บทความวิจัย

สัตวแพทย์มหานครสาร

JOURNAL OF MAHANAKORN VETERINARY MEDICINE

Available online: www.tci-thaijo.org/index.php/jmvm/

การศึกษาจุลินทรีย์ประจำถิ่นในช่องปากลูกม้าแรกเกิด

ชรินทร จันทร์สวัสดิ์[1] ธีรพล ชินกังสดาร[1] และดานัย แสงทอง[2,#]

[1]สาขาวิชาคลินิก คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเทคโนโลยีมหานครกรุงเทพมหานคร 10530

[2]สาขาวิชาสัตวบาลและพื้นฐานวิชาชีพ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเทคโนโลยีมหานครกรุงเทพมหานคร 10530

บทคัดย่อ: โรคภายในช่องปากของม้ามักเป็นสาเหตุหลักของปัญหาที่ส่งผลต่อสุขภาพของม้า เช่น น้ำหนักและความสามารถของม้าที่ลดลงเนื่องจากไม่สามารถกินอาหารได้ ในมนุษย์ สุนัข และแมวนั้นมีการศึกษาเกี่ยวกับจุลินทรีย์ในช่องปากมากมาย แต่ในทางตรงกันข้ามการศึกษาจุลินทรีย์ในช่องปากของม้านั้นกลับมีข้อมูลเพียงเล็กน้อย โดยเฉพาะจุลินทรีย์ในช่องปากของลูกม้าแรกเกิดที่ได้กินเพียงน้ำนมที่มีน้ำตาลในปริมาณมากกลับยังไม่เคยมีผู้ทำการศึกษา ดังนั้นจุดประสงค์ของการศึกษานี้คือ เพื่อศึกษาความหลากหลายทางพันธุกรรมของจุลินทรีย์ในช่องปากม้าแรกเกิดด้วยเทคโนโลยี *16s rRNA* next-generation sequencing โดยทำการเก็บตัวอย่างจากคราบจุลินทรีย์ที่บริเวณรากฟันใต้เหงือก (subgingival plaque) ของลูกม้าแรกเกิดที่ได้รับการดูแลภายใต้สิ่งแวดล้อมและการจัดการเดียวกันเพื่อนำมาวิเคราะห์ชนิดและความหลากหลายของจุลินทรีย์ประจำถิ่น ซึ่งได้ผลจากการวิเคราะห์ได้หน่วยอนุกรมวิธาน (Operational Taxonomic Unit, OTUs) ของจุลินทรีย์ในช่องปากของลูกม้าแรกเกิดจำนวนทั้งหมด 2,017 OTUs ผลจากการวิเคราะห์ทางด้านอนุกรมวิธานสามารถแบ่งจุลินทรีย์ในช่องปากของลูกม้าออกได้เป็นไฟลัมใหญ่ 4 ไฟลัม คือ Proteobacteria (77.74%) Firmicutes (20.98%) Acidobacteria (1.19%) และ Bacteroidetes (0.09%) โดยในทุกตัวอย่างนั้นพบจุลินทรีย์ในแฟมมิลี่ Pasteurellaceae จำนวนมากโดยมี Pasteurellaceae ที่ไม่สามารถระบุชนิดได้และ *Actinobacillus* spp. เป็นชนิดจุลินทรีย์หลักที่พบในส่วนของคลาส Bacilli นั้น พบแบคทีเรียที่มีจำนวนมากที่สุดคือ *Streptococcus* spp. โดยผลจากการศึกษาครั้งนี้มีกลุ่มแบคทีเรียส่วนใหญ่คล้ายกันกับหลายการศึกษาก่อนหน้านี้ทั้งในม้าเต็มวัย ในแมวและในสุนัข อย่างไรก็ตามจุลินทรีย์ที่พบในช่องปากลูกม้าแรกเกิดโดยใช้ตัวอย่างจากคราบจุลินทรีย์ที่บริเวณรากฟันใต้เหงือกในการศึกษาครั้งนี้เมื่อนำผลของ *16s rRNA* next-generation sequencing นำมาวิเคราะห์ความหลากหลายทางพันธุกรรมพบว่ามีความหลากหลายทางพันธุกรรมต่ำ

คำสำคัญ: ลูกม้าแรกเกิด จุลินทรีย์ประจำถิ่นในช่องปาก เทคโนโลยีการวิเคราะห์ลำดับเบสยุคใหม่

[#]ผู้รับผิดชอบบทความ

สัตวแพทย์มหานครสาร. 2562. 14(1): 1-12.

E-mail address: dsangthong@hotmail.com

Submitted date 2018-08-28 Revised date 2018-12-06 Accepted date 2019-05-24

The Study of Newborn Foal Oral Microbiota Diversity

Charintorn Junsawat[1], Teerapol Chinkangsadarn[1], and Danai Sangthong[2,#]

[1]Department of Clinical Veterinary Science, [2]Department of Animal Husbandry & Veterinary Basic Professional Science, Faculty of Veterinary Medicine, Mahanakorn University of Technology, Bangkok 10530, Thailand

Abstract: Equine oral disease is the underlying cause of many general disorders, such as weight loss and poor performance. There are plenty of oral microbiology studies such as human, dog and cat. In contrast, there have been only a few studies dedicated to equine oral microbiology and the investigations of oral bacteria in a neonatal foal which feeding on milk contain a higher amount of sugar has never been reported. The aim of this study was to investigate the microbial diversity in healthy subgingival niches in newborn foal's oral cavities using Next-generation sequencing (NGS) of *16S ribosomal RNA* (*16s rRNA*) gene method. The subgingival plaque of three healthy Thoroughbreds cross newborn foals that are raised in the same environment and management were collected for analysis. A total of 2,017 (Operational Taxonomic Unit, OTUs) presented; 903 OTUs for F1, 495 OTUs for F2 and 709 for F3, representing the four mainly different bacterial phyla; Proteobacteria (77.74%), Firmicutes (20.98%), Acidobacteria (1.19%), Bacteroidetes (0.09%). Pasteurellaceae was highly abundant; unclassified Pasteurellaceae, as well as *Actinobacillus* spp., were observed in all sample. Within the class Bacilli, the most dominant genus *Streptococcus* spp. was observed. The result in this study is similar to the major groups of bacteria that have found in the previous studies in adult horse also, consistent with the result in cats and dogs. However, the microbial diversity in healthy subgingival niches in newborn foal's oral cavities using next-generation *16S rRNA* gene sequencing method was low.

Keywords: Newborn foal, Oral microbiota, Next-generation sequencing

[#]Corresponding author

E-mail address: dsangthong@hotmail.com

J. Mahanakorn Vet. Med. 2019 14(1): 1-12.

Introduction

Horses are large grazing, herbivorous animal. Equine dental abnormalities and oral disease are the underlying cause of many general disorders, such as weight loss, pain, behavior problem, and poor performance. Periodontal disease (PD), one of the most common dental problems in horses (Dixon,

2005), is the polymicrobial infection that affects one or more of the periodontal structure cementum, periodontal ligament, alveolar bone and gingiva. The disease need three main factors: a susceptible host, the presence of pathogenic species or keystone pathogen and foster of other bacterial species to initiated (Hajishengallis and Lamont, 2012, Socransky *et al.*, 1998). According to the explanation from the recent periodontal plaque, theories were that the synergy between different member or genes combination within the microbial community helps each other to the disease-provoking microbiota rather than select periopathogens that initiated periodontitis (Hajishengallis and Lamont, 2012). Untreated PD may lease to exfoliation of affected tooth or periapical infection (Dacre *et al*., 2008, Dixon *et al.*, 2000). Therefore to be able to understand the changes and control in oral microbiota is the key to prevent this disease.

The oral microbiology studies in human have identified more than 500 bacterial species including cultivable and non-cultivable bacteria and many of these are periopathogemic bacteria that involve in the pathogenesis of periodontal disease (Langley and Morris, 2009, Moore and Moore, 1994). There are some studies toward other animals, e.g., dog (Santin *et al*., 2013, Zambori *et al*., 2014), cat (Dolieslager *et al*., 2013), marsupials (Bird *et al*., 2002), even snake (Jho *et al*., 2011). However, around 50% of oral microorganism cannot be identified by conventional culture approaches due most of the oral associated bacteria are facultative anaerobes or obligate anaerobes that require special anaerobic condition with enriched growth medium (Bailey and Love, 1991). Thus, the number and variety of oral microorganism have been considerably underestimated. Until recently, the culture-independent bacteria identification such as *16S rRNA* gene sequencing and cloning methods can provide tremendous information on oral bacteria community in both human and veterinary specimens (Kato *et al*., 2011, Riggio *et al*., 2011, Spratt, 2004). Particularly, Next-generation sequencing that provides in-depth detail of the whole oral microbiota such in human (Vossen *et al*., 2008) and animals such dog (Sturgeon *et al*., 2013), cat (Sturgeon *et al*., 2014) and ovine (Riggio *et al*., 2013).

In contrast, there have been only a few studies dedicated for equine oral microbiology using both conventional culture-dependent and molecular methods. These studies show that the most frequently isolated oral bacteria from supra-gingival or sub-gingival surfaces of horse teeth are *streptococci*, *micrococci*, *Lactobacillus* spp., *Fusobacterium* spp. and anaerobes (Bailey

and Love, 1991, Dorsch *et al*., 2001). Recently, the investigation of the bacteria that populate healthy subgingival niches in the horse oral cavity was acknowledged (Gao *et al*., 2016, Kennedy *et al*., 2016, Chinkangsadarn, 2015). Nonetheless, the investigations of oral bacteria in neonatal foal which feeding on milk contain higher amount of sugar has never been reported, although neonatal foals are very susceptible to opportunistic infections from microorganism that generally being a normal flora which may lead to various diseases; such as severely ill or immunocompromised foal (Ryan and Sanchez, 2005), acute septicemia (Langley and Morris, 2009), wound and respiratory infections including, purulent nasal discharge and abscesses of submandibular nodes in some cases (strangles) and severe pyogranulomatous pneumonia in foals (Venner *et al*., 2007).

Due to the possible etiological roles in equine periodontal disease, as well as other oral infectious disease and soft tissue infection, the aim of this study was to investigate the microbial diversity in healthy subgingival niches in newborn foal's oral cavities using the next-generation *16S rRNA* gene sequencing method.

Materials and methods

Animals

Three healthy Thoroughbreds cross newborn foals (age < 48 hours old) that are raised in the same environment and management at 1st livestock and agriculture division, Veterinary & Remount Department, Kanchanaburi, Thailand.

Transport medium and subgingival plaque sampling

Transport medium was prepared using Wilkins Chalgren Broth (WCB) (Oxoid, Unipath Ltd., United Kingdom) with 10% glycerol. The medium was autoclaved, cooled to room temperature and 1 ml aliquots transferred into cryovials. The vials were stored in 4°C until sample collection. Physical examination will be done in all foals before collection, a subgingival plaque was then collected using sterile universal curette from upper and lower 2nd deciduous premolar. The collected plaque samples were transferred into cryovials containing reduced transport medium, then immediately frozen in liquid nitrogen for transport to the laboratory. Samples were stored frozen at -80°C until required for microbial analysis.

DNA extraction and library preparation

Genomic DNA from bacteria will be prepared from each subgingival plaque samples using the DNA extraction kit (Qiagen, Hilden, Germany), following the

manufacturer's instructions. *16S rRNA* library preparation workflow from Illumina (#15044223 Rev.B) was performed at the Australian Centre for Ecogenomics, School of Chemistry and Molecular Bioscience, The University of Queensland, St. Lucia. Initially, PCR products of ~590bp were amplified according to the specified workflow but using Q5 Hot Start High-Fidelity 2X Master Mix (New England Biolabs) in PCR reactions. The 16S 1406F/1525R primers set (0.4μM) was designed to amplify bacterial and archaeal *16S rRNA* genes: F - GYACWCACCGCCCGT and R - AAGGAGGTGWTCCARCC. The rpsL F/R primer set (0.2μM), used for inhibition control, amplifies Escherichia coli DH10B only: F - GTAAAGTATGCCGTGTTCGT and R - AGCCTGCTTACGGTCTTTA. The PCR was run on the ViiA7 platform (Applied Biosystems).

DNA purification of PCR products at each stage was performed using Agencourt AMPure XP beads (Beckman Coulter). Purified DNA was indexed with unique 8bp barcodes using the Illumina Nextera XT v2 Index Kit A-D (Illumina FC-131-1002) in standard PCR conditions with Q5 Hot Start High-Fidelity 2X Master Mix. Indexed amplicons were pooled together in equimolar concentrations and sequenced on MiSeq Sequencing System (Illumina) using paired-end sequencing with V3 300bp chemistry according to manufacturer's protocol.

Next- generation sequencing and data processing

Next-generation sequencing and subsequent data processing were performed at the Australian Centre for Ecogenomics, School of Chemistry and Molecular Bioscience, The University of Queensland, St. Lucia.

Quality Control was performed using the Illumina MiSeq platform. FastQC files were processed with FastQC (Andrews 2010). Primer sequences were removed, and poor quality sequences were trimmed using Trimmomatic (Bolger *et al*. 2014). All reads were trimmed to 250 bases, reads with less than 250 bp were removed. Operational taxonomic units (OTUs) were selected using QIIME (Walters *et al*. 2010) with a 97% similarity threshold. OTUs with less than 0.05% relative abundance in the OTUs table were removed. Representative OTU sequences were annotated by BLASTing against the Greengenes reference database.

Statistical analysis of next-generation sequencing

The relative abundance of OTUs was normalized by the total cell count within each sample then analyzed for diversity index (Shannon and Simpson) using R- Studio package.

Result

Across all samples, a total of 3,785 OTUs were obtained from three samples. After Eukaryota, Archaea and Cyanobacteria (class Chloroplast) kingdoms were removed, only 2,017 OTUs presented; 903 OTUs for F1, 495 OTUs for F2 and 709 for F3, representing the four mainly different bacterial phyla; Proteobacteria (77.74%), Firmicutes (20.98%), Acidobacteria (1.19%), Bacteroidetes (0.09%) as are presented in table 1. The relative distributions of the OTUs at Phylum level are illustrated in figure 1 and at class/order/family level in figure 2.

Proteobacteria was the most abundant phylum of foal subgingival plaque samples (92.03%, 52.93% and 76.87%, in F1, F2 and F3, respectively). Bacterial taxa belonging to the Gammaproteobacteria were particularly abundant in the horse subgingival plaque samples; especially within F1 (Table 1). Within this class, family Pasteurellaceae was highly abundant. For the Pasteurellaceae this included unclassified Pasteurellaceae (48.84% in F1, 20.00% in F2 and 29.48% in F3), as well as unclassified taxa belonging to the genus *Actinobacillus*, which were observed at 28.02% in F1, 11.92% in F2 and 25.39% in F3.

Firmicutes taxa were abundant in the foal subgingival plaque samples; constituting 7.86% in F1, 46.46% in F2 and 19.89% in F3. In the class Bacilli, the most dominant genus *Streptococcus* were observed at 5.65% in F1, 42.63% in F2 and 13.26% in F3.

Apart from that, Acidobacteria was identified in subgingival plaque samples (0.04% in F2 and 3.24% in F3) also with Bacteroidetes (0.11% in F1 and 0.20% in F2) were presented.

Shannon and Simpson diversity indexes and OTUs numbers are presented in table 2.

Discussion

This study is the first study that uses Next-generation sequencing to identify bacteria in the foal oral cavity. In this study we have observed a total of 4 phyla; Proteobacteria, Firmicutes, Acidobacteria and Bacteroidetes. Proteobacteria and Firmicutes are the 2 major groups of bacteria in the oral cavity (77.74% and 20.98% respectively). These result similar to the major groups of bacteria that have found in the previous studies using next-generation sequencing and 16S rDNA pyrosequencing in adult horse (Chinkangsadarn, 2015, Gao *et al*., 2016, Kennedy *et al*., 2016) also, consistent with the result in cats and dogs (Oh *et al*., 2015, Sturgeon *et al*., 2014). This is highlighting potential commonalities between the oral microbiota of foal, adult horse, dogs and cats.

The most prevalent genus within phyla Firmicutes was *Streptococcus* which related to the theory (Socransky *et al*., 1998) was

described the red complex as microbial species in dental plaque (biofilm) that exist in communities of enclosed matrix relating to one another.

Interestingly, Fusobacteria; one of the common gram-negative, strict anaerobes phyla presented in the oral cavity of horse in several studies (Chinkangsadarn, 2015, Dorsch *et al*., 2001, Gao *et al*., 2016, Trevillian *et al*., 1998) and also cat, dog and human (Gao *et al*., 2016, Oh *et al*., 2015, Sturgeon *et al*., 2014, Sohn *et al*., 2016) and they are considered to play an important role in dental plaque formation by neutralize subgingival environment pH by nitrogenous metabolism (Takahashi, 2005) have not detected in this study. Moreover, Actinobacteria which was identified in several studies of equine oral cavity (Chinkangsadarn, 2015, Gao *et al*., 2016) also, Spirochetes (phylum Spirochaetes); a diverse group of gram-negative, motile bacteria, was previously identified in other study within silver-stained preparations of tissue samples obtained from various sites of periodontal infection (periodontal pockets) (Cox *et al*., 2012) have not presented in subgingival plaque samples from all foals.

Despite the result in this studied was similar to those previous studies, number of the phyla in all subgingival plaque samples was low and the number of taxa in each phylum was not widely presented so we noted that microbial diversity in healthy subgingival niches in newborn foal's oral cavities using next-generation 16S rRNA gene sequencing method was low. The less diverse of microbiota as a foal perhaps the bacterial has not been massive introduced from the environment into newborn foal. Marsh (1994) stated that the stability of bacteria can break down from the environmental factors change, such as low pH level, which responsible for driving these deleterious shifts in the plaque microflora and in periodontal diseases, the consequence of shifting the environment from a neutral one to a more acidic one in the presence of fermentable sugars introduced from the diet inducing exposure of plaque to low pH leads to inhibition of acid-sensitive species, leading to a shift towards community dominance by acidogenic and acid-tolerant Gram-positive bacteria (e.g. *streptococci mutans* and *lactobacilli*).

However, the bacterial composition of plaque remains relatively stable as expected despite regular exposure to minor environmental change as foals did not feed with any diet except milk contain higher amount of sugar from their mother. The type of bacteria ought to similar with the environment they lived in and normal flora on their mother’s nipple or skin should be

Table 1 Bacterial identification in equine subgingival plaque by next-generation *16S rRNA* gene sequencing.

Phylum taxa	**F1**	**%**	**F2**	**%**	**F3**	**%**	**overall**	**%**
Acidobacteria	**0**	**0.00**	**2**	**0.40**	**23**	**3.24**	**25**	**1.19**
rare/unclassified Acidobacteria	0	0.00	2	0.40	4	0.56	6	0.28
Rothia spp.	0	0.00	0	0.00	19	2.68	19	0.90
Bacteroidetes	**1**	**0.11**	**1**	**0.20**	**0**	**0.00**	**2**	**0.09**
rare/unclassified Sphingobacteriaceae	1	0.11	1	0.20	0	0.00	2	0.09
Firmicutes	**71**	**7.86**	**230**	**46.46**	**141**	**19.89**	**442**	**20.98**
rare/unclassified Firmicutes	8	0.89	14	2.83	13	1.83	35	1.66
rare/unclassified Clostridia	0	0.00	0	0.00	3	0.42	3	0.14
Aerococcus spp.	0	0.00	1	0.20	2	0.28	3	0.14
Gemella spp.	12	1.33	2	0.40	8	1.13	22	1.04
Lactobacillus spp.	0	0.00	0	0.00	19	2.68	19	0.90
Staphylococcus spp.	0	0.00	2	0.40	2	0.28	4	0.19
Streptococcus spp.	51	5.65	211	42.63	94	13.26	356	16.90
Proteobacteria	**831**	**92.03**	**262**	**52.93**	**545**	**76.87**	**1638**	**77.74**
rare/unclassified Alphaproteobacteria	0	0.00	2	0.40	3	0.42	5	0.24
rare/unclassified Betaproteobacteria	0	0.00	2	0.40	0	0.00	2	0.09
rare/unclassified Neisseriaceae	0	0.00	66	13.33	2	0.28	68	3.23
Neisseria spp.	0	0.00	8	1.62	1	0.14	9	0.43
rare/unclassified Gammaproteobacteria	93	10.30	19	3.84	75	10.58	187	8.88
Aeromonas sp.	0	0.00	0	0.00	1	0.14	1	0.05
rare/unclassified enterobacteriaceae	9	1.00	0	0.00	4	0.56	13	0.62
Klebsiella sp.	0	0.00	0	0.00	1	0.14	1	0.05
rare/unclassified Pasteurellaceae	441	48.84	99	20.00	209	29.48	749	35.55
Actinobacillus spp.	253	28.02	59	11.92	180	25.39	492	23.35
Haemophilus spp.	31	3.43	3	0.61	6	0.85	40	1.90
Acinetobacter spp.	1	0.11	4	0.81	60	8.46	65	3.08
Moraxella spp.	0	0.00	0	0.00	3	0.42	3	0.14
Pseudomonas sp.	3	0.33	0	0.00	0	0.00	3	0.14
total	903	100.00	495	100.00	709	100.00	2107	100.00

affected to oral microbiota in foals. Beside, some studies have been done about vertical transmission of vaginal *Lactobacillus* species from mothers to newborn infants; these results suggest that infants acquire vaginal lactobacilli from their mothers at birth (Matsumiya, 2002 and Song, 1999). Although there have few studies in vaginal bacteria of a horse (Hoyles, 2002), the association between vertical transmission of vaginal bacteria from mothers to newborn foals never been done. Thus, the further study about the relevance of environmental and vaginal bacteria to oral microbiota in foals should be investigated.

Neonatal foals are very susceptible to opportunistic infections from microorganism that generally being a normal flora which may lead to various diseases; severely ill or immunocompromised foal (Ryan and Sanchez 2005), acute septicemia (Langley and Morris 2009), wound and respiratory infections including, purulent nasal discharge and abscesses of submandibular nodes in some cases (strangles) and severe pyogranulomatous pneumonia in foals (Venner *et al*. 2007). Wilson and Madigan (1989) studied on bacteriologic culture of antemortem blood samples and/or necropsy specimens obtained from 47 foals infected with bacterial septicemia, presenting gram-negative pathogen was the most isolated pathogen at necropsy together with gram-positive bacteria and anaerobic bacteria were isolated only from foals with mixed infections with gram-negative organisms and also, *Actinobacillus* spp. were one of the most frequent isolates in infected foals (34% of foals).

Moreover, Brogden *et al*. (1998) revealed that Pasteurella haemolytica complicated with respiratory infections in sheep and goats, consistent with the studies which show the association of Pasteurella haemolytica and pneumonia in foal (Saxegaard and Svenkrud, 1974, Peet *et al*., 1977). *Actinobacillus* spp. was the most abundant genus in this studied and although there was a high level of unclassified Pasteurellaceae shown in this study, those *Actinobacillus* spp. and unclassified Pasteurellaceae bacteria should be considered in the newborn foal. Sanitary and newborn foal management such as physical examination should have done properly.

Next-generation *16S rRNA* gene sequencing method is a cost-effective method for characterization of oral bacterial communities. Although a substantial number of bacteria were gained from this method, the ability of species identification still has to expand. Besides, despite Next-generation *16S rRNA* gene sequencing method have a capability to process on whether dead or

Table 2 Diversity statistics of bacterial community from 3 foals under the same management as determinate by next-generation sequencing.

	F1	F2	F3
Number of OTUs	903	495	709
Simpson	0.73003	0.86497	0.86003
Shonnon	2.29274	2.53606	2.63791

Figure 1 Relative abundance of bacteria phyla in the oral cavity of foals (F= Foal)

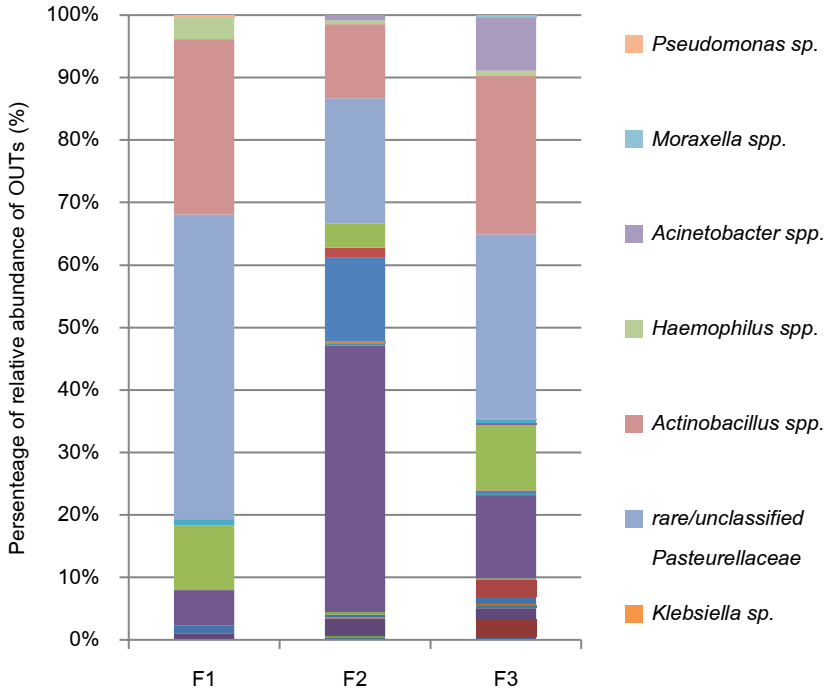

Figure 2 Relative abundance of bacteria class/order/family in the oral cavity of foals (F= Foal)

alive organisms, the phenotypic characterization such as colonial morphology, motility, anaerobic condition and drug sensitivity profiling which one of the keys to successful treatment cannot perform. Thus the Culture-dependent approaches Classic techniques still necessary using to characterize the group of bacteria.

Acknowledgement

The author would like to sincerely thank 1st livestock and agriculture division, Veterinary & Remount Department, Kanchanaburi, Thailand for allowing me collecting the sample and all Facilitation. Moreover, the indispensable people were Rochelle Price, Scientist at microbiological laboratory, Sean Corley, research and development Manager at animal genetics laboratory and Dr. Phil bird, Adjunct Associate Professor at School of Veterinary Science, who supported me, giving many great advices and helped me to understand all the process while I was in a short term placements at the School of Veterinary Science. Also, sincerely thank to the School of Veterinary Science, university of Queensland, Australia that allowed me to had one of the biggest experience to be a student of short term placements at the School of Veterinary Science.

References

Brogden, K. A., H. D. Lehmkuhl, and R. C. Cutlip. 1998. Pasteurella haemolytica complicated respiratory infections in sheep and goats. Vet. Res. 29: 233-254.

Chinkangsadarn, T. 2015. Clinical and microbiological aspects of periodontal disease in horses in South-East Queensland, Australia. The University of Queensland.

Cox, A., P. Dixon, and S. Smith. 2012. Histopathological lesions associated with equine periodontal disease. Vet. J. 194: 386-391.

Dorsch, M., D. Lovet, and G. D. Bailey. 2001. Fusobacterium equinum sp. nov., from the oral cavity of horses. Int. J. Syst. Evol. Micr. 51: 1959-1963.

Gao, W., Y. Chan, M. You, D. C. Lacap-Bugler, W. K. Leung, and R. M. Watt. 2016. In-depth snapshot of the equine subgingival microbiome. Microb. Pathog. 94: 76-89.

Kennedy, R., D. F. Lappin, P. M. Dixon, M. J. Buijs, E. Zaura, W. Crielaard, L. O'donnell, D. Bennett, B. W. Brandt, and M. P. Riggio. 2016. The microbiome associated with equine periodontitis and oral health. Vet. Res. J. 47: 49.

Marsh, P. D. 1994. Microbial ecology of dental plaque and its significance in health and disease. Adv. Dent. Res. 8: 263.

Oh, C., K. Lee, Y. Cheong, S. W. Lee, S. Y. Park, C. S. Song, I. S. Choi, and J. B. Lee. 2015. Comparison of the oral microbiomes of canines and their owners using next-generation sequencing. PloS one. 10: e0131468.

Peet, R., D. Main, J. Cronin, and A. Sier. 1977. Pasteurella haemolytica infection in two neonatal foals. Aust. Vet. J. 53: 152-152.

Saxegaard, F. and R. Svenkrud. 1974. Pasteurella haemolytica associated with pneumonia in a foal. A case report. Acta Veterinaria Scandinavica. 15: 439.

Socransky, S., A. Haffajee, M. Cugini, C. Smith, and R. Kent. 1998. Microbial complexes in subgingival plaque. J. Clin. Periodontol. 25: 134-144.

Sohn, K., K. Sohn, K. M. Kalanetra, D. A. Mills, and M. A. Underwood. 2016. Buccal administration of human colostrum: impact on the oral microbiota of premature infants. J. Perinatol. 36: 106-111.

Sturgeon, A., S. L. Pinder, M. C. Costa, and J. S. Weese. 2014. Characterization of the oral microbiota of healthy cats using next-generation sequencing. Vet. J. 201: 223-229.

Takahashi, N. Microbial ecosystem in the oral cavity: metabolic diversity in an ecological niche and its relationship with oral diseases. International Congress Series, 2005. Elsevier,p. 103-112.

Wilson, W. and J. Madigan. 1989. Comparison of bacteriologic culture of blood and necropsy specimens for determining the cause of foal septicemia: 47 cases (1978-1987). J. Am. Vet. Med. A. 195: 1759-1763.