1 Einleitung

Entzündungen der Nasennebenhöhlen sind bei Pferden häufig beschriebene Erkrankungen. Sie werden meist erst im subakuten bis chronischen Krankheitsstadium diagnostiziert, da das Leitsymptom des einseitigen Nasenausflusses aufgrund der komplexen anatomischen Struktur der equinen Nasennebenhöhlen in der Regel erst bei fortschreitender Erkrankungsdauer auftritt (WAGUESPACK u. TAINTOR 2011; DIXON et al. 2012). Eine gesicherte Diagnose über die zugrundeliegende Ätiologie der Sinusitis erfordert eine aufwendige Kombination mehrerer bildgebender Verfahren (BEARD u. HARDY 2001).

Die wichtigsten diagnostischen Verfahren stellen derzeit, neben der klinischen Allgemeinuntersuchung und der speziellen Untersuchung des Kopfes, die transnasale Endoskopie der Nasenhöhle, der Nasennebenhöhlen sowie röntgenologische Untersuchungen des Kopfes dar. Die invasive Sinuskopie des Sinus conchofrontalis oder Sinus maxillaris rostralis beziehungsweise caudalis bietet nicht nur die Möglichkeit des direkten Einblicks in verschiedene Sinuskompartimente. Über die Trepanationsöffnungen können gegebenenfalls auch direkt Sekretproben oder Biopsien entnommen werden sowie therapeutische Maßnahmen erfolgen (RUGGLES et al. 1993). Darüber hinaus kommt der computertomographischen (CT) Untersuchung der Nasennebenhöhlen eine wesentliche diagnostische Bedeutung zu (HENNINGER et al. 2003), die nicht zuletzt die Wahl des chirurgischen Zugangs und der weiteren Therapie entscheidend erleichtern kann. Weitere am Pferdeschädel eingesetzte bildgebende Verfahren stellen die Szintigraphie und die Magnetresonanztomographie dar, die sich gegenüber der CT Untersuchung allerdings als weniger aussagekräftig herausgestellt haben (BARAKZAI et al. 2006; KAMINSKY et al. 2016).

Zytologische und bakteriologische Untersuchungen der Sekrete aus den Nasennebenhöhlen wurden zuvor nur sporadisch beschrieben und ihre ätiologische Relevanz blieb bislang ungeklärt (TREMAINE et al. 1999; COWELL u. TYLER 2001; TREMAINE u. FREEMAN 2007).

Dagegen liefert die zytologische Analyse des transendoskopisch gewonnenen Tracheobronchialsekrets bei Erkrankungen der tiefen Atemwege wichtige Aussagen über Art und Schwere der Erkrankung und dient darüber hinaus auch der prognostischen Einschätzung (DERKSEN et al. 1989; MAY u. GEHLEN 2009).

Vor diesem Hintergrund werden im Rahmen der eigenen Untersuchungen Sekretproben sowohl transendoskopisch als auch direkt aus den Sinus paranasales von gesunden und kranken Pferden entnommen, um diese zytologisch und bakteriologisch zu untersuchen. Ziel hierbei ist es zunächst herauszufinden, wie sich das physiologische Zellbild und die Keimflora des Pferdes in den Nasennebenhöhlen darstellen, um in einem zweiten Schritt festzustellen, ob sich aus den bei erkrankten Pferden erhobenen Befunden retrospektiv Hinweise auf die zugrundeliegende Erkrankung ableiten lassen. In der abschließenden Diskussion wird der Frage nachgegangen, ob und welchen diagnostischen Nutzen diese Untersuchungen bei der Evaluation von Patienten mit Erkrankungen der Nasennebenhöhlen haben können.

2 Literaturübersicht

2.1 Publikation I

Cytologic and microbiological examination of secretions from the paranasal sinuses in horses and other species

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Abstract

Only sparse literature has been published on the microbiological and cytological examination of secretions from the paranasal sinuses in horses, and the data that is available consists mainly of results in single animals or small case series. Contrarily, the role of bacteria in sinusitis in humans has been the topic of several studies and reviews.
We aim with this review to summarize the current knowledge on the characteristics and microbiological and cytologic composition of paranasal sinus secretions in equids in comparison to humans and selected domestic animals.

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Review Article

Cytologic and Microbiological Examination of Secretions From the Paranasal Sinuses in Horses and Other Species

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Only sparse literature has been published on the microbiological and cytologic examination of secretions from the paranasal sinuses in horses, and the data that are available consist mainly of results in single animals or small case series. Contrarily, the role of bacteria in sinusitis in humans has been the topic of several studies and reviews. We aim with this review to summarize the current knowledge on the characteristics and microbiological and cytologic composition of paranasal sinus secretions in equids in comparison to humans and selected domestic animals.

1. Introduction

Sinusitis is a common disorder in horses with different underlying etiologies [1,2]. Horses are mostly presented in a chronic stage of the disease [3], and an exact etiologic diagnosis may only be achieved by a combination of different diagnostic imaging tools.

There have been quite a lot of retrospective studies, case series, and reviews in recent years regarding historical and clinical features, ancillary diagnostic techniques [1–6], medical and surgical therapy, and long-term results of treatment in horses with sinusitis [7,8]. However, the role of the cytology and microbiology of secretions associated with sinusitis in horses has only been sporadically reported [9–13] or was of unknown importance [11,14].

The purpose of this report is to review pathophysiologic conditions in sinusitis and to summarize findings regarding the cytology and microbiology of secretions to evaluate their diagnostic value.

2. Anatomic and Pathophysiologic Considerations in Sinusitis

2.1. Pathophysiology of Sinusitis

The paranasal sinuses of the horse are air-filled cavities that extent bilaterally within both sides of the skull. Theories on their physiological function include lightening of the skull, humidification, and warming of the inspired air as well as moistening of the nasal olfactory mucosa. All these theories are deniable to some extent so that their precise role remains uncertain [2,15]. Sinusitis is a state of inflammation of the paranasal sinus mucosa [16]. The basic pathophysiological mechanism in the development of sinusitis is the ineffectiveness of mucociliary clearance. The role of mucociliary clearance is to remove physiological and pathologic secretions from the airway and is, therefore, considered to be the primary defense mechanism of the paranasal sinuses [17]. The paranasal sinuses are covered

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by respiratory pseudostratified, columnar, ciliated epithelium, and the latter contains fewer goblet cells and submucosal seromucous glands than the epithelium in the nasal cavity [14]. These glands generate the mucus layer that covers the epithelium. The cilia create a mucus movement to the natural ostia [18].

The beating cilia and specific physical characteristics of the overlying mucus determine the clearance of the physiological and pathologic substances in the mucus. The continuous and coordinated beat of the respiratory cilia is modulated dynamically and can increase to accelerate mucus clearance in times of stress, such as exercise, infection, or fever [17].

The restoration of natural sinus physiology became the goal of medical and surgical treatment of sinonasal inflammatory disease in humans from the time Messerklinger described the sinonasal mucociliary flow patterns in 1966 [17]. He determined that the transport of secretions from the paranasal sinuses is dependent on five factors: (1) The excretory pathways; (2) the secretion; (3) the features of the secretion; (4) the cilia beat; and (5) the resorption [19].

If inflammation occurs, dilation of the venous sinusoids in horses can lead to edematous swelling of the sinus mucosa of up to 15 mm [14] (Fig. 1). Consequent narrowing of the ostia between the sinuses and especially the nasomaxillary aperture can obstruct the drainage pathways completely. This contributes to an intrasinusoidal accumulation of secretions, therefore, impairing local mucosal defense mechanisms further and promoting progression of underlying disease [14,17,18,20]. These conditions predispose for a bacterial infection [14]. The initial seromucous secretion becomes more viscous with the increase in inflammatory cell extravasation. Inflammatory products that cannot be eliminated tend to accumulate, dry out, and promote a chronic inflammatory state [16].

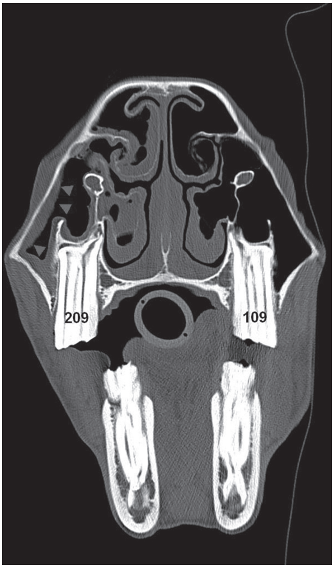

Fig. 1. Computerized tomography scan of a horse with primary sinusitis. Note, the swollen mucosa of the diseased maxillary sinus (red arrowheads, left) compared to the nonaffected side (right). The horse is positioned in right lateral recumbency.

2.2. Sinusitis in Horses

The equine paranasal sinuses represent an anatomically complex, yet well-described system [3,21–27]. The dependency for drainage of secretions of the adjacent paranasal sinuses on the rostral and caudal maxillary sinus [4,25], in addition to the physiologically very narrow nasomaxillary aperture [22], predisposes horses to develop paranasal sinusitis with inspissation of secretions [4,28–30]. In a prolonged clinical course of disease, the inspissated pus can have a caseous character that is only rarely resolved without surgical intervention [28–30].

The most common clinical feature of paranasal sinusitis in horses is unilateral nasal discharge [1,5,31,32] (Fig. 2), followed by ipsilateral mandibular lymphadenitis. However, complete obstruction of the drainage pathways can result in absence of characteristic nasal discharge [6]. Etiologically, paranasal sinus diseases can be classified as primary, with a viral, bacterial, or mycotic origin, or secondary to dental diseases, sinus cysts, neoplasia, and trauma [1,2].

The character of the nasal discharge in horses with primary and dental sinusitis is usually mucopurulent or purulent [1]. In primary sinusitis, it is usually odorless [9,32], but can sometimes be malodourous [6,32]. Schumacher et al [28] found foul-smelling unilateral nasal discharge in five horses with primary sinusitis that was associated with inspissated purulent exudate in the ventral conchal sinus. Most horses with dental sinusitis show malodorous nasal discharge [4,5,12,32,33]. The fetid smell usually suggests the involvement of obligate anaerobic bacteria, which is ascribable to the production of butyric acid in the metabolism of anaerobes [34].

A translucent, honey-colored, odorless, viscous nasal discharge can indicate a sinus cyst (Fig. 2B), which can become more purulent with the occurrence of secondary infection [5,6].

Hemorrhagic nasal discharge or epistaxis is more common with traumatic sinusitis and is typical for progressive ethmoidal hematoma (PEH) [5,6]. The discharge is usually odorless.

It is important to note that unilateral nasal discharge does not necessarily originate from the paranasal sinuses. The nares, the nasal cavity, the guttural pouches, and the lungs display anatomic locations which have to be taken into consideration when a suspected horse is presented [16,35–37].

2.3. Sinusitis in Humans

Most infections of the paranasal sinuses in human medicine are rhinogenic, spreading from the nose into the

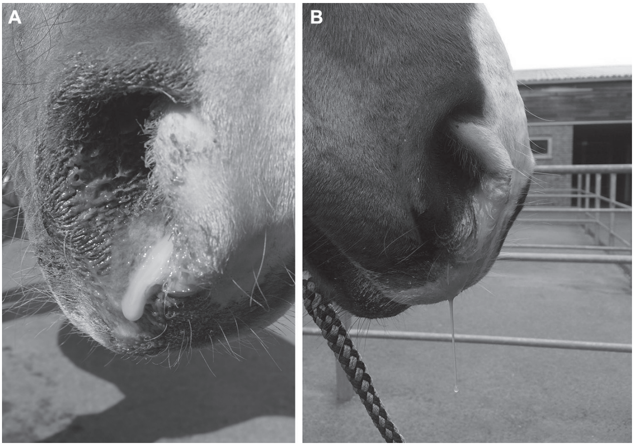

Fig. 2. Unilateral nasal discharge: purulent in a horse with dental sinusitis (A) and translucent, honey colored in a horse with a sinus cyst (B).

sinuses [20]. In contrast to the horse, a sinusitis in humans rarely occurs alone but is mostly associated with inflammation of the adjacent nasal mucosa [38]. Therefore, the term rhinosinusitis replaced the term sinusitis in many expert panels [38–41]. Acute rhinosinusitis (ARS) is usually characterized as a viral upper respiratory tract infection with typical symptoms, such as nasal blockage, discharge, facial pressure, and reduction in the ability to smell [42] lasting 4 weeks or less [38,40,43]. The term chronic rhinosinusitis (CRS) is used by most guidelines if symptoms last 12 weeks or longer [38–40,42]. The estimated incidence of ARS is two to five episodes per year in an average adult [42]. Rhinosinusitis is highly prevalent and constitutes an enormous economic burden [44,45].

Only 0.5%–2% of ARS cases are thought to be complicated by a secondary bacterial infection [39], making proper identification of cases with bacterial etiology one of the primary challenges in the treatment of ARS [44]. It is estimated in the pediatric population that secondary bacterial infection occurs in 5%–13% of viral upper respiratory tract infections [46]. Neither nasal mucus color nor the presence of fever is useful to differentiate bacterial from viral etiology [38,43].

About 10% of sinusitis cases have an odontogenic origin [47], including endodontic infections, periapical pathologies, and iatrogenic complications [48]. In fact, the majority of these cases (up to 64%) develop secondary to dentoalveolar surgical interventions with orosinuidal fistula formation [49], often associated with dislocated foreign bodies in the maxillary sinus [50].

2.4. Sinusitis in Other Domestic Animals

Comparable to humans, rhinosinusitis in cats and dogs occurs more frequently than an isolated inflammation of the parasinusoidal mucosa. The most common cause of chronic mucoid to hemorrhagic nasal discharge in dogs is sinonasal aspergillosis with Aspergillus fumigatus being the species most frequently encountered [51]. The most common causes of chronic nasal disease in cats are idiopathic CRS and neoplasia [52].

The same etiologies for paranasal sinusitis are described in cattle as those in horses [53–56], although they are reported relatively infrequently. Frontal sinusitis often occurs secondary to dehorning [55].

3. Access to the Paranasal Sinuses and Specimen Collection

An upper airway endoscopy is the second diagnostic step after the physical examination if sinusitis is suspected [5]. It is easy to perform in restrained or sedated horses, displays a noninvasive procedure, and is of high diagnostic value in most cases. The anatomic structures of the nasal cavity and the nasomaxillary aperture are usually easily identified. The typical endoscopic appearance of horses affected by sinusitis is discharge from the caudal or rostral maxillary sinus down the drainage angle between the ventral and dorsal nasal concha at the end of the middle nasal meatus via the nasomaxillary aperture (Fig. 3). Transnasal access to the paranasal sinuses is also sometimes possible using a flexible endoscope with a diameter <5 mm, but this requires more experience and is seldom possible for the rostral paranasal sinuses [25,57].

Specific endoscopic diagnosis is aggravated if severe narrowing of the nasal passages restricts proper advancement and positioning of the endoscope due to expansion of the nasal conchal sinuses [5]. The nasomaxillary aperture can be blocked completely by mucosal inflammation,

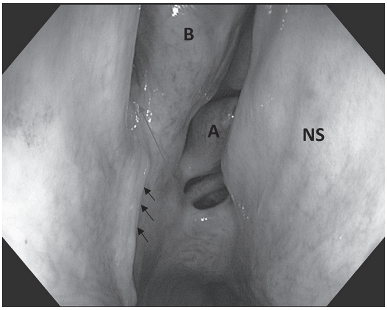

Fig. 3. Endoscopic image the "drainage angle" (red arrow) with purulent, malodorous discharge (black arrows) in a horse with squamous cell carcinoma of the right sinus system. A—middle nasal concha; B—dorsal nasal concha; NS— nasal septum.

resulting in the retention of secretions and absence of characteristic discharge.

Trephination of the paranasal sinuses allows direct access to gain samples of secretions for further evaluation but is obviously a more invasive procedure. It is usually performed with a therapeutic intention but may also have a diagnostic value when a sinuscopy is performed. Although the surgical and cosmetic outcome is most commonly satisfying, opening of the paranasal sinuses holds the risk of wound infection and delayed secondary healing [58].

Anterior rhinoscopy is the first step in examining humans affected by rhinosinusitis, albeit of limited value. Better visualization is achieved by nasal endoscopy [42]. Maxillary sinus puncture has been the traditional gold standard in human medicine to assess the microbial flora associated with bacterial rhinosinusitis [59]. Pursuing alternative harvesting methods with less patient discomfort and minimized risks, nasal cultures, and endoscopically guided middle meatal (EDMM) cultures have been compared to maxillary sinus taps. However, nasal cultures have poor correlation to maxillary sinus cultures [60]. By contrast, studies have shown that there is a satisfying concordance between EDMM cultures and maxillary sinus cultures [60–63], but their validity is limited by small sample sizes. Nevertheless, Benninger et al [64] concluded EDMM cultures to be a feasible, highly sensitive, and accurate culture method for bacterial rhinosinusitis in humans.

4. Microbiology of Secretions From the Paranasal Sinuses

4.1. Microbiology of Secretions From the Paranasal Sinuses in Horses

Despite the paucity of studies focusing on the role of bacteria in equine paranasal sinus diseases, there are some studies and case reports where cultures of exudates from sinusitis have been examined for bacterial growth (Table 1). A wide variety of potential bacterial pathogens was reported to be found on sinusitis mucosae and their etiologic importance often remained unclear [6,14,33].

Anaerobes seem to play an important role in sinusitis with dental origin. They have been found to be part of the normal flora of the respiratory tract and the oral cavity [10,65,66]. The close relationship of the caudal cheek teeth to the maxillary sinuses makes the latter prone to become secondarily infected by dental diseases [66]. These diseases often reveal mixed bacterial growth, with Gram-negative obligatory anaerobes being the predominant group differentiated, with *Prevotella* spp., *Fusobacterium* ssp., and *Bacteroides* ssp. being isolated most frequently [12,67,68]. *Prevotella* and *Veillonella* were the dominating genera in a study on the microbiome associated with equine periodontitis [66]. *Streptococcus* spp. are the aerobic organisms most commonly associated with periodontal diseases [67,68].

Only one publication reports the presence of anaerobes in a case of primary sinusitis [28]. Most of them report mixed aerobic bacteria or pure cultures of *Streptococcus* spp. [5,6,13].

Equine sinus cysts are described as being most commonly bacteriologically sterile [5,14], but secondary bacterial infection may occur [6]. Cysts in horses show similarities to human mucoceles [69].

Table 1
Microbiological findings in horses diagnosed with sinusitis.

Diagnosis	Aerobic and Facultative Bacteria		Anaerobic Bacteria
	Gram Positive	Gram Negative	
Dental sinusitis	*Staphylococcus aureus* [9] β-hemolytic *Streptococci* [9] *Actinomyces* spp. [12]	*Escherichia coli* [9] *Diplococcus mucosus* [9]	*Bacteroides* spp. [10] *Fusobacterium* spp. [10,12] *Peptostreptococcus* spp. [10] *Prevotella* spp. [12]
Primary sinusitis	*Streptococcus* equi var. equi [6,13] Strep. equi var. zooepidemicus [6,27] *Staphylococcus* spp. [27] *Corynebacterium* spp. [28]	Mixed growth [27]	*Bacteroides melaninogenicus* [27]
Sinusitis (nonspecific)	*S. aureus* [9] β-hemolytic *Streptococci* [9,11]	*E. coli* [11] *Pseudomonas* spp. [9,11]	*Bacteroides* sp. [11] *Peptostreptococcus* spp. [11]

An etiologic diagnosis could not be made for all cases.
Tremaine and Dixon (2001) [6]; Mason (1975) [9]; Mackintosh and Colles (1987) [10]; Ruggles et al (1993) [11]; Bartmann et al (2002) [12]; Laverty and Pascoe (1997) [13]; Schumacher et al (1987) [28]; Schumacher and Crossland (1994) [29].

Mycotic pathogens may sometimes be responsible for sinusitis in horses. Aspergillus fumigatus has been described as a common cause of mycotic rhinitis in Great Britain and was also found as a frequent cause of sinonasal mycosis, but heavy growth of other potentially pathogenic fungi is also relevant [6]. Aetiopathogenetically, *Cryptococcus neoformans*, *Coccidiodes immitis*, *Allescheria boydii*, and *Pseudallescheria boydii* have been reported to sporadically cause granulomas in the equine nasal passages and paranasal sinuses [70–74], but these have a more distinct geographical distribution in warmer climates, such as Australia and North America [14]. Transient mycotic infections can occur after sinus surgery [6,14] and after long-term antibiotic administration.

4.2. Microbiology of Secretions From the Paranasal Sinuses in Humans

Bacteria isolated from both healthy and diseased paranasal sinuses have been widely studied in human medicine. Although the most common pathogens for acute and chronic bacterial sinusitis seem to have been identified, there is still some debate about the physiological flora of the paranasal sinuses. Brook [75] reported primarily anaerobic bacteria from normal maxillary sinuses and Jiang et al [76] concluded endoscopically normal maxillary sinuses not to be sterile, whereas other authors described the maxillary or frontal sinuses to be mainly sterile, respectively [77–79]. Kremer et al reported coagulase-negative staphylococci in almost all their samples taken from healthy individuals and found *Staphylococcus aureus* being present in up to one-third of them. However, based on their bacteriological findings, no differentiation could be made between the control group and patients with chronic sinusitis [80].

The predominant organisms found in acute sinusitis are Haemophilus influenzae, *Streptococcus pneumoniae*, and *Moraxella catarrhalis* [64,81–84]. Pure cultures of one pathogen were found in up to 56% of positive samples [82]. Bacteria that were identified less frequently include *S. aureus* and other *Staphylococci*, *Streptococci*, and *Pseudomonae* as well as Enterobacteriaceae [81–84]. *Staphylococcus aureus* and some other species are rather seen as contaminants from the nasal cavity [82]. Obligatory anaerobe bacteria, including *Bacteroides* spp., *Peptostreptococcus* ssp., and *Fusobacterium* ssp., were infrequently isolated in samples, with their occurrence ranging between 0% and 30% [81,83,84].

A greater variation of bacteriological cultures exists in chronic sinusitis [60,81]. Although the organisms found are similar to those in acute sinusitis, the prevalence and quantity when detecting them are significantly higher as well as the occurrence of polymicrobial cultures [81].

Table 2 summarizes some of the bacteria identified more frequently found in humans with chronic sinusitis. These studies differ in their sample collection methods and display conflicting results, making it very difficult to compare the results of various authors. Furthermore, the quantity of bacterial growth was not consistently described.

Mantovani et al [85] collected aspirates from the maxillary sinus punctures in patients with CRS. No bacterial growth was found in 53.2% of the samples, and no anaerobes were detected at all, whereas 45.2% revealed aerobic bacteria and one sample was positive for *C. neoformans*. *Pseudomonas aeruginosa* was identified as the most frequently occurring (27.6% of positive cultures), followed by other Gram-negative bacteria (31%) and *Staphylococcus* spp. (27.6%), and the authors concluded that they represented the bacterial flora found in CRS patients. These results are similar to those from other studies, which also found *P. aeruginosa* and *S. aureus* being cultured most frequently in patients with CRS [86]. *Pseudomonas aeruginosa* was identified more commonly in patients who had undergone functional endoscopic sinus surgery [87].

Table 2
Selection of microbiological findings described frequently in humans diagnosed with chronic sinusitis [68,77–82].

Aerobic and Facultative Bacteria		Anaerobic Bacteria
Gram Positive	Gram Negative	
Staphylococcus aureus	*Pseudomonas aeruginosa*	*Bacteroides* ssp.
Coagulase-negative *Staphylococcus* ssp.	*Enterobacteriaceae*	*Fusobacterium* ssp.
Streptococcus spp.		*Peptostreptococcus* ssp. *Veillonella* spp. *Propionibacterium* spp.

Rombaux et al [88] also found *Staphylococcus* ssp. and Gram-negative aerobes in more than 50% of their cases. Enterobacteriaceae had a high incidence in positive cultures (43.7%), underlining their importance in chronic sinusitis. Mantovani et al [85] regarded Enterobacteriaceae as pathogenic agents with a secondary role.

Microbiological detection of anaerobes varies widely between studies. Doyle and Woodham [89] found no anaerobes when they cultured biopsies from inflamed ethmoid sinuses, whereas Brook [90] detected anaerobes in 88% of the samples from chronically inflamed maxillary sinuses, which were dominated by anaerobic cocci and *Bacteroides* spp. Other studies found anaerobes in 2% and 14% of positive cultures, respectively, including *Bacteroides* spp., *Peptostreptococcus* spp., *Veillonella* spp., *Fusobacterium* spp., and *Propionibacterium* spp. [88,91]. Maxillary sinusitis associated with an odontogenic origin is generally polymicrobial [92], and it is predominated by anaerobic bacteria, including Gram-negative bacilli, *Peptostreptococcus* spp., and *Fusobacterium* spp. in both acute and chronic stages [93].

4.3. Microbiology of Secretions From the Paranasal Sinuses in Other Domestic Animals

Nasal swabs from healthy dogs were dominated by *Moraxella* spp., *Phyllobacterium* spp., *Cardiobacterium* spp., and *Staphylococcus* spp. In diseased dogs, *Moraxella* spp. were significantly less frequently identified, whereas Pasteurellaceae were found significantly more often [94].

Pseudomonas spp. were most commonly found in samples collected from the frontal sinus during surgery in cats with chronic nasal discharge [95] and from nasal brushings in cats with nonspecific chronic nasal disease [96]. Other bacteria cultured included *Escherichia coli*, *Staphylococcus* spp., *Streptococcus* spp., *Pasteurella* spp., *Serratia* spp., *Klebsiella* spp., and *Proteus* spp [96]. Potentially pathogenic bacteria, anaerobic bacteria, and *Mycoplasma* spp. are isolated more often in cats with idiopathic CRS, and the variety of bacterial species found is higher than in healthy cats [97]. Another study revealed *Moraxella* spp. being cultivated most frequently in healthy cats and individuals affected by cat flu. Bradyrhizobiaceae dominated the bacterial microbiome of cats with nasal neoplasia [98].

Bovine paranasal sinuses of clinically healthy individuals were predominantly sterile or did not contain microbes that are detectable with standard cultivating methods. Cultivation of swab samples taken from different sinuses after trephination sporadically revealed *Mannheimia haemolytica*, *Trueperella pyogenes*, *Pasteurella multocida*, and *Bibersteinia trehalosi*, among others [99]. *T. pyogenes* and *P. multocida* were detected in cases of chronic frontal sinusitis [55].

4.4. Assessment of the Microbiome

Studies have shown that many bacteria cannot be cultured using conventional methods due to nutritional and fastidious growth requirements [100]. It has become possible to detect almost the entire community of commensal and pathogenic bacteria of individuals and populations culture independently by using broad platform molecular techniques. This universe of cultivable and uncultivable microorganisms present in a specific ecologic niche, such as the sinus mucosa, is called the microbiome. The assessment of the microbiome in human medicine is already highly prevalent, and sequencing of the bacterial 16S ribosomal RNA has become the standard for identification and phylogenetic classification [101]. The advantages include far greater coverage, accuracy, and sensitivity compared to conventional cultivating methods, allowing a better understanding of host-microbial relations. Wilson and Hamilos [102] summarized the current knowledge on the nasal and sinus microbiome in human CRS and healthy controls. In their review, they highlight the increased burden of certain organisms (e.g., *S. aureus*) associated with CRS. The total bacterial burden in CRS patients is described as similar to healthy individuals. They also refer to studies that revealed anaerobic bacteria to be the most prevalent organisms in CRS patients using pyrosequencing [100,103] and express doubts regarding the validity of earlier culture-based studies that reported a low prevalence of anaerobes in CRS samples.

5. Histological Features of Biopsies From the Paranasal Sinuses in Horses

There are no studies that focus on the cytologic examination of secretions that originate directly from the equine paranasal sinuses.

A large number of neutrophils were found in the nasal secretions from 30 horses with infectious respiratory diseases, especially in those that were in the chronic stage of the disease, whereas eosinophils are the dominant cell population in allergic diseases [104].

Tremaine et al [14] examined biopsies from 79 horses with sinusitis histologically and found mostly chronic inflammatory changes, including mucosal thickening, loss of epithelial integrity, inflammatory cell infiltration, and significant fibroplasia in the sinus mucosa. The mucosal histological features of dental sinusitis were similar to those observed in equine chronic primary sinusitis, including mild-to-moderate infiltration of macrophages and predominantly moderate-to-severe infiltration of neutrophils. Overall, there was no significant correlation between the density of the cellular infiltrate and the severity of the disease [14,105].

Sinus cysts walls showed a mild, chronic, predominantly mononuclear, inflammatory infiltrate and, therefore, indicate a continuous low-grade inflammation [106,107]. Cyst aspirates contained 52%–99% neutrophils [14].

Histological examinations of PEHs often revealed ulcerated stratified squamous epithelium that showed a varying degree of fibroplasia with a localized underlying inflammatory infiltrate of plasma cells, lymphocytes and, less commonly, neutrophils. Numerous siderophages, a few eosinophils and numerous multinucleate giant cells, were sometimes present [14].

6. Cytology of Secretion From the Paranasal Sinuses in Humans and Domestic Animals

Studies in human medicine comparing the results of nasal cytology with those of sinus radiography had good sensitivity in predicting sinus disease using neutrophils counts [108,109]. However, these studies lack the comparison of nasal cytology to sinus aspiration [110].

Araujo et al [91] compared middle meatus secretions collected endoscopically in patients with CRS and healthy individuals and concluded that the microbiology of the middle meatus is similar in both groups. They pointed out the importance of the number of leucocytes to distinguish an infective from a saprophytic organism, as they found only rare or no leucocytes in the samples of the control group compared to numerous leucocytes in the patient group.

Bhattacharyya examined the cytology and microbiology of persistent paranasal sinus secretion in patients who had undergone functional endoscopic sinus surgery and in a control group. The control group demonstrated a higher amount of detached epithelial cells, whereas the diseased group had higher scores of neutrophils, but these results did not reach statistical significance. There was a statistically significant increase in eosinophilic cells in the absence of positive bacterial culture in the diseased group, whereas the number of neutrophils was found to be more distinct when bacteria were present [111].

Gupta et al [112] concluded fine-needle aspiration cytology to be a reliable diagnostic tool in numerous inflammatory and neoplastic sinus conditions, but it did not lead to a diagnosis in 12.6%.

Overall, it is concluded in the *European position paper on rhinosinusitis* that cytology has not been proven to be an adequate tool in the diagnosis of rhinosinusitis but that a biopsy might be indicated to exclude severe and malign conditions such as vasculitides and neoplasia [42].

Nasal mucosal cytology in cats and dogs has been shown to have poor sensitivity and specificity compared to histology in nasal mucosal samples and is often unreliable for detecting chronic changes but may be used to identify fungal or neoplastic lesions in selected cases [96,113].

7. Discussion

Paranasal sinusitis in horses is a well-described condition. So far, only a little or no diagnostic focus was put on the role of the microbiology and cytology of secretions of the paranasal sinuses. There are some case reports, but no comparative studies are published. The clinical significance often remained unclear in those cases reporting microbiological or cytologic findings. Consequently, the diagnostic value is either unknown, limited, or underestimated.

Several studies have been published on the role of microbiological findings in human medicine and some agree with findings in veterinary medicine. To date, there is no consensus on the physiological flora of the paranasal sinuses in humans, and it has not yet been assessed for equids. The spectrum of bacteria in equine primary sinusitis and human ARS include a comparably small number of pathogens differentiated, with some reports of pure cultures. *Streptococcus* species are frequently encountered in equids and humans. Although the main infective agents in ARS have been isolated, there is still no consensus on the bacterial flora in CRS as results vary widely between studies. Nevertheless, there is a tendency in humans and small animals toward a more diverse microbial profile in chronic diseases with higher incidences of *Enterobacteriaceae* and *Pseudomonas*. It seems likely that microbial examinations of horses with sinusitis will show similar results. One should also consider that horses affected by sinusitis are often presented to the referral veterinarian in a chronic stage of the disease and have usually already received antibiotics previously without permanent resolution of the symptoms [1]. These facts are likely to cause a shift in the microbiological composition and complicate the diagnosis and appropriate treatment.

Sinusitis with an odontogenic origin plays an important role in equine medicine but has a smaller incidence in humans, where it often occurs secondarily to dentoalveolar surgical interventions. However, there is little doubt that Gram-negative obligatory anaerobes dominate the bacterial spectrum in dental sinusitis in humans and have also been described in horses. Otherwise, their occurrence has only sporadically been described for equine sinusitis, other than when associated with a dental disease. The cultivation of anaerobes is more delicate, expensive, and labor intensive and might, therefore, be used less frequently in general practice and is possibly more prone to false-negative results due to fastidious growth requirements.

The range of detecting anaerobes in both healthy and diseased human individuals is enormous. Harvesting techniques vary between studies making direct comparisons, especially of microbial findings, difficult. Direct sinus punctures and protected swab insertion and sampling have only a small risk of contamination. However, it is a minimal invasive method and holds the general risks of complications. Comparative studies in human medicine conclude that endoscopically guided aspiration has a higher contamination rate by the endoscope itself due to insufficient cleaning or during nasal passage or unclean handling of the utensils, but the findings did not reach statistical significance [86]. Moreover, studies have shown good concordance between maxillary sinus puncture and EDMM cultures [60,64]. This approach could lead to the use of endoscopically guided transnasal culture methods in horses, avoiding opening of the paranasal sinuses before there is a surgical necessity. Nevertheless, it is essential to note that the assessment and appropriate treatment of the bacteria involved often does not solve the underlying disorder, as many cases of equine sinusitis are secondary to other diseases [6]. Therefore, identification of the bacteria may be used additionally to accurate clinical diagnostics and shall not delay the treatment of the underlying condition.

As previously described, assessment of the microbiome provides greater coverage, accuracy, and sensitivity than conventional cultivating methods [102]. Findings in human medicine indicate that alterations in the microbial community are more likely to be representative for a disease than infections with individual pathogens [100,103]. Notwithstanding this, culture-independent investigations have not yet been securely established in veterinary medicine. They have been used to determine the nasal microbiome in healthy and diseased cats and dogs [94,98] and to determine the microbiome associated with equine periodontitis and oral health [66]. To date, this approach has not been utilized to assess the nasal or paranasal microbiome in horses. Although the technologies are available, the methodical and financial effort is currently likely to exceed the clinical advantage.

It is concluded that the diagnostic value of cytologic examination of secretions from the paranasal sinuses and the nasal cavity is very limited in both human and small animal medicine. Nevertheless, some authors suggest that sample collection for cytologic examination in horses affected from sinusitis [5,114], but they do not comment on their interpretation. There are examples where analysis of secretions in airway diseases has been proven to be a useful tool. Cytologic analysis of tracheobronchial secretion and bronchoalveolar lavage fluid in horses, for instance, helps to confirm a suspected diagnosis by giving further information on character and severity of pulmonary diseases [115,116]. Bronchoalveolar lavage represents a sensitive method to diagnose diffuse diseases, such as chronic obstructive bronchitis and interstitial pneumopathies. Tracheobronchial secretion analysis is useful for generalized diseases by giving insight into the conditions of the respiratory epithelium and the effectiveness of mucociliary clearance [115]. These findings prompt one to suggest whether cytologic examination of paranasal sinus secretions might also be helpful to make an etiologic diagnosis and for follow-up examinations.

8. Prospects

Diagnostics in equine sinusitis can be challenging. In cases where an exact etiologic diagnosis remains unclear,