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REVIEW ARTICLE

The fascinating microbes and their impact on neonatal dogs and cats – A review

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ABSTRACT

Recent literature data indicate that canine and feline neonates are not born in a sterile environment as it was stated previously. The acquisition, colonisation and maintenance of the early life microbiota of healthy fetuses is a rapidly developing research area. In humans, the natural healthy infant microbiome plays an essential role in health and its assembly is determined by the maternal–offspring exchanges of microbes. Even though this topic is becoming more and more important in dogs and cats, the exact role of the neonatal microbiome is not yet fully known in animals. This review summarises the current knowledge of the normal physiological neonatal microbiome in healthy puppies and kittens.

KEYWORDS

neonatology, birth, puppies, kittens, microbiome

INTRODUCTION

Limited information is available on the development of the neonatal faecal microbiome in dogs and cats. During pregnancy, several healthy developmental changes occur in many physiological systems to support the fetal growth. The last decade of research has made it clear that dramatic changes in the composition of the commensal microbiome also occur. However, the role of the natural healthy microbiota is not fully understood yet (Nuriel-Ohayon et al., 2016).

For more than a century, it was assumed that the eutherian fetus inhabits a largely sterile environment *in utero* during pregnancy and is protected from bacterial invasion by the maternal and fetal vascular separation, the immune-privileged status of the placental trophoblast and gestational maternal tolerance mechanisms (De Agüero et al., 2016). However, recent studies in humans and animals have shown that bacterial communities can be found in the uterus, amniotic fluid, placenta and meconium of healthy pregnancies (Bearfield et al., 2002; Jiménez et al., 2005; Steel et al., 2005; Collado et al., 2008; Jiménez et al., 2008; Rautava et al., 2012; Aagaard et al., 2014; Hansen et al., 2015; Collado et al., 2016; Parnell et al., 2017; Zhu et al., 2018; Stinson et al., 2019; Zakošek Pipan et al., 2020). We now know that acquisition of the gut microbiome begins *in utero* and continues to change after birth (Singh and Mittal, 2019). The presence of bacteria in newborn calves (Zhu et al., 2021), foals (Husso et al., 2020), lambs (Bi et al., 2021) and goat kids (Zhuang et al., 2020) has been confirmed in recent studies. Bacteria were present in all tissues or organs, from which samples were taken. In all the species mentioned, the presence of bacteria in the placenta was more evident than in the amniotic fluid. This also has a major impact on the health of the developing offspring. In humans, the gut microbiota is known to be involved in the programming and maturation of the immune system (Dzidic et al., 2018), the utilisation and modification of nutrients from the diet, the shaping of the gut environment through the production of metabolites as by-products of metabolism (Henrick et al., 2021) and the

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prevention of colonisation of the gut by pathogens (Dalby and Hall, 2020). In mice, maternal colonisation has been found to reprogram intestinal transcriptional profiles of the offspring, including increased expression of genes encoding epithelial antibacterial peptides and metabolism of microbial molecules (Bliss and Wynn, 2017). Some of these effects are dependent on maternal antibodies that can retain microbial molecules, and pass them on to offspring during pregnancy. Pups, colonised during pregnancy, have been found to better avoid inflammatory responses and intestinal bacterial invasion than pups born without microbiota (De Agüero et al., 2016).

The purpose of this review is to provide an overview of the composition of the normal physiological microbiota in newborn puppies and kittens, to summarise the latest research in this field and to gather knowledge about the health and development of puppies and kittens based on their microbiome at birth.

MICROBIOTA AT BIRTH

Acquisition of the gut microbiome is now thought to begin *in utero* and to continue to change after birth (Singh and Mittal, 2019). At birth, the microbiome of puppies and kittens consists of a community of microbes likely acquired from their mother through vertical transmission. Later, the microbiome develops and changes during the first month of life. These changes occur primarily in response to a changing nutritional environment, although other external factors such as antibiotics also significantly influence the composition of the bacterial community (Guard et al., 2017). Several studies in humans have found significant effects of the delivery mode on the composition of the neonatal gut microbiota (Dominguez-Bello et al., 2010; Bäckhed et al., 2015; MacIntyre et al., 2015; Rutayisire et al., 2016). The same has been found in our study on puppies, where the delivery mode influenced the diversity and type of bacterial communities in the gut of a newborn puppy (Zakošek Pipan et al., 2020). In humans, a larger cohort study that has examined the delivery method – either vaginal or by caesarean section (C-section) – and its influence on the infant's microbiota, has found difference in the composition of the gut microbiome. They have emphasized the importance of the initial microbiota, to which the infant is exposed to, regarding health later in life (Dominguez-Bello et al., 2010; Shao et al., 2019). Infants delivered by C-section appear to have increased colonisation by opportunistic pathogens from the environment such as *Enterococcus, Enterobacter* and *Klebsiella* species, due to impaired transmission of *Bacteroides* and *Bifidobacterium* from the mother (Shao et al., 2019). It has been suggested that the interruption of bacterial transmission from the vagina to the neonate due to C-section may have long-term health effects and a higher incidence of asthma (Kero et al., 2002) and celiac disease (Mårild et al., 2012) in babies. It has also been found that the gut microbiota of vaginally born infants is composed of bacteria present in the mother's vagina and includes bacteria present in the mother's gut (Mackie et al., 1999; Dominguez-Bello et al., 2010). In contrast, the gut microbiota of infants born by C-section is similar to the maternal skin and oral microbiota in humans (Bäckhed et al., 2015; MacIntyre et al., 2015). Similar observations have also been made in dogs, where puppies, born by C-section, had a meconium microbiota composed of the bacteria present in their dams' oral cavity, whereas puppies, born vaginally, had mainly bacteria present in their dams' vagina. In addition, it has been found that the total microbial diversity was significantly higher in vaginally born puppies compared to puppies born by C-section (Zakošek Pipan et al., 2020).

Additional interesting results were obtained in our previous study when we investigated the microbiota of puppies and their weight gain in the first days of life. Puppies that had bacteria in the meconium or placenta at birth gained more weight than those born without bacteria. Although the difference was not significant during the first two days, from a clinical point of view, puppies with sterile placenta lost an average of 1.9% of their body weight, whereas puppies with placental microbiota gained an average of 0.06%. Moreover, the difference became significant on days 3 and 4 (Zakošek Pipan et al., 2020). This fact is important for better survival of the puppies in the first week of life, when mortality is known to be relatively high (Tønnessen et al., 2012). The higher relative weight gains in puppies born vaginally compared to those born by C-section could be due to a greater diversity of the meconium microbiota in puppies delivered vaginally and, consequently, better food intake from the intestine (Zakošek Pipan et al., 2020).

During pregnancy, the female body undergoes physiological changes to support fetal growth and development (Almeida et al., 2018). Changes in metabolism, hormones, cardiovascular system and immune modulations are observed. Along with the immune changes, there are striking changes in the gut microbiota of dams during pregnancy (Vilson et al., 2018). While *Fusobacterium*, Bacteroidetes and Firmicutes are co-dominant in the faecal microbiome of healthy dogs (Pilla and Suchodolski, 2020), the phyla Firmicutes and Proteobacteria and the genus *Lactobacillus* predominate at the end of gestation. Similar observations have been made in humans, where the gut microbiota changes dramatically from the first to the third trimester, with a dominance of the phyla Actinobacteria and Proteobacteria and a decrease in individual richness (Koren et al., 2012). A change in the relative abundance of different bacteria during lactation and an increase in diversity from pregnancy to the end of lactation have also been observed in the gut of pregnant and lactating dogs (Vilson et al., 2018).

How microorganisms enter the fetal compartment of the placenta remains unclear. Since bacterial translocation via the bloodstream is known to be increased during pregnancy (Aagaard et al., 2014), this could result in microorganisms entering the circulation and colonising the placenta (Pelzer et al., 2017). Another possibility is that bacteria ascend from the vagina to the placenta and/or they are transported from the intestinal lumen to the placenta via maternal dendritic cells (Romero et al., 2015; Zheng et al., 2015). Transmission

of microbes from the mother to offspring is known in animals, including marine sponges, molluscs, insects, domestic chickens, fish, turtles and mice. This suggests that microbes are inoculated into the fetus before birth and that they already play a very useful role during fetal development (Funkhouser and Bordenstein, 2013).

From the data in the literature, it can be concluded that bacteria pass through the placenta mainly when certain non-physiological processes take place in the mother's body, regardless of the time of pregnancy. For example, it has been found in humans, that a 50% reduction in the maternal arterial oxygen tension over a 30-min period resulted in significant overexpression of genes associated with immune responses in the fetal brain 24 h later (Zarate et al., 2017). To address the question of whether bacteria from the environment enter the maternal blood and the fetal tissues, a study has been conducted using a non-catheterised pregnant ewe as a model to determine whether proteins or DNA from the bacteria, introduced into either the maternal mouth or vagina, could be found in the fetus. The mother ewe was inoculated with genetically labelled *Staphylococcus aureus* via three relatively common (intravenous, oral or intravaginal) routes, through which the fetus can be reached. Components of the bacteria were detected in the placenta, amniotic fluid and fetal brain 3–5 days after inoculation. The oral route of transmission was particularly important for the transfer of bacterial cell components to the fetus (Yu et al., 2019, 2021). The importance of the oral route has also been confirmed in other studies in humans, mice and dogs, in which the healthy placental microbiome was found to have a greater similarity to the oral microbiota than to the intestinal and vaginal ones (Jiménez et al., 2008; Pelzer et al., 2017; Zakošek Pipan et al., 2020). The transmission of oral bacteria has been confirmed in a study, in which a group of pregnant mice were orally inoculated with a genetically labelled *Enterococcus faecium* strain. The labelled bacteria were isolated and detected by PCR from the meconium of the inoculated animals obtained by C-section one day before the expected term of delivery. In contrast, it was not detected in samples from the non-inoculated control group (Jiménez et al., 2008).

In both humans and animals, bacteria have been isolated less frequently from the amniotic fluid than from the placenta. In healthy pregnancies, culture-based studies of mid-trimester amniotic fluid have either shown it to be sterile or bacteria could be isolated in only up to 13% (Mandar et al., 2001; Nguyen et al., 2004). Recently, bacterial sequences have been detected in amniotic fluid from healthy births, suggesting that human amniotic fluid harbours a microbial community (Collado et al., 2016). The detection of microbial populations in healthy amniotic fluid suggests that microbial colonisation of the neonate begins before birth. On the other hand, Lim et al. (2018) have failed to isolate live bacteria from healthy human pregnancies. It seems that the amniotic fluid at the time of birth does not usually contain live bacteria, but colonisation with bacteria may have occurred at an earlier stage of pregnancy, especially if there were alterations during pregnancy, since bacteria in the placenta and amniotic fluid have been associated with inflammatory processes, hypoxia, bacteraemia and preterm birth.

Because the intra-amniotic cavity is distinct from the placenta, we cannot rule out the possibility that the human placenta is separately colonised with microbes and that perhaps protective mechanisms prevent these pathogens from entering the amniotic fluid. The cells of the amniotic membrane provide a comprehensive immune defence against potential pathogens and are involved in the immune response by secreting protective molecules such as lipids, peptides and proteins (Šket et al., 2021). The inner part of the amniochorionic membrane could therefore protect the embryo/fetus from environmental dangers, including microbial infections.

In dogs, the most common bacteria isolated from the dams' placenta were *Staphylococcus* spp., *Streptococcus* spp. and *Neisseria zoodegmatis* (Zakošek Pipan et al., 2020). The similarity of bacteria in the placenta and oral cavity suggests the passage of bacteria from the oral cavity into the bloodstream and placenta (Romero et al., 2015). However, microbial density has been found to be very low in humans and dogs (Romero et al., 2015; Zakošek Pipan et al., 2020). Therefore, careful sampling is of great importance and scrutiny is necessary to avoid misinterpretation of results, which may be due to contamination. Samples, collected after vaginal delivery, have a high likelihood of contamination from the birth canal, unlike placental samples, collected under sterile conditions during a C-section.

While these studies have shed some light on the initial colonisation of the infant gut and microbial succession dynamics, much remains to be discovered about the various pathways of microbes into the infant gut. Until now, the research has focused mostly on bacteria, but there is an emerging world of viruses and fungi whose origins, transmission and establishment in the gut and placenta are still unknown.

SHAPING OF THE MICROBIOTA

The health and well-being of companion animals, just as that of their owners, depends on the intestinal microbes. The gut microbiome contributes to the host metabolism, protects against pathogens, educates the immune system and directly or indirectly affects most physiologic functions of the host. A healthy and stable microbiome can simultaneously act as pro- and anti-inflammatory, keeping a balance to prevent excessive inflammation while still being able to correctly respond to infections (Pilla and Suchodolski, 2020). The microbiome is dynamic and many changes during the life of the host occur in response to a variety of factors including diet, environment, medical interventions and disease states. After maternal colonisation of the infant gut, the composition of the microbiota is quickly changed and shaped by the diet and its components, i.e., breast milk or formula (or both). Breast milk is a complex biological fluid with many different nutritional and host components, such as enzymes and

antibodies (Dalby and Hall, 2020). Recently, it has been found that the milk, produced by domestic dogs and cats, is unique in terms of its oligosaccharide profile. Oligosaccharides are important components of the milk, serving as substrates for the intestinal microbiota and act as antimicrobials that prevent pathogen colonisation and support the developing gastrointestinal immune system of neonates (Wrigglesworth et al., 2020). In neonate beagle puppies, a 100-fold increase in lactobacilli by day 21 in the luminal contents of the distal colon has been detected, as the canine milk is a natural source of lactobacilli for the suckling puppy (Martín et al., 2010). Similarly, it has been proven in humans that the breast milk is a source of lactic acid bacteria for the infant gut (Martín et al., 2003). Lactobacilli are therefore the most dominant bacteria present in the early neonatal life and they inhabit commonly all parts of the canine intestine, with *Lactobacillus acidophilus* being dominant (Tang et al., 2012). *Lactobacillus fermentum, L. rhamnosus* and *L. salivarius* have been reported to be present in the healthy canine intestine (Barko et al., 2018). Other canine lactobacilli are represented by *L. murinus* and *L. reuteri* (Tang et al., 2012), *L. animalis, L. sanfranciscensis* and *L. paraplantarum* (da Silva et al., 2013). Lactobacilli found in cats are typical intestinal lactobacilli, e.g. *L. acidophilus, L. salivarius, L. johnsonii, L. reuteri* and *L. sakei*, which can be seen in other animals as well as in humans (Ritchie et al., 2010).

Knowledge on the acquisition and later development of the intestinal microbiome in dogs and cats is limited to a handful of studies in kittens and puppies. In kittens, it has been found that, as in humans, the early faecal microbiome is characterised by a high degree of interindividual variation and that intraindividual diversity and composition change with age. Like in humans, the relative abundance of *Lactobacillus* and *Bifidobacterium* decrease with age, whereas *Bacteroides* and bacterial genes, associated with the ability to metabolise complex carbon sources, increase with age (Deusch et al., 2015).

Another study has found that the faecal microbiome of kittens changed only slightly between 8 and 16 weeks of age (Deusch et al., 2015). These results suggest that, as in humans, the developing gut microbiome converges to a temporally stable configuration as kittens mature. The same temporal instability and substantial interindividual variability have been found in a study on puppies using DNA sequencing methods (Burton et al., 2016). However, it is worth mentioning that the faecal microbiomes of genetically related dogs have been found to be more similar to each other than to those of unrelated dogs, a finding that sheds light on genetics and environmental factors and on their influence (Hand et al., 2013). Vilson et al. (2018) have studied the effect of the environment on the early gut microbiota and found a great impact. In a study, comparing dogs and their owners, significant sharing of the skin and even the faecal microbiota between the dog owners and their pets has been observed (Song et al., 2013). Despite the individual variability, it has been reported that the microbiota of a given individual remains stable over time and that more than 60% of the strains in humans remained stable over a 5-year period (Faith et al., 2013). When studying the microbiome of young dogs, the faecal microbiota of puppies has been found to change between 2 and 56 days after birth. Significant changes have been noted at all taxonomic levels, the most profound of which was a shift from mainly Firmicutes occurring in puppies at the age of 2 days to a co-dominance of Bacteroidetes, Fusobacteria and Firmicutes at the age of 21 days. These shifts were characterised by increased microbial diversity and species richness. It has been suggested that the increase in species richness indicated that the gastrointestinal tract of the puppies was gaining strength and resistance to environmental pathogens. By the age of 42 days, the microbial communities appeared to have reached relative stability (Guard et al., 2017). To change the microbiota of an adult dog, maintenance on a specified diet is required rather than feeding transitional diets (Allaway et al., 2020).

Dogs and cats have several hundred bacterial phylotypes in their gastrointestinal tract. However, both dogs and cats have distinct bacterial species that differ between each other and vary in different dog and cat breeds and geographical areas as well. The microbial differences between dogs and cats are demonstrated in the microbial groups and species levels. Molecular fingerprinting has also revealed that each individual has a unique intestinal microbiota, and its composition is determined by management, diet, genetics, antibiotic exposure and environmental factors. The composition of the microbiota of dogs and cats also changes along the gastrointestinal tract under the influence of nutrient availability and the local microenvironment (Suchodolski, 2011). Microbial diversity and concentration increase along the length of the gastrointestinal tract (Wernimont et al., 2020). For example, the small intestine harbours a mixture of aerobic and facultative anaerobic bacteria, while the colon is colonised almost exclusively by anaerobes (Donaldson et al., 2016). The predominant bacterial phyla in the duodenum are represented by Protobacteria in both dogs and cats versus Firmicutes, Bacteroidetes, Proteobacteria and Fusobacteria in the colon and faeces of both dogs and cats besides *Eubacterium* in cats (Pilla and Suchodolski, 2020).

A recent study, comparing the gut microbiome of 46 cats and 192 dogs using faecal samples, has found that cats had higher diversity in bacterial genera than dogs. Compared to cats, the bacterial genera *Enterococcus, Fusobacterium, Megamonas* and SMB53 were higher in dogs, whereas several genera were more abundant in cats, including *Adlercreutzia, Alistipes, Bifidobacterium, Carnobacterium, Collinsella, Coprococcus, Desulfovibrio, Faecalibacterium, Oscillospira, Parabacteroides, Peptococcus, Peptostreptococcus, Ruminococcus, Slackia,* and *Sutterella* (Jha et al., 2020). The same was observed in a previous study (Handl et al., 2011). Moreover, the cat faeces contains high numbers of obligate anaerobes, and such levels are considered abnormal in dogs and humans (Johnston et al., 2001). Using 16S rRNA analysis, it has been reported that in the feline colon five bacterial phyla are commonly represented with Firmicutes dominating (68%), followed by Proteobacteria (14%), Bacteroidetes (10%),

Fusobacteria (5%) and Actinobacteria (4%) (Ritchie et al., 2010). However, a recent study, using the metagenomic approach, has shown that the gut microbiota of cats is dominated by bacteria of the Bacteroidetes/Chlorobi group, which comprises about two thirds of the total classified diversity, followed by Firmicutes and Proteobacteria (Tun et al., 2012). Similar findings have also been obtained in a recent study, conducted by Alessandri et al. (2020). This high diversity in cats compared to dogs could be because dogs are more omnivorous than cats and can digest a significant amount of carbohydrates. In contrast, cats are obligate carnivores and require foods high in protein to meet their nutritional needs and consume smaller amounts of glucose (Tizard and Jones, 2018).

There is also evidence that members of the microbiome from other parts of the gastrointestinal tract are important reservoirs for the communities found in the human gut. Members of the oral microbiome colonise lower sections of the gastrointestinal tract. In addition, their presence has been associated with disease (Lira-Junior and Boström, 2018).

Altogether, the gastrointestinal microbiome is now known to have a major impact on pet health, both directly and through its influence on dietary response (Suez and Elinav, 2017).

DEVELOPMENT OF THE IMMUNE RESPONSE

The development of the immune system in newborn puppies and kittens is determined by the organisms that colonise the skin and the gastrointestinal and respiratory tracts. The presence of high numbers of microbes provides a rich source of signals to the immune system (Tizard and Jones, 2018). This early microbial exposure determines how the immune system develops. The microbiota generates a complex mixture of microbially associated molecular patterns that act through enterocytic Toll-like receptors (TLRs) to promote the functional development of the immune system (Gensollen et al., 2016). The gut and skin microbiota also contribute to this process as newborns are nursed and cared for by the dam (Brown and Clarke, 2017). The establishment of the gut microbiota is therefore essential for normal immune system development and can potentially be disrupted by prematurity, different types of parturition, diet and use of antibiotics.

The microbiota also modifies the intestinal environment by maintaining a low pH and oxygen tension. There is more immune system activity in the intestine than in all other lymphoid tissues combined. It has been estimated that more than 80% of the body's activated B cells are found in the intestine. Their function is to defend against possible invasion by the microbiota. However, the key to successful accommodation with the intestinal microbiota also depends on the body's ability to regulate inflammation in the gut wall (Kamada et al., 2015). This is achieved by maintaining a balance between proinflammatory Th17 cells and anti-inflammatory Treg cells (Tizard and Jones, 2018).

In mice, it has been shown that the maternal microbiota shapes the immune system of the offspring. Maternal colonisation reprograms intestinal transcriptional profiles of the offspring, including increased expression of genes encoding epithelial antibacterial peptides and the metabolism of microbial molecules. Some of these effects are dependent on maternal antibodies that potentially retain microbial molecules and transmit them to the offspring during pregnancy and in milk. Pups, born to dams transiently colonised in pregnancy, are better able to avoid inflammatory responses to microbial molecules and the penetration of intestinal microbes (De Agüero et al., 2016).

The development of the immune system is pre-programmed in the tissues of the newborn, and is later driven by the exposure to pathogenic and non-pathogenic microbes. The composition of the gut microbiota also has significant effects on the immune function and regulates the local production of antibodies. Although intestinal microbes are usually separated from direct contact with enterocytes by the inner mucus layer and the glycocalyx, intestinal dendritic cells can extend their dendrites into the intestinal lumen and sample the microbiota (Pilla and Suchodolski, 2020). These invading bacteria are usually killed by macrophages and some are also presented to B cells. The B cells produce IgA that is secreted into the lumen, binds to the bacteria and activates the targeted destruction of the bacteria (Tizard and Jones, 2018). Intestinal helper T (Th) cell progenitors can differentiate into either Treg or Th17 cells, depending on the signals received from the microbiota (Tizard and Jones, 2018). In homeostasis, Treg cell production is favoured and Th17 cell production is suppressed, resulting in minimal inflammation within the intestinal wall. In the absence of Treg cells, uncontrolled effector T cells respond to microbial antigens and trigger inflammation (Tizard and Jones, 2018). This process can affected by gut dysbiosis, a condition defined as changes in the composition of the gut microbiota that result in functional changes in the microbial transcriptome, proteome or metabolome (Zeng et al., 2017). The increase in abundance of facultative anaerobic bacteria of the family Enterobacteriaceae is a common marker of dysbiosis (Rivera-Chavez et al., 2017), which has also been observed in dogs (Vázquez-Baeza et al., 2016). Dysbiosis is seen in many pathologies, both local, within the gastrointestinal tract and systemic (Zapata and Quagliarello, 2015), and is associated with obesity (Collado et al., 2008; Kieler et al., 2017), metabolic diseases (Montoya-Alonso et al., 2017), cancer (Zitvogel et al., 2017), neurological (Wu et al., 2020) and many other disorders, in both humans and dogs. Furthermore, the gut microbiome is altered in both acute and chronic diarrhoea. Dysbiosis should always be considered when pathologies of the gastrointestinal tract are present. However, restoration of the composition of the microbiome does not necessarily correlate with clinical recovery, and the long-term consequences of such residual alterations have yet to be discovered. The identification of bacterial compounds, involved in disease pathogenesis, may aid the development of new diagnostic and therapeutic tools and should therefore be further investigated (Pilla and Suchodolski, 2020).

PERSPECTIVES

The acquisition, colonisation, and maintenance of the microbiota in early life, as well as its subsequent interactions with the host, is a rapidly evolving area of research. Dogs and cats are considered to be the most valued companion animals of humans and concern for their health is very important. Since the gut microbiota plays a crucial role in the development of the immune response and the health of the animal, more attention should be paid to its influence.

While the human microbiome has been studied over the past two decades, limited data are available to assess the development of the faecal microbiota of puppies and kittens during the early stages of life and the true impact of microbial changes on host development and overall health remains unknown. Recent advances in DNA sequencing technology, including 16S rRNA analysis, have improved our understanding of the neonatal microbiome and its impact on health in the early months. Metagenomic studies are great for providing information about the healthy oral, intestinal, vaginal and placental microbiome. However, detection of bacterial DNA from the amnion and meconium by PCR does not necessarily mean that culturable bacteria are present in these fetal samples (Rodrigues et al., 2019). Studies in humans and livestock have shown that prenatal transmission of microbes to the fetus is possible, but there is no direct evidence of bacterial transmission *in utero*. Understanding the timing and mechanisms involved in the initial colonisation of the neonatal gut is therefore critical. Further studies are needed to elucidate the effect of the gut microbiota on the development, physiological growth and morbidity of young animals to gain clinical relevance for diagnostic, predictive and therapeutic options and to achieve better neonatal survival of puppies and kittens.

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