# Role of the microbiome in respiratory disease

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## Abstract

"Microbiome" is a term used to describe the community of microorganisms that live on and within vertebrate hosts. These microorganisms primarily live in the gastrointestinal tract and play an important role in nutrient digestion and development of immunity. Understanding the role of the gut microbiome in health and disease is a new and exciting field of study. For decades, the focus on managing infectious disease has been on the pathogen(s) of interest, virtually ignoring the role of beneficial microbes in potentiating or eliminating disease. In addition, initial research evaluating the role of the microbiome in disease was centered on enteric pathogens, due to the inherent proximity of pathogenic and nonpathogenic microorganisms in the digestive tract. However, recent investigations have revealed the potential role of the microbiome in diseases outside the gastrointestinal tract, such as neurologic, respiratory, and autoimmune diseases. Considering the role of the microbiome in clinical disease and decreased performance associated with respiratory infections, this area of research becomes even more relevant to food animal production. The objective of this paper is to review associations between the gut microbiome and outcome during infectious respiratory disease in animals.

**Key words:** microbiome, respiratory disease, respiratory infection

## Résumé

Le microbiome est un terme utilisé pour décrire la communauté des microorganismes qui vivent tant à la surface qu'au sein d'un hôte vertébré. Ces microorganismes vivent principalement dans le tractus gastro-intestinal et jouent un rôle important dans la digestion des aliments et le développement de l'immunité. Mieux comprendre le rôle que joue le microbiome intestinal dans la santé et la maladie est un nouveau champ de recherche très prometteur. Depuis des décennies, la gestion des maladies infectieuses ciblait le ou les pathogènes d'intérêt mais ignorait presqu'entièrement le rôle que les microbes bénéfiques pouvaient jouer dans la facilitation ou l'éradication de la maladie. De plus, les recherches initiales qui évaluaient le rôle du microbiome dans la maladie étaient axées sur les agents pathogènes entériques en raison de la proximité même des microorganismes pathogéniques et non-pathogéniques dans le tractus digestif. Toutefois, des travaux récents ont mis en lumière le rôle potentiel du microbiome dans des maladies à l'extérieur du tractus gastro-intestinal comme les maladies neurologiques, respiratoires et auto-immunes. Compte tenu du rôle que joue le microbiome dans les maladies cliniques et considérant la réduction de la performance causée par les infections respiratoires, ce domaine de recherche devient encore plus pertinent pour les élevages d'animaux destinés à l'alimentation. L'objectif de cette présentation est de faire la revue des associations entre le microbiome intestinal et l'issue des maladies respiratoires infectieuses chez les animaux.

## Introduction

"Microbiome" is a term used to describe the community of microorganisms that live inside and on the surfaces of mammals. These microorganisms are numerous, widely distributed, and diverse, comprised of viral, bacterial, fungal, and protozoan species. Although less is known about the quantity of the non-bacterial microbiome, a recent publication estimated the ratio of bacterial to host cells in the human body as approximately 1:1.[25] Most of these microorganisms reside within the gastrointestinal tract, where they play an important role in development of the immune system and digestion of certain nutrients. The importance of specific microorganisms in the digestive tract of food animals has long been recognized due to their effects on health and production.[17,27] However, for decades, the focus has been on the relationship between the microflora, diet and digestion, with little to no research on the role of these microorganisms in disease.

The relationship and balance between the microbiome and disease is complex and not well understood. However, there is growing evidence indicating the important role that microbiome diversity and composition play in the regulation, elimination, and potentiation of disease. Over the last decade, a significant increase has been seen in studies evaluating the association between the microbiome and diseases outside the gastrointestinal tract, such as respiratory, neurologic, and autoimmune diseases. In humans, diseases such as autism spectrum disorders, chronic fatigue syndrome, obesity, chronic obstructive pulmonary disease, and Alzheimer's disease have been associated with changes in the gut microbiome.[9,13-15,26] For example, reduced gut microbiome diversity and an increase in bacterial species associated with inflammation were found in patients with chronic fatigue syndrome.[9]

## The Microbiome and Respiratory Disease

There has been an increasing amount of research investigating the intricate relationship and complex communication pathways between the gut microbiota and the respiratory tract.[3] Specifically, the gut microbiome can impact systemic and respiratory immunity through the regulation and differentiation of T cells, producing anti-inflammatory molecules such as short-chain fatty acids, development of innate immunity, and homeostasis of adaptive immunity.[11,21,28] This is important for both humans and food animals, where acute and chronic respiratory diseases are a significant cause of morbidity and mortality.

When considering infectious causes of respiratory disease, several recent publications have demonstrated an association between the gut microbiota and a diverse range of infectious respiratory pathogens (Table 1; adapted from Niederwerder, 2017). In mouse models of human disease, outcome following respiratory infections with influenza virus, respiratory syncytial virus, *Burkholderia thailandensis*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Mycobacterium tuberculosis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Aspergillus fumigatus* all have associations with the enteric microbiome composition. Several of these studies have shown that the presence of normal gut microflora are beneficial for pulmonary infections. For example, the presence of endogenous gastrointestinal microbiota was found to be beneficial in response to *Streptococcus pneumoniae* infection in mice. Compared to mice with depleted gut microbiomes due to antibiotic treatment, mice with healthy and diverse microbiomes had less *S. pneumoniae* present in lung, enhanced alveolar macrophage phagocytosis, lower mortality, and less interstitial pneumonia, mediated, in part, by increases in pulmonary IL-10 and TNF-α.[24]

There are similar studies investigating the relationship between the gut microbiome and infectious respiratory pathogens in animals. For example, Schachtschneider et al evaluated the effects of administering the fecal microbiota from an adult boar to piglets prior to challenge with *Mycoplasma hyopneumoniae* on immunological and pathological outcomes. Fecal microbial transplants were delivered orally for 7 consecutive days, increasing the microbiome diversity compared to non-transplanted littermates. After challenge, transplanted pigs had a more rapid antibody response, decreased gross lung lesions and a significant reduction in coughing. However, there were no differences in cytokine level, bacterial load, or weight gain between the two groups.[22] In a study by Whitfield-Cargile et al, there were no fecal microbiome differences between foals that developed clinical or subclinical Rhodococcal pneumonia compared to foals that remained healthy. In this case, microbiome diversity prior to development of disease was not predictive or associated with outcome.[31]

Our research efforts in this area have focused on evaluating the associations between the gut microbiome and disease outcome in weaned pigs following co-infection with porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2). Worldwide, PRRSV and PCV2 are 2 of the most significant pathogens affecting the swine industry, costing billions in lost production over the last 3 decades. Both viruses result in systemic infections with primary lung pathology and reduce weight gain in growing pigs. We use PRRSV and PCV2 in a co-infection disease model for several reasons, including 1) co-infections are common in the field and simulate porcine respiratory disease complex, 2) co-infections enhance pathogenesis and contribute to a wide range of polymicrobial and multisystemic disease syndromes, and 3) both viruses modulate the immune response, increasing susceptibility to other pathogens.

Two of our previous studies have investigated the associations between the microbiome and outcome following

**Table 1.** Associations between the gut microbiome and outcome in infectious respiratory disease.

| Pathogen | Species | Beneficial microbiome characteristic(s) | Reference |
|---|---|---|---|
| PRRSV and PCV2 | Pig | Increased microbial diversity, *Escherichia coli* | 19, 20 |
| *Mycoplasma hyopneumoniae* | Pig | Fecal microbiota transplant, increased microbial diversity | 22 |
| *Rhodococcus equi* | Horse | None detected | 31 |
| Respiratory syncytial virus | Mice | *Lactobacillus johnsonii* | 7 |
| Influenza virus | Mice | Endogenous microbiota, neomycin-sensitive bacteria | 32 |
| *Burkholderia thailandensis* | Mice | *Escherichia coli* | 23 |
| *Streptococcus pneumoniae* | Mice | Endogenous microflora, fecal microbiota transplant, increased microbial diversity | 24 |
| Methicillin-resistant *Staphylococcus aureus* | Mice | Segmented filamentous bacteria | 8 |
| *Mycobacterium tuberculosis* | Mice | Lack of *Helicobacter hepaticus* | 1 |
| *Klebsiella pneumoniae* | Mice | Endogenous microbiota, Fecal microbiota transplant | 4 |
| *Escherichia coli* | Mice | Endogenous microbiota | 2 |
| *Pseudomonas aeruginosa* | Mice | Endogenous microbiota | 6 |
| *Aspergillus fumigatus* | Mice | Vancomycin-sensitive bacteria, segmented filamentous bacteria | 18 |

Adapted from Niederwerder MC. Role of the microbiome in swine respiratory disease. *Vet Microbiol* 2017; doi: 10.1016/j.vetmic.2017.02.017

co-infection with PRRSV and PCV2.[19,20] Improved outcome characteristics included increased average daily gain, reduced lung lesion severity, decreased gross necropsy lesions, and reduced virus replication detected in serum. At the level of the fecal microbiome, 2 significant microbiome characteristics have been associated with improved outcome, including 1) increased microbiome diversity and 2) increased prevalence of *Escherichia coli*. Taken together, both microbiome diversity and composition may play a role in response to PRRSV/PCV2 co-infection.

With regards to bovine respiratory disease, the majority of studies assessing microbiome associations have focused on the upper respiratory tract microbiome. For example, Holman et al investigated the differences in the nasopharyngeal microbiomes of feedlot cattle that were treated for bovine respiratory disease and those that remained healthy. Differences in composition as well as increased microbiome diversity and richness were detected in cattle that remained healthy throughout the feeding period.[10] Another study by Lima et al investigated the pharyngeal microbiomes in the early lives of healthy calves compared to those later diagnosed with pneumonia. At 3 days of age, the total bacterial load detected in swabs was significantly higher in those calves who went on to develop pneumonia.[16] Although these 2 studies seem to be conflicting, age plays a major role in microbiome development and likely contributes to the differences in study conclusions.

### Changing the Microbiome

As pneumonia continues to be a leading cause of morbidities and mortalities in humans and food animals, understanding the factors that shape the microbiome in early life are essential to developing strategies to increase beneficial populations. Summarized in Figure 1, there are numerous factors that impact the colonization of the microbiome in early life. Birth route, diet, environment, genetics, stress, vaccination, medication, and pathogen exposure all play roles in shaping the microbiome in the early lives of humans and animals. One of the earliest factors is birth route, due to initial exposure to the dam's vaginal or skin microflora during C-sections.[29,30] Another important factor is the administration of antibiotics, both injectable and in-feed. To what extent the microbiome changes after antibiotic therapy depends on many factors, including dose, duration, resistance, single drug or combination, and mode of action.[5] However, even a single injection of antibiotic may have long-lasting effects on the microbiome. For example, a one-time amoxicillin injection administered to swine on the day after birth resulted in a significant reduction in microbiome diversity 5 weeks after administration.[12]

### Conclusions

The role of the microbiome in respiratory disease is a developing area of study, with only 888 publications returned
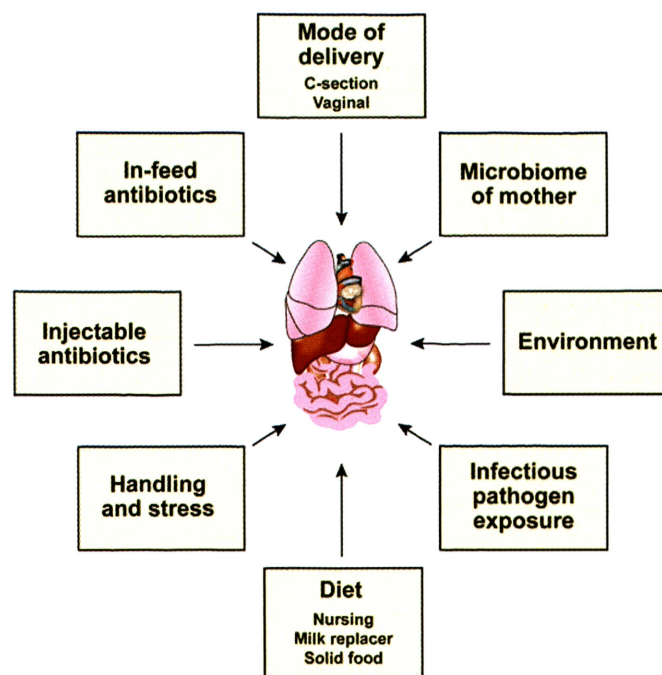


**Figure 1.** Factors affecting microbiome colonization in early life. Adapted from Niederwerder MC. Role of the microbiome in swine respiratory disease. *Vet Microbiol* 2017; doi: 10.1016/j.vetmic.2017.02.017.

in a PubMed search for "microbiome and respiratory" (May 17, 2017). The veterinary literature is even more limited, with 19 publications for "microbiome and respiratory and bovine" and 15 publications for "microbiome and respiratory and porcine." In addition, most microbiome studies to date are focused on the association between the microbiome and disease outcome; the underlying mechanisms by which the microbiota impact respiratory disease for the most part remains unclear. However, the microbiome will likely continue to be an exciting and emerging area of study and provides an alternative approach for reducing the effects of respiratory disease and improving weight gain in food animal production.

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