beekeepers (apiaries) with a 30(+) percent colony-loss

Table 1.

2006/2007 and 2007/2008 Winter Colony-Loss Comparison.  
( $\chi^2$  test,  $p < 0.001$ ).

Year	Number of colonies before winter	Number of colonies after winter	Colony loss* [%]
2006/2007	24790	22347	9.9
2007/2008	26710	22634	15.3

\* The difference significant

Table 2.

Number of apiaries (by size) with a winter 2007/2008 severe colony-loss. Figures with different letters differ significantly (Yates' corrected  $\chi^2$  test,  $p < 0.05$ )

Group of apiaries (by size)	Number of apiaries	Apiaries with severe* losses	
		Number	%
1-20	142	25	17.6ab
21-50	166	38	22.9b
51-100	75	9	12a
Above 100	40	6	15a

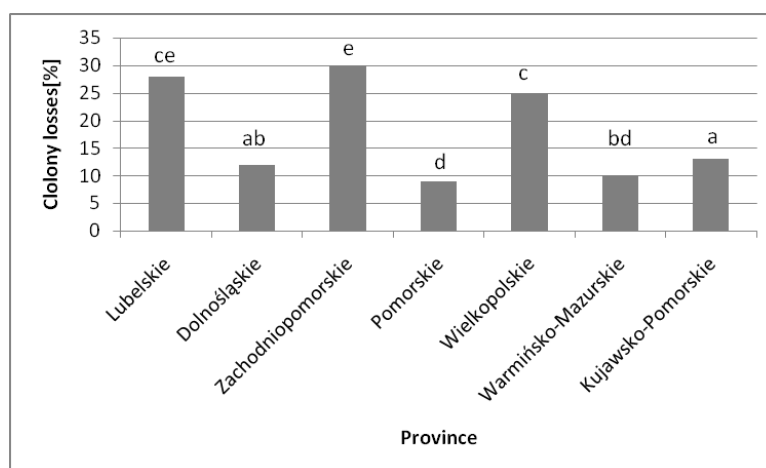
\*at least a 30% colony-loss

was in the group with 21 to 50 colonies (Table 2). Compared to apiaries with 1-20 colonies the difference was not statistically

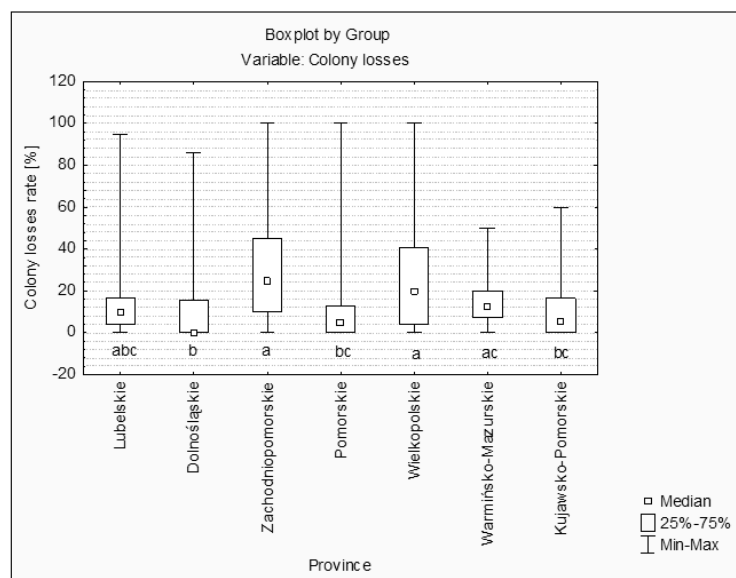
significant. (Yates' corrected  $\chi^2$  test;  $P=0.05$ ).



**Fig. 1.** Polish provinces with number of received questionnaires. The mean percentage loss of colonies in individual apiaries for the winter of 2007/2008 is in brackets. Provinces from which few questionnaires were returned are NOT underlined.



**Fig. 2.** Winter 2007/2008 colony-loss of different provinces. The bars with different letters indicate a significantly different percentage of colony losses (V-square test,  $p < 0.05$ ).



**Fig. 3.** Winter 2007/2008 colony-loss in individual apiaries by province. Median with different letters differ significantly; Kruskal-Wallis test:  $H(6, N=405) = 60,71714, p < 0.05$ .

We received questionnaires from beekeepers from 14 provinces. 7 of those provinces provided such a small response (Fig. 1) that they were not considered in further analyses. The percentage of apiaries with 21-50 colonies was as follows: Lubelskie - 24%, Warmińsko-Mazurskie - 48%, Dolnośląskie - 29%, Zachodniopomorskie - 39%, Kujawsko-Pomorskie - 59%, Pomorskie - 57%, Wielkopolskie - 42%. In Zachodniopomorskie, Wielkopolskie and Lubelskie losses of colonies were significantly higher than in other provinces (V-square test,  $p < 0.05$ ) - Fig. 2. The median percentage of colony loss in individual apiaries was higher in Zachodniopomorskie and Wielkopolskie. In these two provinces 50% of the apiaries showed losses from 10 - 45% and from 4 - 40% respectively. In other provinces, in 75% of apiaries the losses did not exceed 20%. In statistical analysis not all the differences were considered significant (Kruskal-Wallis;  $P = 0.05$ ) - (Fig. 3). In

Wielkopolskie, Zachodniopomorskie Pomorskie, Lubelskie there were some apiaries which lost 100 or almost 100% of their colonies. In Warmińsko-Mazurskie and Kujawsko-Pomorskie the highest losses in apiaries, reached respectively 50 and 65%. In Lubelskie 68.8% of lost colonies in the province belonged to one beekeeper. If we do not take this apiary into account, the percentage of colony-loss in the province falls from 27.5% to 10%.

Each received sample usually contained bees from one colony but sometimes beekeepers sent pooled samples from several colonies. We received from 1 to 20 samples from each apiary. The investigation of bee or brood samples from an apiary which indicated heavy Varroa infestation in at least one of the colonies we treated as an apiary in which a serious problem with Varroa infestation had occurred. The presence of bees with deformed wings or the presence of ABPV - (acute bee paralysis virus) in the samples was also an indicator of serious "Varroa

Table 3.

Infections and infestations in apiaries (found by examination of samples) in different months (winter of 2007/2008).

Month/ year	Number of apiaries	Apiaries with severe infection or infestation						Apiaries with no severe infection or infestation
		<i>Nosema</i> spp.	<i>Nosema</i> spp. + BQCV	<i>Varroa</i> <i> destructor</i>	ABPV	Bees with deformed wings	<i>Varroa</i> problem*	
		no. (%)	no.	no.	no.	no.	no. (%)	
10/07	28	5(18)	0	7	7	8	17(31)	10(30)
11/07	16	1(6)	0	9	3	5	11(69)	4(25)
12/07	8	3(37)	0	4	0	2	6(75)	4(0)
1/08	15	2(13)	1	7	0	2	8(53)	9(53)
2/08	9	2(22)	1	5	3	2	5(55)	3(22)
3/08	20	12(60)	8	4	5	4	10(50)	2(10)
4/08	8	8(100)	5	0	0	0	0(0)	0(0)
Total	104	33(32)	15	36	20	24	57(55)	32(31)

\* - apiary with severe *Varroa destructor* infestation, or with bees with deformed wings, or ABPV  
 ABPV - acute bee paralysis virus, BQCV – black queen cell virus,

problem” in the apiary. If an apiary had even one sample with severe *Nosema* spp. infection, then that apiary was considered to have a serious *Nosema* spp. infection problem. Investigation showed that 55% of the apiaries which sent samples, had a problem with a severe *Varroa* infestation, and 32% had a severe *Nosema* spp. infection. Chronic bee paralysis virus (CBPV) was found in one sample of dead bees and sacbrood virus (SBV) was found in one brood sample. Possible causes of colony-loss were not indicated in samples from 31% of the apiaries.

### DISCUSSION

Collected data indicate that in Poland the colony-loss during the winter of 2007/2008 was significantly higher than during the winter of 2006/2007. It was higher than in Austria, where beekeepers lost about 13.4% of colonies (Crailsheim et al.

2008) but lower than in Canada - 26% (Pernal 2008) and USA - 36% (Penn State 2008). It was also lower than was widely believed. The highest number of beekeepers who had lost 30(+) percent of their colonies were within the group which had 21 to 50 colonies. This suggests that apiary management plays an important role. Apiaries of this size are usually kept to make some extra money and the main source of income lies outside beekeeping. Therefore beekeepers often cannot devote sufficient time to dealing properly with their main problem, *Varroa* infestation, or to prevention and control of other bee diseases.

Analysis of the questionnaires showed that, like in Canada (Pernal 2008), the colony losses were different in individual provinces. The problem seemed to be more serious in Zachodniopomorskie and Wielkopolskie. These are provinces with

fewer (10 - 20) cold days during winter ( $T_{max} < 0^{\circ}\text{C}$ ) than in such provinces as Warmińskie - Mazurskie, Kujawsko-Pomorskie, Pomorskie (Lorenc 2005) where there are more cold days. This corresponds with the findings of the Giray et al. (2007) studies, in which the regional colony losses in Turkey, during the winter of 2006/2007, were related to warmer-than-usual weather conditions. However, in our survey this influence of the climate was observed in those provinces where the percentage of apiaries with 21-50 colonies was high, i.e. in Zachodniopomorskie, Warmińsko-Mazurskie, but not in Dolnośląskie - with the same number of cold days during winter but with a smaller percentage of apiaries having 21-50 colonies. In view of the above the high colony loss in Lubelskie is surprising. Lubelskie had a similar number of cold days as Warmińsko-Mazurskie, and a small percentage of apiaries with 21 to 50 colonies. This situation was generated, however, by very high losses in one big apiary. If we eliminate this apiary from the analysis, the situation in the Lubelskie province fits the model of provinces with rather small losses.

The data gathered in questionnaires may not fully correspond with reality. This is due to the fact that beekeepers are often unwilling to reveal information about colony losses in their apiaries.

The method of estimating Varroa infestation levels in apiaries was far from perfect. It must be realized, though, that it was difficult to perform such assessments if the sample consisted of a handful of dead bees which had remained in the hive after the collapse of the colony. ABPV (acute bee paralysis virus) and DWV (deformed wing virus) multiply intensively in colonies heavily infested by Varroa mites (Nordstrom 2000). For this reason we considered apiaries with ABPV and with

bees with deformed wings – the symptom associated with DWV infection - as having a serious Varroa problem. Analysis of samples showed that Varroa infestation (associated with virus infections) could have been the main cause of colony-loss between October 2007 and February 2008. This is true even though the threat was greater in the fall than in the spring time. If we combine the results from questionnaire analyses and sample investigations, it may be concluded that Varroa infestation could have been an especially important factor in Zachodniopomorskie and Wielkopolskie. These two provinces having fewer cold days during winter, than in the other provinces. During cold days there is usually no brood in colonies and this limits the reproduction of Varroa mites during wintering (Martin 1998). The lowered efficacy of antiviral drugs and a warm winter probably contributed to the situation.

Results of sample investigations for *Nosema* spp. and BQCV (black queen cell virus) show that *Nosema* infection (together with BQCV infection) was probably the next factor, which contributed to colony-loss. It seems that colony-loss due to *Nosema* infection during the late spring months was more visible, although it was also significant during late autumn and winter. After the ban of fumagillin for *Nosema* control in Poland, beekeepers often found it very difficult to deal with this infection. The fact that this is mostly *Nosema ceranae* infection (which according to Martin Hernandez et al. (2007) and Ritter (2008) almost displaced from European apiaries the less harmful *Nosema apis*) makes the situation even more difficult.

In Poland, like in Canada, *Varroa destructor* (with associated virus infections) and *Nosema* spp. played a key role in colony losses during the winter of 2007/2008 (Pernal 2008), but cannot

alone explain the current major losses. We have not been able to determine possible causes of colony-loss in about one third of the apiaries.

### CONCLUSIONS

1. The main causes of big colony losses during the winter of 2007/2008 in Poland were most probably *Varroa destructor* infestation and *Nosema* spp. infection together with associated virus infections. Other factors could also have contributed.

2. Apiaries in provinces with less severe winters suffered more from colony losses than apiaries in the other provinces.

3. In Poland the management system in apiaries of 21-50 colonies was less efficient in protecting colonies from collapsing than in both smaller and larger apiaries.

4. The questionnaire survey system should be improved to increase research reliability.

### ACKNOWLEDGMENT

The research was partially financed by the Ministry of Science and Higher Education. Project number N308 010 32/1204.

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## STRATY RODZIN PSZCZELICH W POLSCE PODCZAS ZIMY 2007-2008

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### S t r e s z c z e n i e

Zimą 2007/2008 roku pszczelarze w wielu rejonach Polski notowali duże straty rodzin pszczelich. Celem pracy było zbadanie skali zjawiska i wskazanie możliwych przyczyn.

Przeanalizowano 431 wypełnionych przez pszczelarzy kwestionariuszy ankietowych z pytaniami dotyczącymi liczby rodzin pszczelich przygotowanych do zimy jesienią 2006 i 2007 roku oraz liczby rodzin, które przeżyły zimę 2006/2007 oraz 2007/2008. Badanie objęło około 1% pszczelarzy oraz 3% rodzin pszczelich w Polsce.

Przebadano 448 próbek martwych pszczół oraz 15 próbek czerwiu, pochodzących z zmarłych lub prawie zmarłych rodzin. Materiał nadesłało 104 pszczelarzy, w okresie od października 2007 do kwietnia 2008 r. W próbkach badano głównie obecność *Varroa destructor*, *Nosema* spp., pszczół ze zdeformowanymi skrzydłami, wirusa ostrego paraliżu pszczół (ABPV), wirusa choroby czarnych mateczników (BQCV), wirusa chronicznego paraliżu pszczół (CBPV) oraz wirusa choroby woreczkowej czerwiu (SBV).

Zebrane dane wskazują, że strata rodzin pszczelich w Polsce zimą 2007/2008 (15,9%) była istotnie większa niż zimą 2006/2007 (9,9%). Procentowo najczęściej pszczelarzy, którzy utracili przynajmniej 30% rodzin było wśród właścicieli 21-50 rodzin (22,9%). Wydaje się, że problem giniecia rodzin był bardziej nasilony w województwach, gdzie ilość chłodnych dni w ciągu zimy ( $T_{max} < 0^{\circ}C$ ) jest mniejsza (10-20), tj. w Zachodniopomorskim i Wielkopolskim, niż tam, gdzie takich dni jest więcej, np. w Warmińsko-Mazurskim, Kujawsko-Pomorskim, czy Pomorskim.

Badanie próbek wykazało, że w 55% pasiek wystąpił „problem z warrozą” (stwierdzono silne porażenie roztocami *V. destructor*, obecność pszczół o zdeformowanych skrzydłach, zakażenie ABPV). W 32% pasiek wykryto silne zakażenie *Nosema* spp. Nie byliśmy w stanie wskazać prawdopodobnej przyczyny zamarcia rodzin w około jednej trzeciej pasiek, z których pochodziły próbki.

**Słowa kluczowe:** straty rodzin pszczelich, zima 2007/2008, Polska, ankieta, martwe pszczoły.