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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

INVESTIGATION of MICROORGANISM CONTAMINATION POINTS in BEEKEEPING EQUIPMENTS WITH CLINICAL SIGNS of FOULBROOD in APIARIES

Yavru Çürüklüğü Klinik Bulguları olan Arılıklarda Arıcılık Ekipmanlarındaki Mikroorganizma Kontaminasyon Noktalarının Araştırılması

Ayşe Ebru BORUM[1*], İbrahim ÇAKMAK[2]

[*1]Balıkesir University Faculty of Veterinary Microbiology Departrment, 10000, Balıkesir TÜRKİYE, Yazışma Yazarı/Corresponding author: E-posta: ebruborum@balikesir.edu.tr, ORCID No: 0000-0002-6916-8982

[2]Bursa Uludag University, Beekeeping Development Application and Research Center-AGAM, Agricultural Faculty, Animal Sciences, Gorukle Campus, 16059, Bursa, TÜRKİYE, E-posta: icakmak@uludag.edu.tr, ORCID No: 0000-0002-8000-5770

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ABSTRACT

The goal of this study was to determine whether colonies with clinical signs of foulbrood in apiaries and hive tools, smokers, gloves, feeders and beekeeper's veils used in the same colonies were a reservoir source for microbial infections. For this purpose, samples were taken from colonies with clinical signs of foulbrood and collected from 29 different apiaries in the Southern Marmara region of Türkiye. The samples were brought to the laboratory under appropriate conditions, and agent isolation and identification were performed. Different microorganisms were isolated from the feeder, hive tool, beekeeper smoker, gloves and beekeeper suit samples collected from each apiary. Bacteria isolated from the samples taken from the hives with clinical signs of foulbrood and from the samples taken from the tools and equipment were isolated as the same species or as a mixture. As a result, an intense presence of microorganisms was detected in the hive tool, beekeeper suit, gloves, feeder, and beekeeper's smoker, used by beekeepers, and it was determined that these materials used in beekeeping were a source of microbial reservoirs.

Keywords: *Apis mellifera*, Microorganisms, Foulbrood, Contamination, Beekeeping equipments

ÖZ

Çalışmada, arılıklarda yavru çürüklüğü klinik bulguları bulunan koloniler ile aynı kolonilerde kullanılan el demiri, körük, eldiven, şerbetlik ve arıcı kıyafetlerinin mikrobiyal infeksiyonlar yönünden bir rezervuar kaynağı olup olmadıklarının belirlenmesi amaçlanmıştır. Bu amaçla Güney Marmara bölgesinde bulunan 29 farklı arılıktan örnekler toplanmıştır. Alınan örnekler uygun koşullarda laboratuvara getirilerek izolasyon ve identifikasyon yapılmıştır. Her arılıktan toplanan yemlik, el demiri, körük, eldiven ve arıcı kıyafetlerinde farklı mikroorganizmalar izole edilmiştir. Yavru çürüklüğü klinik bulguları görülen kovanlardan alınan örnekler ile alet ve ekipmandan alınan örneklerden izole edilen bakteriler aynı tür ya da karışık olarak izole edilmiştir. Sonuç olarak arıcıların kullandıkları yemlik, el demiri, körük, eldiven ve arıcı maske ve tulumlarında zengin bir mikroorganizma varlığı saptanmış ve arıcılıkta kullanılan bu malzemelerin mikrobiyal bir rezervuar kaynağı olduğu belirlenmiştir.

Anahtar Kelimeler: *Apis mellifera*, Mikroroganizma, Yavru çürüklüğü, Kontaminasyon, Arıcılık malzemeleri

GENİŞLETİLMİŞ ÖZET

Amaç: Bu çalışmanın amacı, çeşitli arılıklardaki yavru çürüklüğü klinik bulguları olan koloniler ile aynı arılıklarda kullanılan el demiri, körük, eldiven ve arıcı kıyafetlerinden etken izolasyon ve identifikasyonu yapılarak infeksiyon yönünden bir rezervuar kaynağı olup olmadıklarının tespit edilmesidir.

Gereç-Yöntem: Bu çalışmada Güney Marmara bölgesinde (Bursa, Balıkesir, Bilecik, Yalova ve Çanakkale) yavru çürüklüğü klinik bulguları olan arılıklardaki kolonilerden ölü ve şüpheli larva ve aynı arılıklarda kullanılan şerbetlik, el demiri, körük, eldiven ve arıcı kıyafetlerinden svap örnekleri alınarak mikroorganizma izolasyon ve identifikasyonu yapılmıştır. Yirmi dokuz farklı arılıktaki yavru çürüklüğü klinik bulguları bulunan 43 koloniden yavrulu petek, 43 şerbetlik, 32 el demiri, 29 körük, 30 eldiven ve 29 arıcı kıyafetinden örnekler alınmıştır. Toplanan örnekler uygun koşullarda laboratuvara getirilerek etken izolasyon ve identifikasyonu yapılmıştır. Düzensiz petek gözleri, kapalı yavru gözlerinde delik gibi yavru çürüklüğü infeksiyon bulguları olan kolonilerden yavrulu petekler alınmıştır.

Svap ve larva örnekleri 10 ml. NaCl %0,9 (w/v) içinde süspanse edilmiştir. Süspansiyon ikiye ayrılmıştır. Örneklerin ilk kısmı vejetatif bakterileri öldürmek için 80 °C'de 10 dakika ısıtılmıştır. Süspansiyonun ikinci kısmına ise herhangi bir işlem uygulanmamıştır. Herbir besiyerine süspansiyondan 200 µl inoküle edilmiştir. %5 koyun kanlı Columbia agar (Oxoid CM0331), thiaminli brain heart infüzyon agar (Oxoid CM1136), XLD agar (Oxoid CM0469), MacConkey agar (Oxoid CM0115) ve Nutrient agar (Oxoid CM0003) kullanılmıştır. *Paenibacillus larvae* ve *Melissococcus plutonius* izolasyonu için; MYPGP agara (maya özütü, Mueller-Hinton broth, glucose, K2HPO4, sodium pyruvate ve agar) ekimler yapılmıştır. Tüm besiyerleri 37 °C'de aerobik ve mikroaerofilik koşullarda 48-72 saat inkübe edilmiştir (Nordström ve Fries 1995, Kopcakova vd. 2022). Bütün besiyerlerinde günlük bakteriyel üreme kontrolleri yapılmıştır. İzolatlar, gram boyama ile mikroskopta incelenmiş, katalaz testi yapılmış BBL crystal system ile identifiye edilmiştir.

Bulgular ve Sonuç: Yavru çürüklüğü klinik bulguları görülen koloniler ve arıcılık malzemelerinden alınan örneklerden 69 mikroorganizma ve 28 farklı tür izole edilmiştir. *Bacillus subtilis* (%11,5) en fazla izole edilen tür olarak belirlenmiştir. Klinik bulgu görülen kolonilerden ise 43 yemlik, 32 el demiri, 29 körük, 30 eldiven ve 29 arıcı kıyafetinden svap ile örnekler alınmıştır. Eldiven ve arıcı kıyafetlerinden mikroorganizma izolasyon oranı %100'dür. En az mikroorganizma izolasyonu yapılan arıcılık malzemesi ise körük (%34,38) olmuştur. Örnek alınan kovanlardan ve malzemelerden aynı tür bakteriler izole edilmiştir. Kullanılan arıcılık malzemelerin yavru çürüklüğü görülen kovanlar arası infeksiyonun yayılmasına sebep olduğu bilinmektedir. Araştırmamızda kullanılan arıcılık malzemelerinden birçok mikroorganizma türü de izole edilmiştir. Sonuç olarak arıcılık malzemeleri hem koloniler arasında etkenlerin yayılmasına sebep olurken hem de mikrobiyal bir rezervuar kaynağı olabilmektedir. Bu nedenle arıcılar, arı hastalıklarının koloniler arasında yayılmasını engellemek için alet ve ekipmanın dezenfeksiyonuna önem vermelidir.

INTRODUCTION

Honey bee colonies, *Apis mellifera* produces honey, pollen, bee bread, apilarnil, propolis, royal jelly and bee venom and also has ecological importance in the reproduction of plants. Honey bee products are considered healthy food that provide benefits for people. Honey is known as antimicrobial and can be stored for long years. They also play an important role in the pollination of many economically cultivated plants for food and the economic value of pollination is about 153 billion dollars worldwide (Graham 1991, Gallai et al. 2009, Staveley et al. 2014).

Bees live in close-knit societies where each individual is responsible for the development and survival of the colony. The organization of a bee colony bears many similarities to a multicellular organism often referred to as a "superorganism" (Tautz 2008).

Microorganisms are a factor that negatively affects the health of the entire colony. Microorganisms that affect bees are bacteria, protists and fungi, which are important bee pathogens. Microorganisms generally spread rapidly by beekeeping activities. If left untreated, it causes serious bee deaths and colony losses. Controlling some microorganisms is economically very costly. Sometimes it may be necessary to destroy hives and entire colonies (Cunningham et al. 2022, Leska et al. 2021).

The aim of every beekeeper is to obtain quality and healthy products while avoiding colony losses and

infection problems. There are many different types of microorganisms in the environment. These microorganisms can be found everywhere in apiculture and beekeeping. It is also quite large in number. There are microorganisms that can cause infections under certain conditions, aggravate the course of another infection, cause deterioration in bee products and are harmful to consumer health (Bogdanov et al. 2003).

Sources of contamination can be environmental and beekeeping. Environmental resources can be divided into agricultural and non-agricultural resources (Devillers and Pham-Delègue 2002, Bogdanov et al. 2003). Bees usually fly in a range of 3 km. Therefore, bees and bee products can serve as biomarkers for contamination in this fly area. Contaminants in the flying area can be transmitted to the bee by air and water and carried to the colony with it. They can also be passed to plants through air, water and soil. From here, the plant can pass these contaminants to the bee with nectar and honeydew (Bogdanov et al. 2003).

The larvae are initially sterile, then fed nectar and pollen by worker bees. In this feeding process, their own microbiota is formed with nectar, pollen and worker microflora, or infectious agents are transmitted before the pupal stage (Snowdon and Cliver 1996).

Many microorganisms originate from certain foods or components of the ecosystem. *Actinetobacter*, *Bacillus, Clostridium, Corynebacterium, Pseudomonas, Psychrobacter* and *Vagococcus* are bacteria commonly found in soil. The most important sources of *Bacillus, Clostridium* and *Micrococcus* species are air and dust. *Bacillus* and *Clostridium* species are also bacterial pollutants of sugarcane and beet. *Saccharomyces* and *Torula* have been found in high humidity sugars and *Leuconostoc mesenteroides* sugar refineries. In plants and herbal products, *Brochothrix, Citrobacter, Enterobacter, Erwinia, Flavobacterium, Lactobacillus, Luctococcus, Leuconostoc, Listeria* and *Pediococcus* species are found. In bee intestines: 1% yeast, 29% gram-positive bacteria species (*Bacillus, Bifidobacterium, Streptococcus* and *Clostridium*) and 70% gram-negative bacteria (*Achromobacter, Citrobacter, Enterobacter*, *Erwinia, Escherichia coli, Flavobacterium, Klebsiella, Proteus* and *Pseudomonas*) found (Snowdon and Cliver 1996).

Honeybee diseases and pests, which cause colony losses in the beekeeping sector, cause the destruction of thousands of colonies every year. Especially American foulbrood (AFB) and European foulbrood (EFB) are common, important and dangerous bacterial diseases all over the world. Beekeeping equipment also plays an important role in the transmission of these infections between apiaries and colonies (vanEngelsdorp et al. 2013).

Paenibacillus larvae (American foulbrood), *Melissococcus plutonius* (European foulbrood), *Serratia marcescens*, *Aspergillus* spp. (Stonebrood), *Ascosphaera apis* (Chalkbrood) are important bacterial and fungal infections frequently seen in bees (Leska et al. 2021). However, apart from these infections, there is a common minor foulbrood infection, which is quite common and is confused with AFB and EFB by beekeepers. This disease shows the same clinical findings as AFB and EFB and causes concern in beekeepers. The causative agents of this infection are very diverse. *Bacillus* spp., *Corynebacterium* spp., *Staphylococcus* spp. and *Streptococcus* spp. are one of the most common factors. These factors are; human, animal and environmental origin. Beekeeping tools and equipments that are not sterilized and disinfected can infect the colonies and cause significant losses.

The aim of this study was to determine whether colonies with clinical signs of foulbrood in various apiaries with hive tools, smoker, glove and beekeeper suits-veils used in the same apiaries were a reservoir source in terms of infection by isolating and identifying the agents.

MATERIALS AND METHODS

In this study, microorganisms were isolated and identified by taking dead and suspicious larvae from colonies in apiaries with clinical signs of foulbrood and swab samples from the feeder, hive tool, beekeeper smoker, gloves and beekeeper suits used in the same apiaries. Samples of honeycomb with brood, 43 feeders, 32 hive tools, 29 beekeeper smokers, 30 gloves and 29 beekeeper suits were taken from 43 colonies with clinical signs of foulbrood in 29 different apiaries in Southern Marmara region of Türkiye. The collected samples were brought to the laboratory under appropriate conditions and agent isolation and identification were made. Honeycombs with brood were taken from colonies with irregular comb eyes, holes in

closed brood cells, and signs of foulbrood infection (Beekeeping equipments, Picture-1).

Picture 1. Hive tool, feeder, gloves, smoker and bee suit

Swab and larval samples were suspended in 10 ml of NaCl 0.9% (w/v). The suspension was divided into two. The first portion of the samples was heated at 80 °C for 10 minutes to kill vegetative bacteria. No treatment was applied to the second part of the suspension. 200 µl of the suspension was inoculated into each medium. 5% sheep blood Columbia agar (Oxoid CM0331), brain heart infusion agar with thiamine (Oxoid CM1136), XLD agar (Oxoid CM0469), MacConkey agar (Oxoid CM0115) and Nutrient agar (Oxoid CM0003) were used. For isolation of *Paenibacillus larvae* and *Melissococcus plutonius*; Inoculations were made on MYPGP agar (which contains yeast extract, Mueller-Hinton broth, glucose, K2HPO4, sodium pyruvate, and agar). All media were incubated at 37 °C under aerobic and microaerophilic conditions for 48-72 hours (Nordström and Fries 1995, Kopcakova et al. 2022). Bacterial growth controls of all plates were performed daily. The isolates were examined with light microscopy after gram staining and catalase test and were identified with the BBL crystal system (BBL Crystal Enteric/Nonfermenter ID and Gram Positive ID Kits -Becton Dickinson and Company, USA) (Özakın et al. 2003, Forsgren et al. 2013, De Graaf et al. 2013).

RESULTS

Samples were collected from 29 different apiaries in the Southern Marmara region. In the study, bacterial and fungal agents were isolated and identified by taking samples from feeders, hive tools, beekeeper smokers, gloves and beekeeper suits used in colonies with clinical signs of foulbrood in apiaries. Species isolated and identified from honeycomb and material samples collected from 29 different apiaries are given in Table 1.

Honeycomb samples with brood were taken from 43 colonies with clinical signs of foulbrood in 29 different apiaries in the study. The agents isolated from honeycomb samples are shown in Table 2. A total of 69 isolates were obtained from all samples. Twenty-eight different species were isolated from samples taken from colonies and beekeeping materials with clinical signs of foulbrood. *Bacillus subtilis* (11.5%) is the most isolated species.

Table 1. Samples isolated from different tool and equipment samples used in apiaries and from honeycomb samples in hives with clinical findings

Tablo 1. Arılıklarda kullanılan farklı alet ve ekipman örneklerinden ve kovanlardaki petek örneklerinden izole edilen örnekler

Apiary No	Sampled beekeeping equipment and isolated microorganism species					Microorganism species isolated from hive samples
	Feeder	Hive tool	Beekeeper smoker	Gloves	Beekeeper suit	
1	1 (-) 2 *Bacillus subtilis*	*Enterococcus faecalis*	*Enterococcus faecalis* *Bacillus brevis*	1 *Enterococcus faecalis* 2 (-)	*Bacillus licheniformis*	1 *Bacillus brevis* 2 *Enterococcus faecalis* *Bacillus subtilis*
2	1 (-) 2 *Bacillus subtilis*	*Enterococcus faecalis*	(-)	*Corynebacterium jeikum*	*Corynebacterium jeikum*	1 *Corynebacterium jeikeium* 2 *Bacillus subtilis* *Enterococcus faecalis*
3	*Bacillus subtilis*	*Staphylococcus epidermidis*	(-)	*Staphylococcus aureus*	*Bacillus circulans*	*Staphylococcus aureus* *Staphylococcus epidermidis* *Bacillus subtilis*
4	1 *Bacillus pumilus* 2 *Bacillus subtilis* 3 *Bacillus licheniformis*	*Staphylococcus epidermidis*	(-)	*Staphylococcus aureus*	*Corynebacterium jeikum* *Acinetobacter lwoffi*	1 *Bacillus pumilus* *Corynebacterium jeikum* 2 *Acinetobacter lwoffi* 3 *Bacillus licheniformis*
5	1 *Bacillus subtilis*	1 *Staphylococcus epidermidis* 2 *Bacillus subtilis*	*Bacillus brevis*	*Bacillus brevis* *Bacillus subtilis*	*Bacillus subtilis*	*Corynebacterium aquaticum* *Bacillus brevis* *Bacillus subtilis*
6	1 (-) 2 *Bacillus subtilis* 3 *Bacillus subtilis*	*Corynebacterium jeikum*	*Bacillus brevis*	*Corynebacterium aquaticum* *Corynebacterium jeikum*	*Corynebacterium jeikum*	1 *Corynebacterium pseudodiphteriticum* *Corynebacterium jeikum* 2 *Corynebacterium pseudodiphteriticum* *Corynebacterium jeikum* 3 *Corynebacterium aquaticum* *Aerococcus urinae*
7	1 (-) 2 *Bacillus brevis*	*Staphylococcus epidermidis* *Bacillus pumilus*	(-)	*Corynebacterium aquaticum* *Bacillus subtilis*	*Corynebacterium aquaticum*	1 *Corynebacterium aquaticum* *Bacillus pumilus* 2 *Lactococcus lactis ssp. Cremoris* *Micrococcus luteus*
8	(-)	*Bacillus brevis*	(-)	*Bacillus brevis* *Staphylococcus aureus*	*Staphylococcus aureus*	*Bacillus licheniformis* *Bacillus brevis*
9	*Enterococcus faecalis*	*Staphylococcus saprophyticus* *Enterococcus faecalis*	*Staphylococcus saprophyticus*	*Enterococcus faecalis*	*Staphylococcus saprophyticus*	*Staphylococcus saprophyticus* *Enterococcus faecalis*
10	(-)	*Bacillus cereus*	(-)	*Corynebacterium bovis* *Bacillus subtilis*	*Bacillus subtilis*	*Corynebacterium bovis* *Bacillus cereus*
11	*Bacillus subtilis*	1 *Bacillus cereus* *Staphylococcus epidermidis* 2 *Staphylococcus epidermidis*	*Bacillus subtilis*	*Staphylococcus epidermidis*	*Bacillus subtilis*	*Staphylococcus epidermidis* *Bacillus subtilis*
12	1 (-) 2 (-)	*Corynebacterium pseudotuberculosis*	*Corynebacterium*	*Corynebacterium*	*Corynebacterium*	1 *Corynebacterium pseudotuberculosis*

			pseudotuberculosis	*pseudotuberculosis*	*pseudotuberculosis*	2 *Corynebacterium pseudotuberculosis*
13	1 *Bacillus subtilis* 2 (-)	*Corynebacterium renale*	(-)	*Corynebacterium renale*	*Bacillus subtilis*	1 *Rhodococcus equis* *Corynebacterium renale* 2 *Bacillus subtilis*
14	1 *Bacillus subtilis*	*Staphylococcus aureus*	(-)	*Staphylococcus aureus* *Staphylococcus epidermidis*	*Staphylococcus epidermidis* *Bacillus subtilis*	*Staphylococcus aureus* *Staphylococcus epidermidis* *Bacillus subtilis*
15	1 (-)	*Staphylococcus aureus* *E.coli*	(-)	*E.coli*	*Staphylococcus aureus* *E.coli*	*E.coli* *Morganella morgani*
16	(-)	*Corynebacterium jeikum*	*Staphylococcus epidermidis*	*Corynebacterium jeikum*	*Corynebacterium jeikum* *Staphylococcus epidermidis*	*Corynebacterium jeikum*
17	1 *Bacillus subtilis* 2 *Bacillus subtilis*	*Bacillus subtilis*	*Corynebacterium bovis*	*Corynebacterium bovis* *Bacillus subtilis*	*Bacillus subtilis*	1 *Corynebacterium bovis* *Bacillus subtilis* 2 *Corynebacterium bovis*
18	1 *Staphylococcus simulans* 2 *Bacillus subtilis*	*Staphylococcus simulans* *Staphlococcus warneri*	(-)	*Staphylococcus simulans* *Bacillus subtilis*	*Staphylococcus warneri*	1 *Staphylococcus simulans* *Staphylococcus warneri* 2 *Providencia stuartii*
19	*Bacillus subtilis*	*Bacillus cereus*	*Bacillus cereus*	*Staphylococcus epidermidis* *Bacillus cereus*	*Bacillus cereus*	*Bacillus cereus*
20	(-)	*Escherichia coli* *Enterococcus faecalis*	(-)	*Enterococcus faecalis*	*Escherichia coli*	*Enterococcus faecalis*
21	1 (-) 2 (-)	*Bacillus pumilus*	*Bacillus licheniformis*	*Enterococcus faecalis*	*Escherichia coli*	1 *Bacillus licheniformis* *Bacillus pumilus* 2 *Bacillus pumilus*
22	1 (-) 2 *Bacillus brevis*	*Bacillus brevis* *Enterococcus faecalis*	(-)	*Enterococcus faecalis*	*Enterococcus faecalis*	1 *Bacillus brevis* 2 *Enterococcus faecalis*
23	(-)	*Bacillus brevis* *Bacillus cereus*	(-)	*Klebiella oxytoca*	*Klebiella oxytoca*	*Bacillus brevis* *Klebiella oxytoca*
24	(-)	1 *Staphylococcus epidermidis* *Bacillus cereus* 2 *Staphylococcus epidermidis*	(-)	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*	*Staphylococcus epidermidis* *Sphingomonas paucimobilis*
25	(-)	*Corynebacterium bovis* *Bacillus cereus*	(-)	*Corynebacterium bovis*	*Bacillus cereus*	*Corynebacterium bovis*
26	*Bacillus subtilis*	*Corynebacterium striatum*	(-)	*Corynebacterium striatum*	*Corynebacterium striatum* *Bacillus cereus*	*Corynebacterium striatum*
27	1 (-) 2 (-)	*Corynebacterium pseudodiphteriticum*	(-)	*Corynebacterium jeikum* *Enterococcus faecalis*	*Corynebacterium jeikum*	1 *Corynebacterium pseudodiphteriticum* 2 *Corynebacterium jeikum*
28	*Bacillus pumilus*	*Staphylococcus saprophticus*	(-)	*Staphylococcus saprophticus*	*Staphylococcus saprophticus*	*Bacillus pumilus* *Staphylococcus saprophyticus*
29	(-)	*Staphylococcus epidermidis* *Staphylococcus saprophticus*	(-)	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*

Table 2. Bacterial species isolated from honeycomb samples taken from hives with foulbrood clinical signs

Tablo 2. Yavru çürüklüğü klinik bulguları görülen kovanlardan alınan petek örneklerinden izole edilen bakteri türleri

Isolated microorganisms	Positivity rate
Bacillus subtilis	8 (11.5%)
Bacillus brevis	5 (7.24%)
Bacillus pumilus	5 (7.24%)
Bacillus cereus	2 (2.89%)
Bacillus licheniformis	3 (4.34%)
Staphlococcus aureus	2 (2.89%)
Staphylococcus epidermidis	5 (7.24%)
Staphylococcus saprophyticus	2 (2.89%)
Staphylococcus simulans	1 (1.44%)
Staphylococcus warneri	1 (1.44%)
Corynebacterium jeikum	6 (8.69%)
Corynebacterium aquaticum	3 (4.34%)
Corynebacterium pseudodiphteriticum	3 (4.34%)
Corynebacterium striatum	1 (1.44%)
Corynebacterium bovis	4 (5.79%)
Corynebacterium renale	1 (1.44%)
Corynebacterium pseudotuberculosis	2 (2.89%)
Klebiella oxytoca	1 (1.44%)
Sphingomonas paucimobilis	1 (1.44%)
Enterococcus faecalis	5 (7.24%)
Escherichia coli	1 (1.44%)
Acinetobacter lwoffi	1 (1.44%)
Morganella morgani	1 (1.44%)
Providencia stuartii	1 (1.44%)
Rhodococcus equi	1 (1.44%)
Lactococcus lactis ssp. Cremoris	1 (1.44%)
Micrococcus luteus	1 (1.44%)
Aerococcus urinae	1 (1.44%)
Total	69 (100%)

From the colonies with clinical signs, samples were taken from 43 feeders, 32 hand irons, 29 smokers, 30 gloves and 29 beekeeper suits by swab. Microorganism isolation rates from equipment used by beekeepers are shown in Table 3. The microorganism isolation rate from gloves and beekeeper suits was 100%. Beekeeper smoker was determined as the beekeeping material with the lowest microorganism isolated. (34.38%).

Table 3. Beekeeping equipment and microorganism isolation rates

Tablo 3. Arıcılık ekipmanları ve mikroorganizma izole edilme oranları

Beekeeping Equipments	Number of samples	Microorganism isolation rates
Feeder	43	22 (51.16%)
Hive tool	30	30 (100%)
Beekeeper smoker	29	10 (34.48%)
Gloves	30	29 (96.66%)
Beekeeper suit	29	29 (100%)

DISCUSSION

Microorganisms were investigated predominantly on honey bees, and partly on nectar, pollen and have been reported in research and review studies. Particulary pathogenic microorganisms were intensively studied and reported in a number of research papers around the world since they cause colony losses in honey bees (Snowdon and Cliver 1996, Gillliam 1997).

In a recent study by Bayrakal et al. (2020) honey, bee and bee larva were examined from 900 samples in 300 colonies by molecular method for bacterial, fungal, viral and parasitic factors. They reported a number of bacterial, fungal and parasitic agents from those samples. Another study by Cunninham et al. (2022) reports bees as bioindicators of the environment and analyzed plant pathogens carried by honey bees in the environment.

On the other side, contamination of microorganisms in beekeeping pieces of equipment has not been studied and it is difficult to compare these data to other studies and assess the rate of contamination by those materials used in beekeeping activities. Hive tools, beekeeper suits, gloves, feeders, and beekeeper smoker were determined as the source of microorganisms in this study as 100%, 100%, 96%, 51% and 34% respectively. This explains the reason for the fast and high rate of microorganism contamination in apiaries. These results also provide a good dataset to demonstrate the source of microbial reservoirs of apiaries in beekeeping.

In this study, a total of 69 microorganisms and 28 different bacterial species were isolated from samples taken from colonies and beekeeping materials showing clinical signs of foulbrood as a result of isolation and identification. The same species of bacteria were isolated from the sampled hives and materials. THe high number of microorganisms particularly bacteria underlines the importance of hive materials for the source of contamination and this should be considered in beekeeping practices.

Honeybees can be affected by a variety of bacteria, fungi, viruses and parasites and disease management is an important part of beekeeping activities. Good beekeeping and biosecurity practices are very important to control bee pathogens (Arbia and Babbay 2011, Al-Waili et al. 2012, Borum 2022, Rasovic 2021). Pathogenic microorganisms often spread rapidly due to beekeeping activities and some of them can be fatal to bees if left untreated. In addition, some infections such as American foulbrood are very hard to treat or expensive to treat. Sometimes, it may require the destruction of infected hives or even entire colonies (Leska et al. 2021). Some practices by beekeepers can be a source of pathogen contamination. Especially foulbrood agents can be transmitted by beekeeping tools and pieces of equipments (Fries and Camazine 2001, CFIA 2013).

The bacterial species isolated from the materials were the same as the agents isolated from the brood combs taken from the hives with clinical signs of foulbrood and bacteria grew at different rates at samples taken from the feeders, hive tools, gloves, beekeeper smokers and beekeeper suits. This gives an idea of the route of contamination in apiaries. The continuous use of these materials without disinfection will cause contamination and this should be avoided in apiaries (Locke et al. 2019, Tomljanović et al. 2020). Hygiene is of great importance for maintaining the health of bees and bee products (vanEngelsdorp et al. 2013, Rasovic 2021). In particular foulbrood diseases such as American foulbrood (no effective treatment available) and European foulbrood cases will

increase among colonies and apiaries without disinfection and this will cause economic losses.

In conclusion, the data provided here may help to improve disease management and hygienic applications to avoid pathogenic infections in honey bee colonies or apiaries. Beekeepers should be informed about contamination routes of pieces of beekeeping equipments and apply disinfection procedures during beekeeping applications to avoid pathogenic infections. *Since beekeeper smoker has less infection compared to other beekeeping equipments due to high temperature in burning smoker beekeepers are advised to disinfect the hive tool with smoker before the beekeeping practices in the field to reduce infection rates of other colonies.* More research is needed in this area to reduce or avoid contamination of bee colonies with equipments and use less medications in beekeeping.

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