CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

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Department of Animal Science & Food Processing in Tropics and Subtropics

Evaluation of the Effectiveness of the Control Varroa Mites (*Varroa destructor*) at Increased Risk of Mite Infestation

M.Sc. Diploma Thesis

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Supervisor: doc. MVDr. Daniela Lukešová, CSc.

Co - supervisor: Ing. Ivan Pavlásek, DrSc.

Author: Bc. Lukáš Staněk

Declaration

I declare, that I have elaborated with my diploma thesis “Evaluation of the Effectiveness of the Control Varroa Mites (*Varroa destructor*) at Increased Risk of Mite Infestation” independently and I have used only quotations listed in References.

Prague, 18.4.2012

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Lukáš Staněk

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Abstract

The aim was to evaluate the extension of varroasis, an infectious disease of bees caused by *Varroa destructor* mite species in the Czech Republic, where in 2011 total of 12600 samples were tested of which were 7256 positive, while in 2012 there was 14951 samples examined with 11238 samples were positive for the present of *V. destructor* mite. Based on the laboratory diagnosis of presence of mites in bee debris, there was simultaneously monitored the effectiness of preventive measures against *V. destructor* occuring in bee colonies kept in individual regions and districts in the Czech Republic. Similarly were evaluated nationwide results from affected bee colonies in the period from 2005 to 2011, which were published by the Bee research institute at Dol.

Results of prevalence of varroasis in the years 2006-2011 pointed to a fact, that the expansion of *V. destructor* is slightly increasing since 2010 which can be attributed to increasing resistance of the mites to the treatment products based on pyrethroids.

Key words: *varoasis, Varroa destructor,* prevalence, effectiveness of control, Czech Republic

Abstrakt

Cílem práce bylo zhodnotit rozšíření varroázy, infekčního onemocnění včel, způsobeného roztočem druhu *Varroa destructor,* v České republice, kdy v únoru roku 2011 bylo celkem vyšetřeno 12600 vzorků, z toho 7256 pozitivních, zatímco v roce 2012 se jednalo o vyšetření 14951 vzorků, kdy 11238 vzorků včelí měli bylo pozitivních na přítomnost roztoče *V. destructor*. Na základě laboratorní diagnostiky přítomnosti roztočů ve včelí měli, byla současně sledována účinnost preventivních opatření proti roztoči *V. destructor* vyskytujícím se u včelstev chovaných v jednotlivých krajích a okresech na území České republiky. Obdobně byly vyhodnoceny celorepublikové výsledky postižených včelstev za období 2005-2011, které byly zveřejněny Výzkumným ústavem včelařským v Dole.

Výsledky prevalence varroázy v letech 2006 - 2011 poukázaly na skutečnost, že se rozšíření *V. destructor* od roku 2010 mírně zvyšuje, což se dá přičíst nárůstu rezistence roztočů k ošetřujícím přípravkům na bázi pyretroidů.

Klíčová slova: varoáza, *Varroa destructor*, prevalence, efektivnost kontroly, Česká republika

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A	Apis
Fig	Figure
V	Varroa
SVI	State Veterinary Institute
SVA CR	State Veterinary Administration in the Czech Republic
SVS ČR	Státní Veterinární Správa České republiky
ZO ČSV	Základní organizace Českého svazu včelařů
BRI	Bee Research Institute
ČIA	Český institut pro akreditaci
OIE	World Organization for Animal Health
WAHID	World Animal Health Information Database
Coll	Collection

Content

1. Introduction

The genus *Varroa* (family Varroidae) is in present represented at least by the four species of ectoparasitic mites. The *Varroa* mites feed on haemolymph of bees (*Apis spp.*) in whole world. *Varroa jacobsoni* was first described in Java on *A. cerana* (Oudemans, 1904), *V. rindereri* described on Borneo from *Apis koschevnikovi* (De Guzman and Delfinado-Baker, 1996), *Varroa underwoodi* was described in Nepal from *A. cerana* (Delfinado-Baker and Aggarwal, 1987) and *Varroa destructor* (new species) was described on *Apis cerana* and *Apis mellifera* (Anderson and Trueman, 2000).

This thesis is focused on Varoosis caused by *V. destructor*. This species is originally from Asia, where his host was an *A. cerana* and further shifted to *A. meliffera* and was spread over the world. Currently is the greatest threat for apiculture in the whole world out of Australia and some countries in western and central Africa (Rosenkranz *et al.*, 2010; OIE, 2012). *V. destructor* spread in a short time period. In the Czech Republic this mite was discovered in the year 1978 by Hanko. Further gradually spread throughout the Republic.

Till this time, it was found that the only hosts of the mite *Varroa destructor* are two species: Eastern honeybee and Western honeybee. *A. cerana* is naturally equipped with many biological defense mechanisms and therefore honeybee is a better host for this mite. It was not found attacked bumblebees, wasps and other *Hymenoptera* (Veselý *et al.*, 2003). At *Apis cerana*, the mite *Varroa* reproduces only on the drone brood. Adult females may also be present on female workers brood, but do not lay eggs here. Worker bees of *Apis cerana* are able to skillfully dispose of mite females by their mandibles. Even attacked worker bee can call to help other mates for this purpose they use unique dances (Veselý *et al.*,2003; Rosenkranz *et al.*, 2010).

Varroosis in Czech Republic since 2008 is decreasing due to the principles increase the efficiency of the control *Varoosis*, which issued by State veterinary administration. This thesis focused on evaluation of the effectiveness of the control varroa mites (*Varroa destructor*) at increased risk of mite infestation.

2. Literature review

2.1 Taxonomy

The species *Varroa destructor*, which was assumed to be *Varroa jacobsoni* until the end of last century (Anderson and Trueman, 2000), is the mite which is responsible for the clinical signs of Varoosis in *A. mellifera*. In most articles from 20th century about *Varroa* refer to *Varroa jacobsoni*, but the research subject was *Varroa destructor* (Rosenkranz *et al.*, 2010).

Phylum: ARTHROPODA "arthropods"

Subphylum: CHELICERATA

Class: Arachnida "arachnids"

Order: Acarina "tick and mites"

Suborder: Mesostigmata

Family: Varroidae

Genus: *Varroa*

Varroa destructor

Varroa jacobsoni

Varroa underwoodi

Varroa rindereri

(According Lukešová *et al.*, 2007)

At present the genus *Varroa* is represented by at the least four species of ectoparasitic mites:

- ***Varroa jacobsoni* Oudemans** - described on *Apis cerana*, as a natural ectoparasitic mite, in Java (Oudemans, 1904), later wide-spread throughout Asia on this bee (Koeniger *et al.*, 1981) and in Indonesia on *Apis nigrocincta* (Anderson and Trueman, 2000; Hadisoesilo and Otis, 1998)

- ***Varroa destructor*** - described on *Apis cerana* and *Apis mellifera* (new host), formerly erroneously classified as *V. jacobsoni* until the year 2000 (Anderson, 2000; Anderson and Trueman, 2000)
- ***Varroa rindereri*** - described on Borneo from *Apis koschevnikovi* (De Guzman and Delfinado-Baker, 1996)
- ***Varroa underwoodi*** - described in Nepal from *Apis cerana* (Delfinado-Baker and Aggarwal, 1987)

Varroa jacobsoni from the Java, which was described by Oudemans in 1904, completely lack the ability to reproduce on Western honey bee *Apis mellifera* (Anderson, 1994; Anderson and Sukarsih, 1996), therefore these mites is not worldwide serious pest of *A. mellifera*. Mitochondrial DNA (mtDNA) cytochrome oxidase I (CO-I) gene sequences is differ from phenotypically similar mites that can reproduce on *A. mellifera* in Europe (Anderson and Fuchs, 1998). Other reports confirm the variation among populations of *Varroa jacobsoni* (De Guzman *et al.*, 1998; De Guzman and Rinderer, 1999; Kraus and Hunt, 1995), therefore it was suggested that *V. jacobsoni* may not be only one species (Table 1) and this hypothesis was confirmed by Anderson and Trueman (2000).

V. destructor is represented by the seven haplotypes (Table 1), of which only two (the Korean and the Japanese/Thailand haplotypes) infests and reproduces on *A. mellifera* (Rosenkranz *et al.*, 2010). The Japanese/Thailand haplotype is a more restricted distribution than the former and is considered less virulent (De Guzman and Rinderer, 1999). This one has only been reported from *Apis mellifera* in North and South America, Thailand and Japan, while Korean haplotype parasitize western honeybee worldwide widespread out of Australia and S. America (Anderson and trueman, 2000; De Guzman *et al.*, 1998; Garrido *et al.*, 2003; Muñoz *et al.*, 2008). The haplotype of economic importance is Korean type due to its high reproductive potential (Strapazzon *et al.*, 2009).

V. jacobsoni was redefined by body size and mtDNA gene sequences. This species is only a guest on *Apis mellifera*, all of 9 haplotypes of this species parasitizing *Apis cerana* (Rosenkranz *et al.*, 2010).

Table 1: *Varroa* species and their haplotypes parasitizing on honey bees. Economic importance is two haplotypes of *Varroa destructor*. (Rosenkranz *et al.*, 2010)

Parasite	***Host***	***Haplotype***	***Pathogenicity***
Varroa jacobsoni	*Apis cerana*	Ambon	-
		Bali	-
		Borneo	-
		Flores	-
		Java	-
		Lombok	-
		Malaysia	-
		Sumatra	-
		Sumbawa	-
Varroa destructor	*Apis cerana*	Japan/Thailand	-
		Nepal	-
		Vietnam	-
	Apis mellifera	Japan/Thailand	*
		Korea	**
		China	-
		Korea	-

* Low pathogenicity

** High pathogenicity

\- No pathogenicity

2.2 Origin and distribution of *Varroa destructor*

2.2.1 Origin and the worldwide situation

The mite *Varroa destructor* originally was attacking only on Eastern honeybee, the original host. The first *Varroa* mites were discovered in Java in 1904 on the *Apis cerana*, formerly known as *Apis indica* (Oudemans, 1904). Further was successfully shifted to the Western honeybee. To the host shift most likely occurred in the first half of the last century, when *Apis*

mellifera colonies were transported to the Far East or Eastern USSR. This situation led to a sympatric distribution of both honey bee species (Oldroyd, 1999) and due to that the parasite could infest the new host. The first infestation of *A. mellifera* was recorded in People's Republic of China in the year 1954. Later *Varroa* mites were found in the Pakistan (1955), in Japan in 1958 (Mikawa, 1986), in the 60th has spread from Asia and Far East across the former USSR, due to transport honey bee colonies, to Europe. In 1967 was reported from Bulgaria, further in Romania (1975), Poland (1976), Germany (1977, Ruttner and Ritter, 1980*), Czechoslovakia (Hanko, 1978), Austria (Nixon, 1983). South America reported *Varroa* mites in 1971 in Paraguay, where mites have arrived on queens of *Apis mellifera* from Japanese beekeepers (Bailey & Ball, 1991; De Jong *et al*., 1982b). In the United States of America was discovered in 1987 (De Guzman and Rinderer, 1999). In New Zealand this disease was identified in 2000 (OIE). The varroasis was spread gradually in the whole world except Australia, South America, Some countries in Asia, central and some countries in western Africa (Figure 1) and became the main problem of world beekeeping. Worldwide spread of disease according to the WAHID (World Animal Health Information Database) is shown in Figure 1.

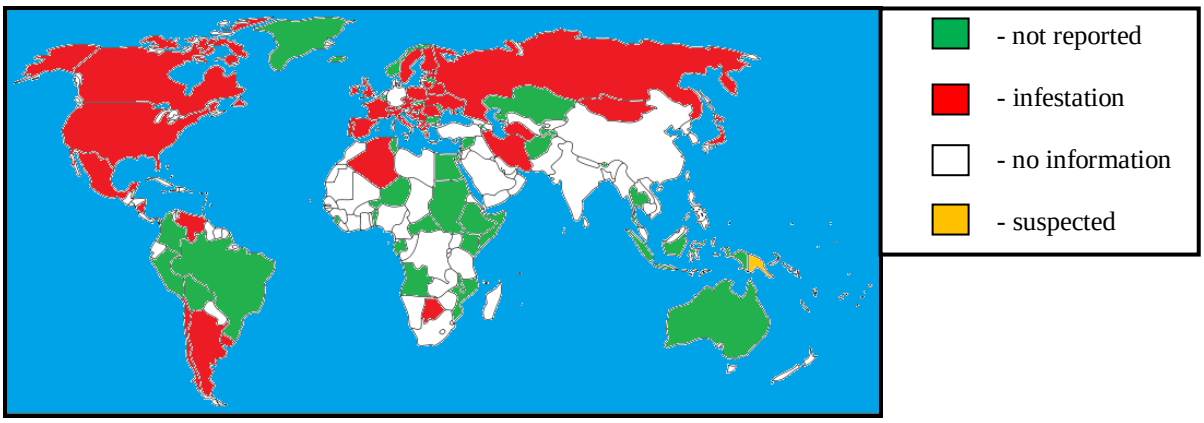


Figure 1: worldwide prevalence according to World Animal Health Information Database (OIE)

* Author provided evidence for earlier existence of mites.

2.2.2 Situation in the Czech Republic and at neighbours

The first Varroa mites in the former Czechoslovakia were discovered by Hanko in 1978 in a systematic verification of debris from the border areas of the USSR. A serious situation arose in the spring of 1981, when it was found out, that the mite *Varroa destructor* was introduced into the region of Ústí nad Orlicí. From there it gradually spread throughout the Republic (Veselý *et al.*, 2003). Currently in Czech Republic are 42% sites without mites. The most endangered districts in the republic, in number more than 3 mites on the colony, are Břeclav, Brno, Kolín, Praha, Příbram, Kladno, České Budějovice, Strakonice, Tachov and Jindřichův Hradec. The worst situation in the Czech Republic was in the years 2007 and 2008, loss of bee colonies was recorded about 40% (State Veterinary Administration CR, 2012). Germany reported losses 18% of the bee colonies, caused by Varroasis, in 2009/2010, 15% in the last year and in spring 2012 up to 30% (State Veterinary Administration DE, 2012).

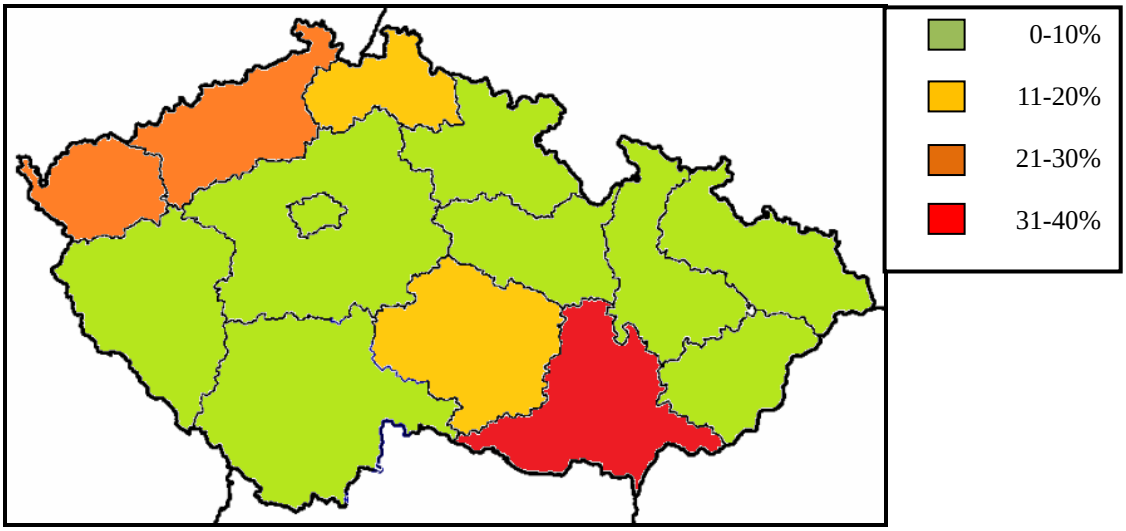


Figure 2: level of infestation in Czech Republic in 2009

2.3 Biology Description

2.3.1 Morphology

Varroa mites according to Indatidis (1983) show a distinctive sexual dimorphism and depending upon the host, mites have many morphological adaptations. The parasite has body divided into two well-defined parts. The larger part idiosoma comprises different ventral shields and one dorsal shield. Idiosoma at the female mites is ellipsoidal and flattened with greater width than length. For adherence to the host, females have special structures, the apoteles on strong and short legs (De Ruijter and Kaas, 1983). The legs of the females are shorter in relation to the body size than the legs of males. Their body is transversely oval, wide 1.5 to 1.9 mm and length 1.1 to 1.5 mm. In adulthood the ventral and shiny dorsal shields are sclerotised and at first show a brownish-yellow and later reddish brown coloration. The mite has flexible membranes between the shields enable to increase the body during egg formation and feeding (Rosenkranz *et al.*, 2010).

The mouthparts, which consist of two chelicerae and two sensory pedipalps, are formed by anteroventrally placed gnathosoma. The second part of mite body. The chelicerae are formed by the basal, the middle and distal part (Rosenkranz *et al.*, 2010).

The female has two systems of genitalia. A vagina, a uterus and ovary are formed the first one. The ovary, with two lyrate organs, is situated ventral to the spermatheca (De Ruijter and Kaas, 1983), reservoir for the spermatozoa. Inside the ovary are developed the oocytes and the lyrate organs has a nutritional function (Alberti and Zeck-Kapp, 1986). The second part of the genital tract is formed by solenostomes, pair of pores, and it is adapted to receive and maturation of sperm. These organs are situated between 3rd and 4th pair of legs and open up into ductus. In the center of the female body ductus associated in single tubule and pass into the sperm duct. The sperm duct continues to a large sac organ, spermatheca, which serves as a container for the spermatozoa until the fertilization of eggs. The eggs are released through the genital orifice and the genital opening is located between the 2nd pair of legs (Alberti and Hänel, 1986).

Males are smaller in all developmental stages in compared to the females. Their body is pear shaped and measured 0.8 mm in diameter. Throughout the life cycle they are off-white coloration and have weakly sklerotisation than females, especially on the dorsal shield and the

legs. The male reproductive system is not divided into two parts opposed to female. It is composed by unpaired ejaculatory duct, two vasa deferentia and by a single testis, which is situated in the rear of the body. Between the second pair of legs, at the edge of the sternal plate, is located the ejaculatory duct opening. According to Alberti (1980a, 1980b) the sperms pass through eight stages of maturation and belong to the ribbon type.

The whole body of the ectoparasite is covered by different types of hairs, which have chemo- and mechanoreceptive functions (Milani and Nannelli, 1988). The first front legs are very frequently lifted in the air like as feeler of the insects (Rickli *et al*., 1992).

Figure 3: *Varroa* mite parasitizing on the bee larvae; **Source**: FAO

2.3.2 Reproductive biology

The female mite begins own reproductive cycle that leaves an adult bee and enters into unsealed cell in which is found a worker larvae or drone larvae at the age of 5 days. The first egg is laid 70 hours after the cell capping (Infatidis, 1983; Steiner *et al*., 1994). From the first egg hatches the male, due to the haplo-diploid sex determination system and from all following eggs hatches the females (Ifantidis, 1990; Martin, 1994; Rehm and Ritter, 1989).
Capping cell contains one or more females and offspring in various stages of development. After 1 – 1,5 days from the laid eggs are hatches six-legged larvae, other stages are protonymph (duration 1,5 to 2,5 days) and deutonymph (3 – 3,5 days) (Martin, 1994).

2.3.3 Life cycle

If the only one female get to the cell, her offspring is fertilized by only one male present in the cell, thus own brother. When gets into the cell two or more females, may occur outbreeding. When a drone bee or worker bee stops their development in the cell, adult female mites attach themselves to them and leave the cell along with them (Veselý *et al.*, 2003). Males and others still immature mites, which did not complete their development, remain in the cell and die, because lacks a free living stage. There are two phases in the life cycle of *Varroa destructor* females (Rosenkranz *et al.*, 2010). The first one is phoretic phase on adult bees. Female mites stay on adult bees usually 4-13 days before they get into another cell, where once again begin their reproductive phase. *Varroa* females usually hidden on the adult bees under the sternites of the bee (Fernández *et al.*, 1993) On the wintering long-lived bees the female mites holds 5 – 8 months. At that time they do not lay eggs, because in the hive is not brood.

Adult females and developmental stages of Varroa mites feed on haemolymph of bees and bee brood. On the dead bees mite can survive 16 to 17 days, without bees only 6 – 7 days and on the combs can survive up to 40 days (Fries *et al., 2001)*.

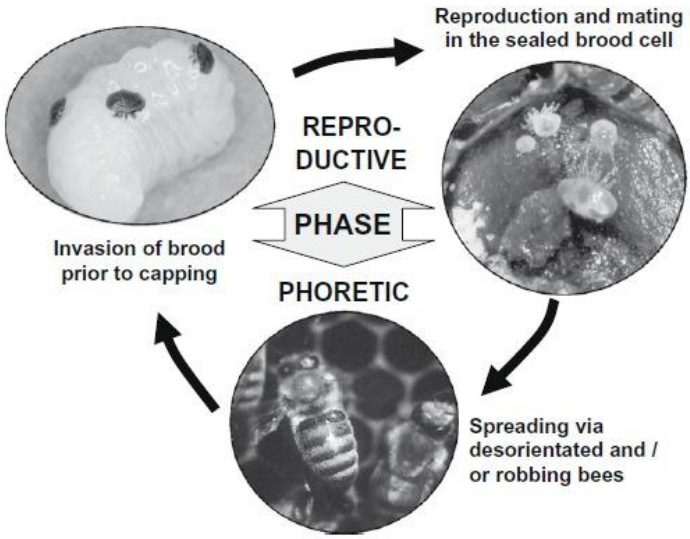


Figure 4: Life cycle of female *Varroa destructor*; **Source:** Rosenkranz *et al.*, 2010

2.4 Spread the disease

The mite *Varroa destructor* is spreading in many ways, but quickly spread in the hive itself, because there is a close contact between bees and so move to the neighboring bees is very easy. Drones, who are the most attacked in the colony, carry this parasite while flies foreign colonies. Further transmission also bees cause by swarming and robberies. In this way the disease spreads annually from 5 to 10 km depending on terrain. The largest share of the spread is however moving diseased hives. Varroa mites can also spread successfully sending mothers. Although mites attacks the queen at least, but the spread the disease causes by accompanying bees. The spread of the varroa mite by other *Hymenoptera* have not yet been demonstrated. Similarly, there is no evidence that the mite moved from one bee to another bee on the flower or on the mother returning from the wedding flight (Veselý *et al.*, 2003).

2.5 Clinical signs

Due to parasitosis bees are discomfort and weakened. During the winter they can even die. If house bee within 10 days of age is attacked, shorten its life to half. Older bee shortens life less. Mite is vector for viruses as a Deformed wing virus (DWV) and Acute bee paralysis virus (ABPV). From contested brood bees hatch with various deformities such as incompletely developed wings, incompletely developed abdomen, crooked legs or reduced the number of legs (Bowen-Walker *et al.*, 1999; Yue and Genersch, 2005; Yue *et al.*, 2006). Healthy worker bees bring affected bees from hives, where the bees die. In a stronger infestation also die bee pupae.

Without treatment the amount of mites reaches the thousands and more, and a colony dies within one to three years. Sometimes a hive will die even with a lower level of infestation, whereas the colonies of *Apis cerana* are able to survive infestation by *Varroa destructor* without visible damage (Korpela *et al.*, 1993; Fries *et al.*, 2006). When occurs to massive proliferation of mites during the summer, hive dies as early as in autumn.

Causative agent *varroa* mite could be confused with bee louse *Braula coeca* Nitzsch, 1818. Bee louse has, like mite, reddish brown body color. However, unlike mites don’t parasitize the bee brood. The body is divided on head, chest and abdomen and has three pairs of legs.

Mite *Varroa destructor* is also involved in the spread of diseases such as chalk brood (*larvae Ascosphaerosis apium*), American Foul Brood (*larvae Histolysis infectiosi pernicosa apium*), European foulbrood (*Putrificatio polybacteria larvae*), acute paralysis virus, cloudy wing virus and more.

2.6 Prevention against *Varroa destructor*

Since the *V. destructor* attacked the *Apis mellifera,* many colonies has destroyed. Beekeepers have started to use acaracides against the mites to keep the population under control. Beekeepers in Europe to control *Varoosis* have used since the nineties the coumaphos and synthetic pyrethroids. Unfortunately, mites rapidly acquire the resistance to these substances. It was needed the new strategy for battle with *Varroa destructor*, so development of new veterinary medications (Rademacher, 2006). Resistance to the pyrethroids in Europe monitored by Trouiller (1998) and his data advert to, that the resistant susceptibility originated in Italy in the 1990s and then spread to Slovenia, Switzerland, France, Belgium and Austria.

In the Czech Republic are using pharmaceuticals, which are made in Bee Research Institute at Dol. Produced medications are Formidol, Varidol 125, MP–10, M1–AER, Gabon PA-92 and Gabon PF-90. Each medication has different application in the time of the year.

Water emulsion of M1-AER serves to early springs. The active substance in this medicine is the tau-fluvalinatum. Product is intended for the treatments of bees at a time when in the colony is not sealed brood or surfaces are negligible. The active substance is spreading in the hive space like a mist of microscopic particles of diluted cells or brood caps penetrated at the coating of the brood. Aerosol affects only the mites which are presence on adult bees, to the sealed brood cells do not penetrate. At the coating the brood, are cursed adult mites and their instars in the brood cells, even mites on adult bees, which the active substance gains by contact with the surface of sealed brood and its transfer between them (Bee Research Institute at Dol) .

Treatment by M1-AER is associated with fumigation by Varidol 125. It is applied by fumigation or aerosol. It is governed exclusively for use in bees. In Czech Republic is not proved the resistence of *Varroa destructor* against Varidol. Application is proceed from 1. October till 15. April. The active substance is spreading in the hive space by smoke from fired

up strips or by the mist from microscopic particles of the diluted product. It affects mites present on adult bees, to the sealed brood cells do not penetrate. The active substance in this medicine is the Amitrazum (State Veterinary Administration).

Only Formidol needs veterinary recipe. Evaporation pad, which is made from short fibre cellulose contains 40ml technically pure formic acid with a concentration of 85% according to ČSN 66 1471. Evaporation pad with formic acid is intended to treat summer colonies. If the daily maximum temperatures exceed 25 °C do not deposit or remove regulatory container in the afternoon, but in the morning or evening after the colonies are settled. In particularly strong colonies in high-capacity hives choose repeated treatment. It is less effective than a Gabon PF-90 and Gabon PA-92 (Bee Research Institute at Dol).

Gabon PA-92 is used to kill *Varroa* mites present on bees, especially for protection the winter generation of bees and bees in the autumn, at a time when the sealed brood is in the colony. This preparation kills also Bee louse *Braula coeca*. Not used at the time, when the ware honey is present in the colony. In 2010 the distribution of Gabon PA-92 was changed because of European Union (EU) regulation, when Gabon PA-92 can no longer be classified as veterinary medicine, but must be registered as a veterinary medicinal product. The effective substance Acrinathrin is not registered in the EU as a medicine. Registration precedes clinical evaluation, the monitor was set Bee Research Institute at Dol (State Veterinary Administration, 2012).

Gabon PF-90 contains same as Gabon PF-92, strips from Gabon wood. But an active substance in this medicine is the tau-fluvalinatum.

Obligatory directs of the State Veterinary Administration of the Czech Republic quote:

Summer

State Veterinary Administration of the Czech Republic ordered beekeepers area treatment the bee colonies by medicines Gabon PA 1,5 mg or Gabon PF 90 mg in cases where the winter investigation of winter bee drop had more than 25% of ZO ČSV average finding more than three females of *V. destructor* in the beehive; when the natural summer mite fall more than five per day and females is indicated by a high intensity of varroasis by inspection of bee colonies. Monitoring the natural fallout of mites at the strongest bee hive in the period from July to September the best indicates mite infestation. To reduce the risk of residues the Gabon

PF 90 mg is applicated in two consecutive years. It can be used again after one year or replacement for Gabon PA 1,5 mg. For Gabon PA 1.5 mg are not restrictions about residues creation. Preparations Gabon PA 1,5 mg and Gabon PF 90 mg are applied as soon as possible after the final collection of honey. At lower intensities varroasis would suggest that beekeepers use of Formidol 40 ml due to the low dose of the active substance and short time of duration does not leave residues in honey even in the wax. In areas with evidence of resistance of mites to pyrethroids is applied the Formidol 40 ml in case of necessary repeatedly, or veterinary medicinal preparations based on thymol.

Autumn and winter

In the period from 10 October to 31 December 2011, ordered all three treatments by Vvaridol 125 mg/ml. The treatment is carried out fumigation or aerosol. In the bee colonies mustn't be at second and third treatment sealed bee brood. Recommended intervals between treatments are 14 to 21 days. This treatment is important to avoid creating resistance. Regional Veterinary Administration orders the investigation of winter bee drop of mixed sample after made obligatory autumn treatment of bee hives from each station. The samples of the bee debris are sent for investigation to 15 February 2012. Diagnostics are carried out at the State Veterinary Institutes and Bee Research Institute at Dol. Other treatments by Varidol 125 mg/ml are recommended in areas with evidence, where mites have resistance to pyrethroids.

For spring treatment is not possible to authorize products Gabon PF 90 and Gabon PA 1,5 mg.

2.7 Diagnosis of *Varroa destructor*

For diagnostics of *Varroa* destructor in the Czech Republic are used accredited laboratories, five State Veterinary Institutes, Bee Research Iinstitute at Dol and Private Veterinary Laboratory Testing Laboratory Náchod No. 1221 accredited by ČIA (see at Appendix 4).

State Veterinary Institute have accreditation in accordance with standard ČSN EN ISO/IEC 17025, and its activity also complies with ČSN ISO 9002. SVI can be found in cities Prague, Hradec Králové, Jihlava, České Budějovice and Olomouc.

BRI at Dol is private company engaged in the research, development, production and education in the branch of beekeeping and bee products. The company can be found in Dol

near village Máslovice. The institute has Testing laboratory No. 1203 with accreditation in accordance with standard ČSN EN ISO/IEC 17025, validity to 2012.

PVL at Nachod since 1999 is holding a certificate of accreditation issued by the national accreditation authority, Czech Institute for Accreditation. The current CA is No. 434/2009. The quality of services meets the requirements of ČSN EN ISO/IEC 17025.

Exist several ways to assess the status of bee colony infestation. Main laboratory diagnostic method in the Czech Republic, is investigating winter bee drop in February in laboratories with accreditation. Next methods are Investigation of drone brood, Investigation of mature bees with using a shaker and macroscopic investigation of adult bees.

Verification of winter bee drop means presence of female mites in the debris. In winter, when broods are not in the hive, females live on adult bees. Over winter a part of the mites will die and these mites can be proved in the debris (Veselý *et al*., 2003). Diagnostic by winter bee drop is described later in chapter material and methods.

Investigation of drone brood consist in uncapping honeycomb cells and takeout the broods. Carefully look at the brood and the bottom of cells for the presence of adult and developmental stage of *Varroa destructor* (State Veterinary Institute at Prague, 2008).

Investigate of adult bees with using a shaker. The sample of killed bees is poured to a suitable Erlenmeyer flask and in proportion 1:1, is poured by water at temperature 50-55 °C. The sample is shaken on a shaker for 1 5 minutes and then the contents of the flask gradually poured through a colander strainer equipped with double gauze. Material caught up in gauze is several times washed in the sink and then transferred on the bigger plastic tray. The captured material is put out and inspected by the eye or magnifying glass for the presence of *V. destructor* (State Veterinary Institute at Prague, 2008).

In the Macroscopic investigation of adult bees, the sample of dead bees spread out on filter paper and carefully inspecting at least 50 bees. Determination of mites is done using flat magnifying glass or stereomicroscope.

Another method how to diagnose the Varroasis, in the world, is guanine visualization. This is the indirect method for diagnosing tracheal mites of honey bees, in which a purine compound was used to determine the presence of *Acarapis woodi* (Mozes-Koch and Gerson, 1997). But

Varroa jacobsoni also excreted guanine (Ericson *et al*., 1994), it can be used to diagnose for *Varroa destructor*.

Beekeepers should monitor the level of infestation during the year themselves in field conditions.

3. Aims of the thesis

This thesis is focused on the two main aims. The first one is the assessment for expansion of *Varroa destructor* in the Czech Republic in period from 2011 till 2012 and the second aim is evaluation efficacy of biosecurity *Varroa* mites under the conditions of Czech Republic.

4. Hypothesis

1. Prevalence of *Varroosis* in the Czech Republic decreases.
2. Spread the disease is preventing measures by ordered of State Veterinary Administration.

4. Materials and methods

4.1 Materials for detection of the *Varroa destructor*

Bee debris and drop were sent for testing to SVI Prague from selected regions of the Czech Republic and during February 2011 and 2012 the mite diagnostic was made (Figure 5) using the flotation method for its morphological observation. Samples of bee debris (winter bee drop) were sent according to legislation of SVA CR from 26 districts (seven regions) of CR. The varroasis diagnostics is a state contract and is fully funded by the state since the year 2009.

Figure 5: State Veterinary Institute in Prague; **Source:** Author

4.2 The procedure of mite diagnosis

Flotation method is based on different specific wheight of the debris, oil and dead females of the *V. destructor*. This method uses medicinal oil for its specific attributes and further directly uses glass rods, beakers, Erlenmayers flask (see at Appendix 3), a strainer, funnel, gauze and cotton cellulose. The sample sent to the laboratory was unapacked and its content was poured in a beaker or a plastic cup, depending on the amount of the sample. Sample of bee debris (Figure 7) was at first flooded with a small amount of medicinal oil and mixed with a glass rod. It was necessary to ensure a thorough crushing of the lumps of the debris since mites won't float on the oil with the debris adhering to their surface. The mixed sample of debris (drop) was then flooded with oil of a specific weight of 0,9 g/cm^3, in a proportion debris to oil

approximately 1:10. The evaluated sample was thoroughly and repeatedly mixed with a glass rod always 3 times in a row in 1 to 2 minute intervals. After 5 minutes of standing still in the beaker or plastic cup it was possible to determine from the surface of the oil the amount of floated female *V. Destructor* (Figure 6 and 10).

In case there was received a bigger amount of debris, the given sample was divided to series of beakers to ensure the proportion of debris to oil (1:10). Next step of the examination was same (see above). The total number of *V. Destructor* females in the sample examined is given by the sum of all the beakers used.

After the examination of a given sample of debris (drop) was the content of the beaker (female mites, bee debris or dead bees) with oil transfused through a triple layer of gauze in a strainer that was placed in a glass funnel of diameter minimally 1,5 cm to Erlenmeyer's flask and the beaker was then carefully cleaned with the cotton cellulose repeatedly. The flotation method or the test of debris and drop was selected with regards to the season, namely February. There are three other examination methods, Examination of drone brood, Adult bee examination using a shaker and Macroscopic of examination of adult bees. However the flotation method is bound and ordered by the SVA CR.

All packaging material was stored in paper bags which were then sterilized in a HS 201 A sterilizer at a temperature of 120-130 °C for 20-30 minutes. The examination material was stored in barrels and their contents were disposed by the carcass disposal institute ASAVET Biřkov.

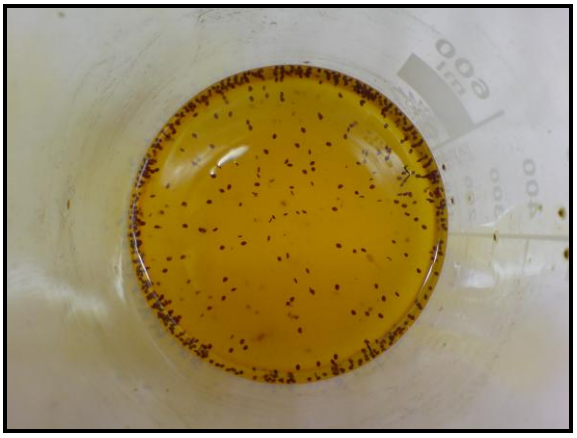


Figure 6: Positive sample with females on the surface; **Source**: Author

Figure 7: Sample with winter bee drop (debris) in plastic pot; **Source:** Author

4.3 The result of examination of Parasitology

Registration of the received material and the corresponding result of Parasitological examination for the presence of *Varroa destructor* was processed using Labsystem. Written documents were sent to corresponding District Veterinary Inspectorates, veterinarians sending debris samples, Bee keepers and local ZO ČSV (Základní organizace českého svazu včelařů).

4.4 Statistical evaluation

The data from diagnostic of varroa mites in State Veterinary Institute Prague labs were analyzed using nonparametrics descriptive statistics and ANOVA in statistic program STATISTICA 10 (see graph No. 1 showing average prevalence of varroasis in seven regions in the Czech Republic in years 2006 - 2012).

5. Results

In 2011 there was 12600 samples of winter drop (see Table No.2) sent to the parasitological laboratory of the State Veterinary Institute Prague representing 125 407 hives (see Table No.5) from the thirty districts of the Czech Republic. In the following year 2012 was in the same laboratory examined 2 351 more samples of debris (see Table No.3), which represented 133 581 bee colonies (see Table No.4). Samples of bee debris taken in 2011 and 2012 originated from seven region - Carlsbad region, Pilsen region, Liberec region, Usti region, Central region, capital city of Prague and Hradec Kralove region (see Table No.2 and No. 3). Number of positive findings according to the regions was in the range from 53% (Liberec region) do 100% (Hradec Kralove region). While in 2012 was the highest recorded number of positive colonies in the Carlsbad region (91%) and the least affected was Liberec region (75%). The annual increase was in the years 2011 and 2012 was in almost all the regions (see Table No.4). In the Carlsbad region increased by 28%, Pilsen by 26%, Liberec by 22%, Usti by 20%, Central by 25%, the city of Prague recorded increase only by 2% and Hradec Kralove had a decrease by 23%.

Table 2: The results of investigated samples for varroasis 2011

Region	**Investigated samples (n)**	**Positive samples (n + %)**
Carlsbad	1272	788 (62%)
Pilsen	1846	1106 (60%)
Liberec	1867	817 (44%)
Usti	1658	1050 (63%)
Central	5601	3222 (58%)
Prague	355	272 (77%)
Hradec Kralove	1	1 (100%)
TOTAL	12600	58%

Table 3: The results of investigated samples for varroasis from different regions of the Czech republic in 2012

Region	**Investigated samples (n)**	**Positive samples (n + %)**
Carlsbad	1177	1045 (89%)
Pilsen	1922	1621 (84%)
Liberec	1867	1288 (69%)
Usti	1753	1457 (83%)
Central	6372	5107 (80%)
Prague	1280	317 (25%)
Hradec Kralove	580	403 (69%)
TOTAL	14951	75%

Table 4: Results of investigated bee colonies in regions in years 2011 and 2012

Region 2011	Investigated bee colonies (n)	Positive bee colonies (n + %)	Region 2012	Investigated bee colonies (n)	Positive bee colonies (n + %)
Carlsbad	14633	9168 (63%)	**Carlsbad**	12724	11528 (91%)
Pilsen	15129	9694 (64%)	**Pilsen**	16171	14539 (90%)
Liberec	16218	8544 (53)	**Liberec**	16448	12392 (75%)
Usti	18997	12692 (67%)	**Usti**	17853	15553 (87%)
Central	57698	35521 (62%)	**Central**	62182	54174 (87%)
Prague	2720	2232 (82%)	**Prague**	3013	2537 (84%)
Hradec Kralove	12	12 (100%)	**Hradec Kralove**	5190	3980 (77%)
TOTAL	125407	62%	**TOTAL**	133581	86%

From the table 4 we can see that the spread of varroasis prevalence increased in 2012 compaired to 2011 by 24%. Prevalence of varroasis spread in the affected regions is marked in the Figures No.8 and No.9

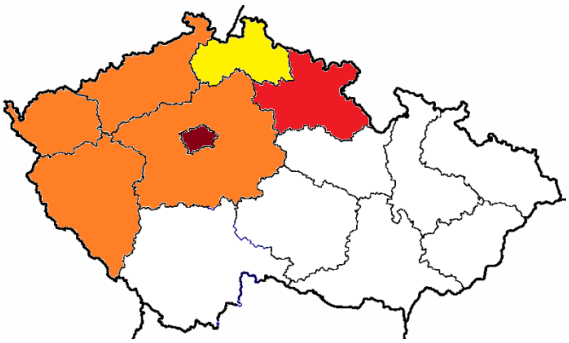


Figure 8: Varroasis in investigated regions 2011

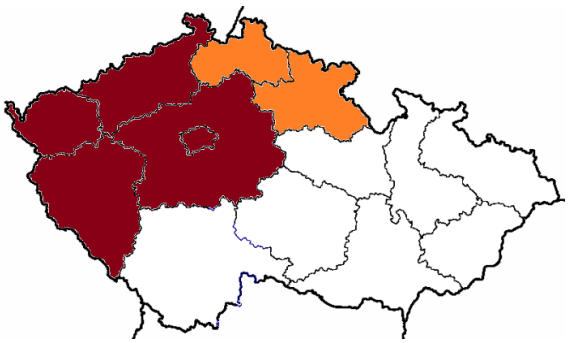
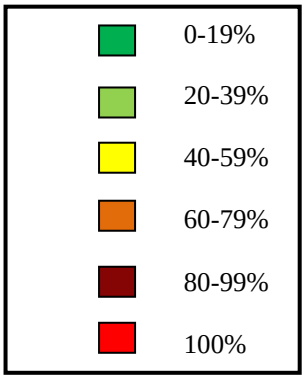


Figure 9: Varroasis in investigated regions 2012



Among the most vulnerable districts to varroasis in 2011 were among others Kolin, Kladno and Pribram (see Table No.5) which all belong to the Central region (SVA CR, 2011). In the district of Kolin, of the total number examined 4 905 in 2011 was found a total of 3 709 colonies positive for the presence of *V. destructor* mites. While in 2012 there was an increase of 17% (see Table No.5). Similar situation occured in two other regions Kladno and Pribram, where there was an increase in the incidence of varroasis by 15% and 12% (see Table No.5).

Table 5: Results of investigated bee colonies in endangered townships in 2011 (according SVS CR) and 2012 (own results)

Township 2011	**Investigated bee colonies (n)**	**Positive bee colonies (n + %)**	**Township 2012**	**Investigated bee colonies (n)**	**Positive bee colonies (n + %)**
Kolin	4905	3709 (76%)	**Kolin**	5633	5238 (93%)
Kladno	3007	2532 (84%)	**Kladno**	3161	3037 (96%)
Príbram	7758	5643 (73%)	**Príbram**	8176	7234 (88%)

Among the samples under investigation by State Veterinary Institute Prague were also districts Semily and Rakovník, which were last year evaluated by the State Veterinary Administration of the Czech Republic as districts with minimal mite findings. However, when comparing 2011 and 2012 it must stated that the number of colonies positive with varroasis has rapidly increase. Specifically in Semily by 33% and in Rakovnik by up to 47% (Table 6).

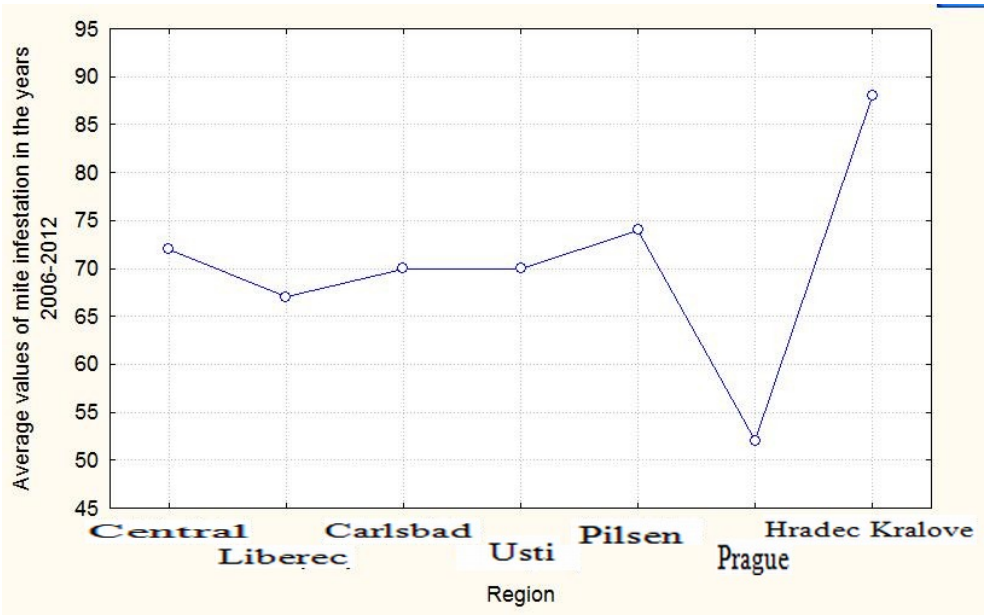
Table 6: Results of investigated bee colonies in townships with the lowest infestation in 2011

Township 2011	**Investigated bee colonies (n)**	**Positive bee colonies (n + %)**	**Township 2012**	**Investigated bee colonies (n)**	**Positive bee colonies (n + %)**
Semily	3883	1562 (40%)	**Semily**	2589	1881 (73%)
Rakovnik	5153	2288 (44%)	**Rakovnik**	6095	5520 (91%)

The most serious situation of the prevalence of varroasis and bee colony losses have been announced by the State Veterinary Administration of the Czech Republic in the years 2007 and 2008. Table No. 7 shows that the situation in 2009 was significantly improved..

Table 7: Infestation levels through the years 2005 - 2011

Year	Total Samples (n)	Infestation level (%)
2005	43495	69
2006	41128	73
2007	40267	**79**
2008	42131	**81**
2009	48910	26
2010	39546	54
2011	52001	58



Graph 1: Average prevalence of varroasis in seven regions in the Czech Republic in years 2006 - 2012

6. Discussion

Western honey bee *Apis mellifera* is not an original host for mite *Varroa destructor.* This ectoparasite moved from Eastern honey bee *Apis cerana* to *A. mellifera* colonies imported to Asia during the last century. Varroa mites have spread to almost on all continents, where *A. mellifera* are kept. This parasite gains unique worldwide status due to lack balanced relationship (Walter and Procter, 1999) between host and parasite (new pest of *A. mellifera*), pest has spread fast almost worldwide and at present it may be difficult to find a *Varroa* free western honey bee colony (Rosenkranz *et al.*; Finley *et al.*).

The mite which is causing *Varoasis* in *A. mellifera* belongs to the species *Varroa destructor*, which was formerly erroneously known as *Varroa jacobsoni*. The new species *V. destructor* is represented by seven haplotypes, of which only Korean and Japan/Thailand haplotypes are economic important. Mites of Korean haplotype parasitize *Apis mellifera* worldwide, whereas Japan/Thailand haplotype is found only in Asia (Anderson and Trueman, 2000; Rosenkranz *et al.*, 2010). The mite *V. destructor* is now the major pest of *A. mellifera* worldwide except some countries in South America, Austrila, Greenland and more, see Figure 1 in literature review.

The ectoparazites are seen by naked eye, their body has transversely oval shape and dorsoventrally flattened shape, length 1.1 to 1.5 mm and wide 1.5 to 1.9 mm. In adult the mites are reddish brown in color and shiny (Veselý *et al.*, 2003; Rosenkranz *et al*., 2010). Due to size of the mite it can be used several methods for diagnostic without a microscope. In the literature review is describe these methods, Investigation winter bee drop by Flotation method, Investigation of drone brood, Investigation of mature bees with using a shaker and macroscopic investigation of adult bees. According to Ericson (1994) it can be used a guanine visualization for diagnostic of mites *V. jacobsoni* Oudemans. In SVI Prague was used the Flotation method (more information in Material and methods), where skilled staffs counting the female of ectoparasites by naked eye (Figure 6, 10 and 11).

Figure 10: positive sample with hundreds of *varroa* mites; **Source:** Author

Figure 11: *varroa* mites in the gauze; **Source:** Author

Varroa destructor is in the Europe since 1967, when first report was from Bulgaria. At the present the mite is in the whole Europe even in the United Kingdom. In neighboring Germany, according to State Veterinary Administration DE, were reported bee colonies losses 18% in the year 2009/2010, 15% in the last year and now in 2012 were losses, caused by varroasis, up to 30%.

In 1997 takes the European Union measures to support beekeepers in the fight against varroasis. 25th June 1997, adopted Regulation EC No 1221/97, which summarizes the commands to improve the conditions of production and marketing of honey. This regulation

has still validity and was even on 26th April 2004 by EC Regulation No. 797/2004 updated and reissued. Further about honeybees are the most relevant three European Community regulations, namely Council Regulation (EC) 1804/99, Council Regulation (EC) 434/97 and Council Regulation (EEC) 2377/90 (EU, 2011).

In the Czech Republic, each beginning beekeeper must realize the following basic legislative duties. Registering in the Czech-Moravian Breeders Association, be recorded in the central register and have a veterinary certificate to transport bought bee colonies. Movement of bee colonies is in, quoting: compliance with the provisions of § 134 paragraph 1 of the notice of the Ministry of Agriculture No. 299/2003 Coll. Measures for prevention and control of diseases and diseases transmissible from animals to humans, in valid statutory text, may be permanent transfer of bee colonies only permitted to area with the same or worse health situation. Moved can be only tested bee colonies for *varroa* and medically treated under the preventive and control conditions in accordance with them. The moving bee colonies must be accompanied by a veterinary certificate issued by a veterinary inspector.

Assessment the prevalence in the years from 2005 till 2011. The most serious situation of the prevalence of varroasis and bee colony losses have been announced by the State Veterinary Administration of the Czech Republic between 2007 and 2008. From the Table 7 we read that the situation has improved significantly in 2009 (drop from 81% to 26%), due to the order by State Veterinary Administration of the Czech Republic, when it was necessary to realize radical measures in apiculture in the Czech Republic (see prevention in literature review). In the following years the prevalence of varroa recorded follow-up increase, which can be explained by the gradually onset of resistance of mites against to pyrethroids in bee-keeping not only in this country, but also worldwide, as previously shown for example in Great Britain in 2001, France, Belgium, Austria and US in 20th century (Baxter *et al.,* 1998; Troullier, 1998; Thomposon *et al.,* 2002). Based on the results of investigation it cannot be said, that the first hypothesis concerning the decreasing prevalence of varroasis in the CR is confirmed, but prevalence of *Varroa destructor* is still lower than years 2007 and 2008 (see Tab. No.7).

The second hypothesis was confirmed and relates to the prevalence of varroasis which is prevented by the measures of State Veterinary Administration of the CR, which is annually enacting the obligation (see Prevention in literature review) and in cooperation with the

Czech-Moravian Breeders Association and the Bee Research Institute at Dol protect the Czech Republic.

7. Conclusions

Based on the results, it can be concluded, that prevalence in the Czech Republic is not decreasing, but since the year 2009 the prevalence is still lower than before. The prevalence is slow increasing, but it may be explained by the gradually onset of resistance of mites against to pyrethroids.

It was proved, that the prevention measures of the State Veterinary Administration of the Czech Republic due to regulation, which directs each year and in cooperation with the Czech-Moravian Breeders Association and the Bee Research Institute at Dol, reduced the prevalence of varroasis in the Czech Republic.

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APPENDICES

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NÁRODNÍ AKREDITAČNÍ ORGÁN

Český institut pro akreditaci, o.p.s.
130 00 Praha 3, Olšanská 54/3

vydává

OSVĚDČENÍ O AKREDITACI

č. 455 / 2011

Státní veterinární ústav Praha
se sídlem Sídlištní 136/24, 165 03 Praha 6 - Lysolaje, IČ 00019305,
zřízen Zřizovací listinou Ministerstva zemědělství

pro zkušební laboratoř č. **1176.3**
Zkušební laboratoř diagnostiky infekčních nemocí zvířat

Předmět akreditace:

Preventivní a diagnostické vyšetřování klinického a sekčního materiálu ze zvířat zdravých, nemocných, uhynulých nebo ulovených či poražených s cílem průkazu respektive izolace původců infekcí zvířat, jejich typizace, a průkazu protilátek proti infekčním agens v rozsahu uvedeném v příloze tohoto osvědčení.

Toto osvědčení o akreditaci vydal Český institut pro akreditaci, o.p.s. na základě posouzení splnění akreditačních požadavků podle

ČSN EN ISO/IEC 17025:2005

a po zjištění, že zkušební laboratoř je odborně způsobilá objektivně a nezávisle vykonávat činnosti uvedené v rozsahu předmětu akreditace.

Adresát tohoto osvědčení je oprávněn používat při své činnosti v rozsahu tohoto osvědčení a po dobu jeho platnosti vedle svého názvu označení „zkušební laboratoř akreditovaná ČIA č. 1176.3", pod podmínkou, že bude vždy postupovat v souladu s příslušnými předpisy vztahujícími se k činnosti akreditované zkušební laboratoře, a to zejména ČSN EN ISO/IEC 17011, čl. 8.1, ČSN EN ISO/IEC 17025, zákona č. 22/1997Sb., o technických požadavcích na výrobky, ve znění pozdějších předpisů, včetně navazujících předpisů vydaných Českým institutem pro akreditaci, o.p.s.

Prokáže-li se, že adresát tohoto osvědčení neplní akreditační požadavky rozhodné pro jeho vydání a nedodržuje závazky podmiňující akreditaci, může Český institut pro akreditaci, o.p.s. účinnost tohoto osvědčení pozastavit nebo osvědčení o akreditaci zrušit.

Toto osvědčení je vydáno v souladu s ustanovením § 16 odst. 1 zákona č. 22/1997 Sb., o technických požadavcích na výrobky a v souladu s ustanovením § 151 zákona č. 500/2004 Sb., správní řád.

Toto osvědčení je platné do **02.11.2016**

V Praze dne 15.11.2011

Ing. Jiří Růžička, MBA
ředitel
Českého institutu pro akreditaci, o.p.s.