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Prevalence of honeybee diseases in Incheon area in 2011

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Abstract

This study investigated the occurrence of honeybee diseases in Incheon area, at the point of great widespread of sacbrood disease in the country. Sixteen resident beekeeping apiaries; 3 native honeybee and 13 European honeybee apiaries were selected for this research. Over 20 adult bees were evenly collected from the most colonies of each apiary three times (March, June, November) within a year. In this work, 13 honeybee diseases including 7 viral diseases, 2 bacterial diseases, 2 fungal diseases, and 2 parasitic diseases were detected by preliminary inspections and PCR. As a result, viral infections were confirmed at 34 among 48 apiaries (70.8%) over the entire examination period. Parasitic diseases showed the highest detection rate of 45.8%, which are detected in 44 among 96 cases. In the seasonal prevalence, 30 cases (15.6%) of 7 pathogens were detected from 14 apiaries in March, 50 cases (24.0%) of 9 pathogens and 56 cases (26.9%) of 9 pathogens were detected from all apiaries in June and November, respectively. Nosema was shown to be the most prevalent pathogen from March to November, followed by sacbrood virus (SBV) and stonebrood. The spread of SBV infection in Incheon would be underestimated by the increasing of detection rate over the time. Especially, Chinese sacbrood virus was detected from 4 European honybee apiaries, but clinical symptoms were not found. No chalkbrood, acute bee paralysis virus, and chronic bee paralysis virus were detected in this study. The effective therapy and preventive measures should be prepared for beekeeping industry.

Key words : Honeybee diseases, PCR, Nosema, Sacbrood virus

INTRODUCTION

Honeybees are threatened by various pathogens because of their crowded and warm conditions in social interactions such as mutual grooming and food sharing (Liu et al, 2010). Honeybee diseases can be largely categorized as infectious and parasitic diseases. Infectious diseases are divided into three groups; 6 bacterial diseases, 18 viral diseases, and 2 fungal diseases. Parasitic diseases have been reported 3 internal protozoal diseases and 8 external parasitic diseases (Yoo and Yoon, 2009). Prevalence of viral diseases is estimated to 1.4% among honeybee diseases, but little has been known about the accurate mechanism causing disease (Yoon, 2001). In general, honeybee viruses are widespread and most of them persist as latent infections. So, it is difficult to diagnose with clinical signs only. However, some diseases not only collapse bee colonies but damage productivity directly. Actually, Chinese sacbrood virus disease emerged in 2009 has caused the collapse of *Apis (A.) cerana* colonies over 90% due to infection to larvae and adults in South Korea. Apart from SBV, acariasis, parasitic disease such as nosema, chalkbrood, and foulbrood can cause damage to the domestic beekeeping industry. It is possible to diagnose these parasitic diseases or chalkbrood by their clinical symptoms which make it easier to control the diseases but the early and accurate diagnosis is vital to control other dis-

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eases which show extensively non-specific symptoms.

To date, the diagnosis of honeybee diseases have been largely dependent on experiences and intuitions which cause misdiagnosis in many cases and result in extensive uses of antibiotics, and the emergences of antibiotic resistant bacteria. It is reported that most apiarists virtually tend to self-diagnose when honeybee colonies are collapsed. This fact makes it difficult to apply appropriate treatment and measure because they are unaware of accurate causes of diseases (Chung et al, 2011).

The current situation of the honeybee diseases in Incheon area has never been reported, although national reports about the severity of honeybee diseases and prevalence are available. For this reason, this study intends to diagnose 13 different honeybee diseases and aim to provide the foundation in order to establish appropriate treatments and proper preventive measures against epidemics about the outbreak of honeybee diseases and their distribution in Incheon area.

MATERIALS AND METHODS

Sample collection

This study was conducted in 16 apiaries including 3 native honeybee apiaries and 13 European honeybee apiaries among resident beekeeping farms in Incheon in 2011. The herd size of native honeybee apiaries is in the range from 10 colonies to 30 colonies, and that of European honeybee is 200～300 colonies. More than 20 adult bees were evenly collected from the most colonies of each apiary, respectively. In order to examine the characteristics of the onset of diseases according to the activity periods of honeybees, samples were collected three times; March (immediately after the wintering), June (major honey flow period), and November (immediately before the wintering).

Extraction of nucleic acid and PCR analysis

Viral RNA and genomic DNA were extracted from

Table 1. List of primers for honeybee diseases

Pathogen	Oligonucleotide	Sequence (5'→3')	Product (bp)	Reference
Sacbrood virus	SBV-F	ACCAACCGATTCCTCAGTAG	487	Grabensteiner et al, 2007
	SBV-R	CCTTGGAACTCTGCTGTGTA		
	SBV-R_2	TCTTCGTCCATCCTCATCAC	258	Yoo et al, 2007
	CSBV-F	GGATGAAAGGAAATTACCAG	426	Liu et al, 2010
	CSBV-R	CCACTAGGTGATCCACACT		
Acute bee paralysis virus	ABPV-PF	TTATGTGTCCAGAGACTGTATCCA	901	Benjeddou et al, 2001
	ABPV-PR	GCTCCTATTGCTCGGTTTTTCGGT		
Chronic bee paralysis virus	CBPV-F	AGTTGTCATGGTTAACAGGATACGAG	455	Ribière et al, 2002
	CBPV-R	TCTAATCTTAGCACGAAAGCCGAG		
Deformed wing virus	DWV-F	TCATCTTCAACTCGGCTTTCTACG	479	Lee et al, 2005
	DWV-R	CGAATCATTTTCACGGGACG		
Black queen cell virus	BQCV-F	TCGTCAGCTCCCACTACCTTAAAC	700	Benjeddou et al, 2001
	BQCV-R	GCAACAAGAAGAAACGTAAACCAC		
Kashmir bee virus	KBV-F	GATGAACGTCGACCTATTGA	415	Choi et al, 2008
	KBV-R	TGTGGGTGGCTATGAGTCA		
Paenibacillus larvae (American foulbrood)	AFB16s NF	GTGTTTCCTTCGGGAGACG	233	Lee et al, 2004a
	AFB16s NR	CTCTAGGTCGGCTACGCATC		
Mellissococcus pluton (European foulbrood)	EFB-NSF	AAGAGTAACTGTTTTCCTCG	564	Ha et al, 2005
	EFB-NSR	ACGCCTTAGAGATAAGGTTT		
Ascosphera apis (Chalkbrood)	Asco18S-F	GGCTGTAGGGGGGAACCAGGA	994	Lee et al, 2004b
	Asco18S-R_1	CGGGTGGTCGTTTCCAGCCTC		
Aspergillus flavus (Stonebrood)	Asp 18S-F	ATCGGGCGGTGTTTCTATG	312	Lee et al, 2004b
	Asp 18S-R	ACCGGGCTATTTAAGGGCCG		
Nosema apis	Nosema-F	CTGCCTGACGTAGACGCTAT	592	Yoo et al, 2007
	Nosema-R	CTTCGATCCTCTAGCTTACG		

bee homogenates using DNeasy Blood & Tissue Kit and RNeasy Mini Kit (Qiagen, Holland). The DNA and RNA were immediately used for PCR. Specific primer pairs were selected to amplify each pathogen (Table 1). Amplifications were carried out in 20 μl reaction mixtures employing the Accupower PCR premix or RT-PCR premix (Bioneer, Korea). RT-PCR thermal reactions proceeded with an initial reverse transcription incubation at 50°C for 30 min, followed by incubation at 95°C for 15 min. This was followed by 40 cycles of denaturation at 94°C for 30 sec, annealing at 45～55°C for 30 sec, and extension at 72°C for 1 min. A final extension step was performed at 72°C for 10 min. PCR products were electrophoresised in a 1.5% agarose gel containing 0.05 μl/ml RedSafe (iNtRON, Korea) and photographed under UV light. Honeybee mites were examined through the preliminary inspection and a microscope.

RESULTS

The results according to the time of the examination

30 cases of disease were reported from 14 apiaries in March, 50 cases were reported from all apiaries in June, and 56 cases were reported in all apiaries in November (Table 2).

The results according to the group of pathogens

Parasitic disease detected in 44 among 96 cases from 33 apiaries showed the highest detection rate of 45.8%, and bacterial disease detected in 19 among 96 cases showed the second highest detection rate of 19.8% over the entire examination period. Viral disease detected in 57 among 336 cases from 34 apiaries was the third one of 16.8%, and fungal disease detected 16 among 96 cases showed the lowest detection rate of 16.6% (Table 3).

The results according to the type of diseases

Seven pathogens and 30 cases including 7 cases of stonebrood (SB), 6 cases of nosema disease, 6 cases of black queen cell virus (BQCV), 5 cases of European foulbrood (EFB), 3 cases of sacbrood virus (SBV), 2 cases of American foulbrood (AFB) and 1 case of acariasis were detected in March. During the honey flow period (flower season), in June, total 9 pathogens and 50 cases including 15 cases of nosema, 9 cases of SBV, 9 cases of AFB, 6 cases of acariasis, 3 cases of deformed wing virus (DWV), 3 cases of stonebrood (SB),

Table 2. Seasonal prevalence of the honeybee diseases

Period of honeybees	No. of apiary		No. of pathogen		No. of disease	
	Test	Positive (%)	Test	Positive (%)	Test	Positive (%)
March (Immediately after wintering)	16	14 (87.5)	13	7 (53.8)	208	30 (15.6)
June (Honey flow period)	16	16 (100.0)	13	9 (69.2)	208	50 (24.0)
November (Immediately before wintering)	16	16 (100.0)	13	9 (69.2)	208	56 (26.9)
Total	48	46 (95.8)	39	25 (69.4)	624	136 (21.8)

Table 3. Detection rate according to the group divided by pathogen

Group divided by pathogen	No. of pathogen		No. of apiary		No. of disease	
	Test	Positive (%)	Test	Positive (%)	Test	Positive (%)
Parasite	2	2 (100.0)	48	33 (68.8)	96	44 (45.8)
Bacteria	2	2 (100.0)	48	19 (39.6)	96	19 (19.8)
Virus	7	5 (71.4)	48	34 (70.8)	336	57 (16.8)
Fungi	2	1 (50.0)	48	16 (33.3)	96	16 (16.6)
Total	13	10 (76.9)	192	102 (53.1)	624	136 (31.4)

Table 4. Frequencies of the various diseases according to the species

Breed	Season	Parasite		Bacteria		Fungi		Virus							Total	
		Mite	Nosema	AFB	EFB	CB	SB	ABPV	CBPV	DWV	BQCV	KBV	SBV	CSBV	No.	%
Apis cerana	March	-	-	-	-	-	2	-	-	-	-	-	3	-	5	12.8
(3 apiaries)	June	1	3	-	-	-	1	-	-	-	-	-	3	-	8	20.5
	November	-	1	-	-	-	3	-	-	-	-	-	3	-	7	17.9
	Subtotal	1	4	-	-	-	6	-	-	-	-	-	9	-	20	17.1
Apis mellifera	March	1	6	2	5	-	5	-	-	-	6	-	-	-	25	14.8
(13 apiaries)	June	5	12	9	-	-	2	-	-	3	1	2	6	2	42	24.9
	November	5	10	-	3	-	3	-	-	9	3	5	9	2	49	29.0
	Subtotal	11	28	11	8	-	10	-	-	12	10	7	15	4	116	22.9
Total		12	32	11	8	-	16	-	-	12	10	7	24	4	136	21.8

AFB: American foulbrood, EFB: European foulbrood, CB: chalkbrood, SB: stonebrood, ABPV: acute bee paralysis virus, CBPV: chronic bee paralysis virus, DWV: deformed wing virus, BQCV: black queen cell virus, KBV: kashmir bee virus, SBV: sacbrood virus, CSBV: Chinese sacbrood virus.

Table 5. Single and mixed infection according to season

Season	N.D.*	Single infection	Mixed infection			Total
			2	3	> 4	
March	2	4	7	1	2	16
June	0	1	6	4	5	16
November	0	0	4	4	8	16
Total	2	5	17	9	15	48

*Not detected.

2 cases of kashmir bee virus (KBV), 2 cases of Chinese sacbrood virus (CSBV), and 1 case of black queen cell virus (BQCV) were identified. Over the period before wintering, total 9 pathogens and 56 cases including 12 cases of SBV, 11 cases of nosema, 9 cases of DWV, 6 cases of SB, 5 cases of KBV, 5 cases of acariasis, 3 cases of EFB, 3 cases of BQCV, and 2 cases of CSBV were detected. Nosema disease was the most prevalent followed by SBV and SB (Table 4). Acute bee paralysis virus (ABPV), chronic bee paralysis virus (CBPV) and chalkbrood (CB) were not identified in this study.

The results according to the species

The pathogens for 4 different diseases including sacbrood, acariasis, nosema, and stonebrood were identified in three native honeybee apiaries and among these, sacbrood virus were shown in the highest detection rates of 100% by being identified in all the apiaries. In 13 European honeybee apiaries, all the honeybee disease excluding acute bee paralysis virus, chronic bee paralysis virus and chalkbrood were identified and the prevalence of nosema disease, sacbrood and American foulbrood were 71.8%, 38.5% and 28.2% respectively over the entire period (Table 4).

Infection aspect according to the number of disease

Reviewing the aspect of the infections, the single infection was 10.4% (5 apiaries), double infection was 35.4% (17 apiaries), and the mixed infection with more than 3 diseases was 50.0% (Table 5).

DISCUSSION

Damage by honeybee diseases has been thought to be one of the reasons to reduce the productivity in domestic beekeeping industry. In general, honeybee diseases

caused by mites, nosema and foulbrood have been reported frequently, but most viral diseases persist as inapparent infections (Ball and Bailey, 1997). Environmental factors and parasite infestations, however, may activate virus infection, which may lead to clinical symptoms (Grabensteiner et al, 2007).

Nosema (N.) apis was first identified by Zender as a bacteria causing nosema disease in honeybee in 1905 and a new species of *N. ceranae* was found among Chinese native honeybee in 1995 (Fries et al, 1996). This pathogen was detected in Spain, Germany and Switzerland, and it has been confirmed to distribute throughout Europe (Higes et al, 2006). It had been also detected in the United States by examining the honeybees in 12 states between 1995 and 2007 (Chen et al, 2008). According to the domestic studies on nosema disease, 56% of incident rate was reported in 18 apiaries in 2002 (Lee et al, 2003) and Kim et al (2010) reported 73% of incident rate during the flowering season and 85% during the season of no blossom of *Robinia pseudoacasia* L. in 2009. This study examined 16 apiaries in Incheon area in 2011 and the incident rate was 66.7% in average which ranged between the result of Lee et al (2003) and the result of Kim et al (2010). However, the incident rate during the flowering season was higher than those of non-blossom with the percentage of 93.7% and 68.8% respectively in this study showing different from the study result of Kim et al (2010), and this was thought to be stemmed from the fact that nosema disease is the parasitic disease with the characteristics of local epidemic and this study was conducted within Incheon area only.

SBV of *A. cerana* was first observed in Gangwon Province, Korea in 2009 and subsequently spreaded throughout the rest of Korea. SBV is the a most dangerous virus associated with the collapse of *A. cerana* colonies due to its infection of larvae and adults with high epidemic ability (Ongus et al, 2004). Larvae with sacbrood that failed to pupate, full of ecdysial fluid and rich in SBV beneath their unshed skin, and the body color of infected larvae changed from pearly white to pale yellow.

76.7% of native honeybee apiaries were affected by sacbrood virus in 2010. Especially, farms around Jiri Mountain were severely damaged. In 2011, 126 cases of sacbrood virus infection were reported throughout the country, and it was presumed that occurrences were caused by carrier bees passed the winter. There was not significant cases in Incheon area until 2010 and there was no cases reported among European honeybees until March, but SBV was identified from 6 among 13 subject farms in this study over the activity period and 9 among 13 subject farms in November suggesting that sacbrood virus was spreading among European honeybees over the time. CSBV was not identified from native honeybees at all in this study, but it was identified from 4 European honeybee apraries in the sample of June and November totally. However, these CSBV infections were supposed to be inapparent infections because there were no clinical symptoms in the all positive apiaries. The infection rate of SBV and CSBV was 50.0% which was significantly higher than the previous report by Choi et al (2008) with the figure of 5.6% and the report by Yoo and Yoon (2009) with the figure of 17.6% and this was thought to be resulted from the proliferation of virus secondary epidemics in 2010～2011.

Foulbrood can be classified into American foulbrood and European foulbrood according to pathogens and it is known that the symptoms of American foulbrood are severer. Foulbrood was first occurred in the middle land of South Korea in 1950's which almost completely destroyed domestic apiculture and it is still occasionally reported in some areas with less severe degree (Yoon, 2002). Foulbrood showed 39.6% of detection rate in this study and the infection rate was 56.3% during the activity period by being identified in 9 among 16 farms which was similar to the reported figure of 58.8% by Yoo and Yoon (2009) and this was thought to be caused by early rainy season.

Chalkbrood caused by *Aspergillus* spp. and *Aspergillus flavus* is the most commonly identified pathogen. Stonebrood is frequently confused with chalkbrood as the characteristics resemble each other. Pathogenic spores germinate and infest the gut of the larvae resulting in mummification of the brood of bee appearing chalky (Lee et al, 2004b). Despite the fact that it has been perceived as minor honeybee disease comparing foulbrood or parasitic diseases, the infection rate of stonebrood in

this study was 33.3% and it seemed to be more problematic in the native honeybee apiaries.

Deformed wing virus, black queen cell virus and kashmir bee virus were identified and their detection rates were 25.0%, 20.8% and 14.6% respectively. These values appeared to be lower comparing the results of 33%, 35.6% and 27.9% by Yoo and Yoon (2009) and 66.67%, 33.3% and 11.11% by Choi et al (2008). Especially, deformed wing virus which is known to be occurred by *Varroa* mites, its detection rates have been reported depending on the area and Incheon area were shown in lower infection rate compared to the other areas. Overall characteristics of infection according to the farms showed that 10.4% were suffered from a disease, 35.4% were suffered from 2 different diseases, 22.9% of them were suffered from 3 different diseases and 27.1% of them were suffered from more than 4 different diseases. These results suggested that more than 50% of subjects became infected from more than 3 different diseases similar to the study results by Yoo and Yoon (2009).

The current situation of honeybee diseases in Incheon area was firstly confirmed in this study and it showed that a great number of apiaries were exposed to honeybee diseases. It was also confirmed that viral diseases were the case of multi-infection caused by more than 2 pathogens although they were latent infection without clear clinical symptoms. Continuous monitoring of the diseases is necessary for the correct diagnosis of the diseases which changes diversely during the different active periods and for the establishment of the prompt control measures. The accurate diagnosis of honeybee diseases would only facilitate to settle appropriate control measures contributing to the productivity and the quality improvements of apiculture products.

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