LITERATURE REVIEW

Obligate anaerobic bacterial infections in dogs and cats

Regarding obligate anaerobic bacteria (OAB) infections in dogs and cats, many outstanding questions remain: How do we define causation in clinical cases? What range of diagnostic testing do we undertake? How do we treat them? The aim of this literature review is to summarize the existing veterinary scientific literature, with regard to the questions above.

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ABSTRACT
Obligate anaerobic bacteria (OAB) are commensals of many body sites in animals. However, OAB can also behave as opportunistic pathogens. There is a paucity of veterinary literature on the role of OAB in dogs and cats with only a low number of specific surveys on this topic, the majority of which stem from the 1970s and '80s. This literature review collates the available data and shows the most common OAB genera of dogs and cats to be *Bacteroides,* followed by *Clostridium, Fusobacterium, Porphyromonas, Peptostreptococcus,* and *Prevotella.* This review also synthesizes the data for the in vitro antimicrobial sensitivity spectrum for the widest range of OAB, confirming susceptibility to metronidazole, chloramphenicol, amoxicillin-clavulanic acid and clindamycin that appears to be stable over time, within the confines of the studies. This review also describes the methodological problems seen in the canine and feline reports and emphasizes a cautious interpretation of data that is of limited scientific value. It briefly outlines the main characteristics of OAB diagnostics in human medicine.

INTRODUCTION

Anaerobic bacteria were reported as early as 1680 by van Leeuwenhoek but in veterinary medicine, surveys on obligate anaerobic bacteria (OAB) were not conducted until the 1970s (20,26). They are frequently part of the inherent bacteria flora and veterinarians are challenged to decide whether these organisms are natural inhabitants, contaminants or true pathogens in infectious diseases (6,33,34,41,58). When considered in infections, they are typically part of a polymicrobial process (7,15,17,26,27,31,35,48). Additionally, they require advanced laboratory methods for their identification, which makes diagnosis difficult, time-consuming and expensive (44). Regarding OAB infections in dogs and cats, many outstanding questions remain: How do we define causation in clinical cases? What range of diagnostic testing do we undertake? How do we treat them? The aim of this literature review is to summarize the existing veterinary scientific literature, with regard to the questions above. Important information is outlined from human bacteriology, where veterinary data is missing.

LITERATURE REVIEW

The database of PubMed and Google Scholar were searched to find surveys in canine, feline and human medicine regarding OAB infections using the main terms: "anaerobic bacterial infection" or "anaerobic bacteria" or "anaerobe" and combining them systematically with organ systems and respective diseases. By this means, 49 veterinary articles could be included in the following work. Information from human medicine was included on specific topics where the author found the comparison to be of significant value and veterinary data was absent.

PREVALENCE OF OAB INFECTIONS IN DOGS AND CATS

Eight out of the 49 veterinary articles were classified as bacteriological based surveys on OAB prevalence in various veterinary species including dogs and cats (7,15,17,26,27,31,35,48). Three of the eight articles were solely canine and feline studies (7,15,31). The overall prevalence of OAB infections was between 9–37% with around 1,1–3,1 OAB isolates per positive bacterial culture (TABLE 1).

The highest prevalence according to infection site was seen in abscesses followed by draining tracts, respiratory tract and pleural effusions as seen in TABLE 2 where six out of eight surveys provided comparable data.

Identical prevalence for infection sites was found by Jang & Hirsh and Love et al. although their surveys focused entirely on *Fusobacterium* species pluralis (spp.) respective *Bacteroides* spp. (30,37). Most common isolates were members of the genera *Bacteroides* followed by *Clostridium, Fusobacterium, Porphyromonas, Peptostreptococcus,* and *Prevotella* (TABLE 3).

Although regarded as facultative anaerobes, *Actinomyces* spp. were included in this literature review as anaerobic conditions are widely used for their analysis (6).

Infection sites found in bacterial isolate and case studies are outlined alphabetically below.

BIOFILM - König et al. analyzed bacteria DNA in suture biofilms in postoperative surgical site infections from three dogs. Biopsy samples were collected from one ovariohysterectomized uterus stump, one post-castration skin wound, and one surgical skin wound. *Fusobacterium* spp. and *Porphyromonas* spp. were existent in all samples (34).

BLOOD - Twenty-three percent out of 292 cats and 24% out of 938 dogs, all with suspected sepsis, had positive blood culture results in two studies by Greiner et al. Twelve respective 10% of the samples included OAB (21,22).

BONE - Case series of osteomyelitis in dogs and cats have identified OAB in degrees between 17–69% (5,43,58). OAB which were found to be involved in this disease process included genera of *Actinomyces*, *Fusobacterium*, *Bacteroides*, *Clostridium*, *Wolinella* and *Peptostreptococcus* (5,11,32,43,58).

CENTRAL NERVOUS SYSTEM - Dow et al. presented four dogs and cats which were diagnosed at necropsy either

TABLE 1. Prevalence of OAB in infections as found in eight veterinary OAB surveys [a]only publish date exists [b]number represents %

Study	Hirsh et al. (26)	Prescott (48)	Berg et al. (7)	Hirsh et al. (27)	Dow et al. (15)	Jang et al. (31)	Even et al. (17)	Lawhon et al. (35)
Study period	1975-1978	1979[a]	1979[a]	1980-1983	1983-1985	1991-1995	1996-1997	2005-2009
Species	Various animals	Various animals	Dogs & cats	Various animals	Dogs & cats	Dogs & cats	Various animals	Various animals
Total no. specimen	3167	72	304	3133	153	1510	247	4018
No. cultures positive for OAB	583	72	111	823	53	268	247	368
Prevalence of OAB positive cultures (%)	18	-	37	26	35	18	-	9
No. of pure OAB cultures	-	8-9	39	9[b]	27	53	-	100
No. anaerobe isolates per OAB positive culture	-	3,1	1,7	1,9	1,7	1,1	1,7	-

TABLE 2. Prevalence of OAB positive cultures with regards to infection sites (%) as found in six out of eight veterinary OAB surveys [a] blood, ears, eyes, hooves, intestines, liver, mammary glands, oral cavity, pancreas, pericardial effusion, skin, spleen, urinary tract. [b-c]were reported as one in the original article.

	Hirsh et al. (26)	Prescott (48)	Berg et al. (7)	Hirsh et al. (27)	Jang et al. (31)	Even et al. (17)
Species	Various animals	Dogs & cats	Dogs & cats	Various animals	Dogs & cats	Dogs & cats
Cutaneous abscess	45,80	28,57	46,85[b]	51,88	17,16	10,87
Draining tract	4,80	21,42		6,07	7,46	32,60
Respiratory tract	14,75	7,14	6,31	12,88	15,31	21,74
Pleural effusion	12,86	-	2,70	12,52	16,42	4,35
Peritoneal effusion	6,35	14,29	7,21	9,36	7,46	4,35
Bone	2,92	14,29	13,51[c]	2,92	2,61	-
Joint	1,72	-		-	-	-
Genital tract	2,74	14,29	4,50	1,22	-	4,35
Central nervous system	1,54	-	0,90	0,36	-	-
Miscellaneous[a]	6,52	-	18,02	2,79	33,58	21,74

TABLE 3. Prevalence of OAB species (%) as found in eight veterinary OAB surveys [a]*Actinomyces spp., Eubacterium spp., Propionibacterium spp., Peptococcus spp.*

Study	Hirsh et al. (26)	Prescott (48)	Berg et al. (7)	Hirsh et al. (27)	Dow et al. (15)	Jang et al. (31)	Even et al. (17)	Lawhon et al. (35)
Species	Various animals	Various animals	Dogs & cats	Various animals	Dogs & cats	Dogs & cats	Dogs & cats	Dogs & cats
Bacteroides spp.	46,21	55,90	31,01	43,98	30,21	23,45	29,85	13,09
Clostridium spp.	7,73	4,61	30,48	10,02	13,54	8,62	-	54,36
Fusobacterium spp.	5,68	9,74	10,70	21,00	29,17	14,83	35,82	6,38
Porphyromonas spp.	-	-	-	-	-	11,72	16,42	-
Peptostreptococcus spp.	15,30	6,15	7,49	12,03	11,46	19,66	-	5,70
Prevotella spp.	-	-	-	-	-	7,24	17,91	3,36
Miscellaneous[a]	25,08	23,60	20,32	12,97	15,62	14,48	-	17,11

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with meningomyelitis or subdural empyema. All were infected with OAB, most commonly with genera of *Bacteroides* and *Fusobacterium* (16).

EYES - OAB were found in 14% of all canine and 8% of all feline samples reviewed for ulcerative keratitis by Ledbetter and Scarlett. Predominant bacteria genus was *Clostridium* followed by *Peptostreptococcus* and *Actinomyces* (36). For a survey on orbital abscess isolates Wang et al. investigated 41 dogs and cats. Fifty-nine percent of dogs had a positive bacterial culture including 35% aerobic-anaerobic mix and 20% pure anaerobic infections. Seventy-one percent of cats had a positive bacterial culture including 14% aerobic-anaerobic mix and 14% pure anaerobic infections. Predominant OAB were *Bacteroides* spp. and *Clostridium* spp. (59). Further canine case studies on eye diseases found *Bacteroides*, *Fusobacterium* and *Peptostreptococcus* spp. in orbital cellulitis, *Actinomyces* spp. in endophthalmitis and ulcerative keratitis and *Prevotella* spp. in an orbital abscess (6,12,28,50).

GASTROINTESTINAL TRACT - The enteropathogenic bacteria *Clostridium difficile* and *Clostridium perfringens* are widely accepted in literature for potentially causing large or small bowel diarrhea (40,41). Seventy-four out of 108 Parvovirus infected dogs proved to be infected with *C perfringens* at necropsy by Turk et al. (54). Cultures from liver and gallbladder from 248 dogs and cats were investigated by Wagner et al. Fourteen percent of liver and 36% of bile samples from cats were culture positive. In dogs, 5% of liver and 28% of bile samples were culture positive. Seventeen percent of feline and 52% of canine samples had mixed aerobic-anaerobic culture results. Most common OAB belonged to *Bacteroides* spp. and *Clostridium* spp. (56).

HEART - Joseph et al. reported a case of a dog that died directly after explorative thoracotomy for surgical inspection of a mass located at the heart base. The mass was classified as an abscess and culture discovered *Prevotella oralis* (33).

ORAL CAVITY - Almansa Ruiz et al. found OAB in root canals of dogs with complicated crown fractures with an incidence of around 4% including *Prevotella melaninogenica* and *Clostridium acetabulyticum* (1). These bacteria may give rise to endodontic infections and can cause systemic infections in humans (1,29). A study by Thengchaisri et al. on feline gingivitis found 29% of the subgingival bacteria belonging to OAB, predominantly *Bacteroides* spp. (52). No difference between the subgingival flora between dogs and cats was found in the literature (24). Dental plaque bacteria that form biofilms lead to periodontal disease like periodontitis in humans and animals. Many similarities exist regarding pathogenesis and bacteria species in these cases (23,25,60). In dogs, the OAB genera of *Peptostreptococcus, Actinomyces* and *Porphyromonas* were found to be causative for periodontal disease (23,47). Periodontopathic bacteria have been described to cause systemic disease in humans (4,46). In veterinary medicine, periodontal disease was related to histopathologic changes in the heart, liver, and kidney of dogs (14).

Anaerobe blood agar plate with Gentamicin and Metronidazole susceptibility test disc.

PERITONEAL EFFUSION - The prevalence of OAB in primary peritonitis was found to be 20% for cats respective 33% for dogs by Culp et al. *Clostridium spp.* and *Propionibacterium* spp. were commonly found (13).

RESPIRATORY TRACT - Macdonald et al. found only two out of 39 feline pneumonia cases to be infected with OAB including genera of *Fusobacterium, Peptostreptococcus,* and *Porphyromonas* (39). On the contrary, OAB have been found with a prevalence of over 20% in canine lower respiratory tract infections by Angus et al. (3). Out of 98 pyothorax patients, Walker et al. found 36% dogs and 44% cats to be infected with OAB including genera of *Peptostreptococcus, Bacteroides, Fusobacterium, Porphyromonas* and *Prevotella* (57). On the other hand, all of the 19 investigated feline pyothorax patients studied by Love et al. were infected with OAB, most frequently *Bacteroides* spp. (38).

WOUNDS - Seventeen out of 104 infected bite wounds were cultured positive for OAB in the study of Meyers et al. including isolates from *Prevotella* spp. and *Clostridium* spp. (42). Eight out of 57 isolates from infected canine wounds of traumatic origin, which were collected during the post-operative period, belonged to OAB in the study of Urumova et al. All isolates belonged to the species *P melaninogenica* (55).

OAB are able to spread from local infections via blood and lymph due to impaired local defense mechanisms, trauma like foreign body penetration or simply daily activities like tooth brushing or chewing (2,6,8,11,12,21,36,45,53,59). The final role of OAB had frequently yet to be determined. Since the majority of these organisms belong to endogenous bacteria, they might be incidental findings or opportunists (6,33,34,41,58).

DIAGNOSTIC TESTS FOR OAB

Only one veterinary article provided

guidelines for sample sites (7). In human medicine, suppuration and abscess formation and tissue destruction with gas formation is indicative for OAB infections (10). In humans, infection sites with a prevalence of 70–100% include brain, dental, peritonsillar, lung and pelvic abscesses, head and neck space, orofacial, intraabdominal and obstetric infections, aspiration pneumonia, and diabetic foot ulcers (18,19,44). Only 24 of the 49 veterinary scientific articles described their sampling techniques and transport methods (1,3,7,8,9,22–24,30–32,34,37–39,42,43,45,47,52,55,56,58,60). For bacterial culture, pre-reduced anaerobically sterilized (PRAS)-brucella media at different types of anaerobic atmospheres was mostly used (15,26,27,30,31,56,57). For bacterial identification (ID), different biochemical tests or commercial identification kits were used (22,33,35). In human medicine, it is recommended that OAB culture starts within two hours after sampling (44). Concerning time efficiency and species diversity, advanced diagnostics like matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and 16S rRNA gene-sequencing are preferred for ID compared to often used biochemistry-based identification kits (44). For more information, the reader is advised to confer with Nagy et al. (44).

TREATMENT OPTIONS FOR OAB

Only twelve of the 49 veterinary scientific articles performed antimicrobial susceptibility testing (AST) (6,7,12,17,21,27,31,32,35,40,50,55). TABLE 4 outlines AST information from veterinary OAB surveys, where four out of eight surveys provided data. For presentational purposes, the table shows the data artificially split into two major categories – most recent data (Lawhon et al. 2013), versus historical data (surveys from 1985 to 1998), in a crude display of sensitivity data over time.

The highest susceptibilities for OAB could be found for metronidazole, chloramphenicol, amoxicillin-clavulanic acid and clindamycin (17,27,31,35). In humans, physicians are advised to perform AST at least for specimens collected from suspected bacteremia, brain abscesses, endocarditis, infections of prosthetic devices and joints, osteomyelitis, and vascular grafts (44). *Bacteroides* spp. were frequently resistant to ampicillin and penicillin (6,33,34,41,58). OAB are naturally resistant to aminoglycosides and quinolones except for pradofloxacin (31,51,57). *Bacteroides* spp. frequently have beta-lactamase activity (52,58,59). Trimethoprim-sulfonamide combinations, tetracyclines, and ceftizoxime have unpredictable activities against OAB in vivo (21,22,57,59). It is suspected that antimicrobials that eliminate aerobic bacteria minimize anaerobic growth as well due to erasing synergistic effects between aerobic and OAB (36,50). Dental procedures are performed against periodontal diseases that frequently involve OAB. Although known to cause oropharyngeal bacteremia, these procedures could not be linked to infections or clinical illness in dogs and even for humans, prophylactic systemic antimicrobial treatment is hardly ever warranted for these procedures (2,8,9,45,49,53).

DISCUSSION

Veterinary literature provides only limited information on OAB infections in dogs and cats (7,15,17,26,27,31,35,48). OAB were found to be involved in infectious diseases in the entire body with different prevalence (7,15,17,26,27,31,35,48). Cutaneous abscesses were found to be the most common infection site for OAB in the surveys (7,17,26,27,31,48). Whether or not these results were accomplished simply due to easier sampling possibilities was never being argued by the authors of the OAB surveys. Scientific data revealed mainly polymicrobial disease processes with the predominant occurrence of *Bacteroides* followed by *Clostridium, Fusobacterium, Porphyromonas, Peptostreptococcus* and *Prevotella* spp. (7,15,17,26,27,31,35,48). Sample collection and transport methods were rarely described and ID was often based on biochemical reactions for which many OAB are known to be insensible (7,22,23,30–35,39,42,44,45,52,55,56,58,60). This circumstance makes the presented ID results frequently questionable. AST was performed by only a few veterinary articles (6,7,12,17,21,27,31,32,35,40,50,55). Results confirmed susceptibility to metronidazole, chloramphenicol, amoxicillin-clavulanic acid and clindamycin and moreover even quite stable in vitro susceptibilities over time (17,27,31,35). However, the insecurity remains regarding the existing scientific value, whether AST was performed on true infectious agents. Through wrong sample selection and techniques, laboratory diagnostics would frequently investigate simply natural flora or contaminants.

TABLE 4. Antimicrobial susceptibilities (%) as found in four out of eight veterinary OAB surveys Mz=metronidazole Cm=chloramphenicol A/C=amoxicillin-clavulanic acid Ap=ampicillin Cl=clindamycin Pc=penicillin [a]only found in (31) [b]only found in (27) [c]only found in (17)

	Mz	Cm	A/C	Ap	Cl	Pc	Study
Bacteroides spp.	98	100	100	50	100	21	Recent data: *Lawhon et al.*
Clostridium spp.	91-100	93-100	97-100	93-100	85-100	90-100	
Fusobacterium spp.	93	100	100	100	100	77	
Porphyromonas spp.	100	100	-	100	-	100	
Peptostreptococcus spp.	88	100	100	100	100	100	
Prevotella spp.	100	100	83	100	100	57	
Bacteroides spp.	97-100	100	100[a]	34-100	83-98	0-100	Historical data: Hirsh et al. (27) Jang et al. (31) Even et al. (17)
Clostridium spp.	100[a]	100[a]	100[a]	100[a]	80[a]	-	
Fusobacterium spp.	100	100	100[a]	100	95-100	100	
Porphyromonas spp.	100	93-100	100[a]	100[a]	88-100	95[c]	
Peptostreptococcus spp.	100[a]	100	100[a]	100	100	97[b]	
Prevotella spp.	100	93-100	100[a]	100[a]	88-100	95[c]	

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Anaerobe pouch system with CO2 generator and anaerobic atmosphere indicator.

This circumstance could seriously falsify AST results as possible existing virulence factors are being missed.

Focus for veterinarians should not lie on the development of new diagnostic methods. Instead human medicine recommendations should be applied to collect own research results and to develop veterinary expertise. These studies must have a prospective design with a focus on clinical and laboratory bacteriology to provide high diagnostic standards. Clinicians must learn about the pathophysiology of these bacterial infections and improve their knowledge in clinical bacteriology. Laboratory diagnostics should be left to microbiologists in large reference laboratories that have the resources to implement advanced methods like MALDI-TOF MS and 16S rRNA gene-sequencing for ID (44). Close working relationships must be initiated between clinicians and microbiologists to improve diagnostics. It is recommended that bacteria processing starts within two hours after sampling (44). As the majority of clinics will not have reference laboratories in close reach, the initial obstacle will be to secure bacteria survival during frequently unfavorable transport conditions (44). One option could be that clinicians start cultivating sample material in their clinics and sending it during incubation in devices providing a suitable environment and temperature. It is crucial to solve this issue as it is known that poorly performed incubation will result in detecting fewer OAB (44). Concerning antimicrobial resistance developments, veterinarians must no longer rely on outdated susceptibility data for OAB, often received under questionable standards.

CONCLUSION

Each scientific article not describing or following strict precautions for sample selection and laboratory techniques fails to provide reliable information regarding clinical relevance, ID and antimicrobial susceptibilities of OAB. As a consequence, existing information on OAB in the majority of veterinary scientific articles must be questioned in order to proceed in the process of finding higher levels of evidence. This review highlights that we have an outdated and limited literature base, with low confidence in the data quality. Clinicians have to improve their knowledge regarding the pathophysiology of OAB involvement in infection to gain true clinical bacteriological understanding. Microbiologists in reference laboratories should implement advanced diagnostics for quicker and more secure ID.

SAMMANFATTNING

Obligatoriska anaeroba bakterier (OAB) ingår i den naturliga bakteriefloran i många delar av kroppen hos hund och katt men kan också uppträda som opportunistiska patogener. Det finns endast en liten mängd litteratur inom veterinärmedicin rörande OAB:s roll hos hundar och katter, där den mesta litteraturen är från 1970- och 1980-talen. Denna litteraturstudie sammanfattar tillgänglig data och visar att de vanligaste OAB-genera från hundar och katter är *Bacteroides,* följt av *Clostridium, Fusobacterium, Porphyromonas, Peptostreptococcus* och *Prevotella.* Förekomst av OAB i respektive organsystem redovisas och

vanligaste kliniska infektionen med OAB är kutana abscesser. Vidare sammanfattas resistensläget för antibiotika in vitro, som inom rapporternas begränsningar verkar ha varit stabilt över tid med vanligt förekommande känslighet för metronidazol, kloramfenikol, amoxicillin-klavulansyra och klindamycin. Denna studie beskriver också de omfattande metodiska problem som ses i de befintliga hund- och kattrapporterna och betonar en försiktig tolkning av dessa data, som är av begränsat vetenskapligt och kliniskt värde. Där veterinärmedicinsk data saknas presenteras det humanmedicinska perspektivet. ■

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