Research article

Molecular characterization of the cloacal microbiota of wild and captive parrots

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ABSTRACT

The gastrointestinal microbiota plays a fundamental role in health and disease. Only limited data are available about the composition of the intestinal microbiota of captive animals compared to those of wild animals. The aim of the present study was to characterize the cloacal microbiota of apparently healthy wild and captive parrots.

A total of 16 parrots, 8 wild and 8 captive, belonging to 3 different species, were used in this study. Cloacal material was collected via cloacal swabbing. DNA was extracted and 16S rRNA genes were amplified using universal bacterial primers. Constructed 16S rRNA gene clone libraries were compared between groups.

A total of 518 clones were analyzed, and 49 operational taxonomic units (OTUs) were identified. The OTUs were classified in 4 bacterial phyla: *Firmicutes* (72.9%), *Proteobacteria* (14.9%), *Actinobateria* (12%), and *Bacteroidetes* (0.2%). Bacterial diversity was significantly lower in wild birds than in captive birds. Principal component analysis based on the Unifrac distance metric indicated that the cloacal microbiota differed between wild and captive parrots. *Staphylococcus saprophyticus* was significantly more abundant in wild birds, while *Escherichia coli* was significantly more abundant in captive birds. In conclusion, wild and captive parrots appear to have differences in the composition of their cloacal bacterial microbiota. The clinical significance of these differences remains to be determined.

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1. Introduction

Although parrot keeping has been known to occur for many thousands of years, large parrots have never become domesticated (McKusick, 2001). Today, few generations separate large parrots kept as pets from those found in the wild, and in many cases, captive large parrots are wild-caught.

The gastrointestinal microbiota reflects the co-evolution of microorganisms with their animal host, and is believed to play a fundamental role in the animal's health, growth, immune response, and disease (Eckburg et al., 2005). The gastrointestinal microbiota varies greatly among species and is highly conserved within species, although significant inter-individual variation exists even within the same species (Stevens and Hume, 1998; Suchodolski et al., 2004). The gastrointestinal microbiota is affected by many factors such as diet, environment, antibiotic administration, and infection with pathogenic organisms (Lu et al., 2003). Most of these factors differ

substantially between wild and captive parrots. It is suspected that a sudden change of environment, such as that taking place during transfer from the wild to captivity, may lead to changes in the intestinal microbiota.

Efforts have been made in humans and other animal species to characterize the intestinal microbial communities using molecular methods (Leser et al., 2002; Suchodolski et al., 2004, 2008; Eckburg et al., 2005; Ritchie et al., 2008). To date, no molecular data are available about the composition of the cloacal microbiota in parrots. Because captive parrots live in different environments, the characterization of their intestinal microbiota might not accurately reflect the normal intestinal microbiota of wild parrots. The aim of the present study was to characterize and compare the cloacal microbiota of apparently healthy wild and captive parrots using molecular methods.

2. Materials and methods

2.1. Animals and samples

A total of 16 parrots, 8 wild and 8 captive, were used in this study. The wild parrots consisted of 4 Mealy Parrots (*Amazona farinosa*), 3 blue-and-yellow Macaws (*Ara ararauna*), and 1 red-and-green Macaw (*Ara chloropterus*) captured at the large clay lick adjacent to the Tambopata Research Center in the lowlands of southeastern Peru (13°07′S, 69°36′W) (Brightsmith et al., 2008). The captive parrots belonged either to a breeder located in Texas (4 Mealy Parrots) or the Schubot Exotic Bird Health Center at Texas A&M University (3 blue-and-yellow Macaws and 1 red-and-green Macaw).

Cloacal content was collected via cloacal swabbing using sterile cotton-tipped swabs from all parrots. Wild parrots were all free-ranged and captured using nylon foot snares set on prominent perches 10–30 m from the large clay lick. Birds were wrapped in towels during sample collection in order to avoid contamination with material outside the cloaca. Cloacal swabs were placed into DNA-free sterile tubes and initially kept in refrigeration (4 °C), then shipped to the laboratory on dry ice. Upon arrival, samples were stored at −80 °C. This work was conducted with permission of the Peruvian government's Instituto Nacional de Recursos Naturales (INRENA) and samples were imported with permission from USDA APHIS.

The same collection technique was used to obtain samples from captive parrots. All captive birds were on similar diets, which consisted of ZuPreem AvianMaintenance™ FruitBlend (ZuPreem, Mission, Kans.), fresh fruits and vegetables, and/or mixed seeds. No antibiotics had been given for several months prior to sample collection. The 4 macaws belonging to the Schubot Exotic Bird Health Center were carriers of Psittacid Herpesvirus, but had no clinical signs at the time of sample collection. All 16 birds were adults, but their exact ages were unknown.

2.2. Isolation of DNA

All samples were analyzed separately. DNA was extracted using a bead beating method followed by phenol:chloroform:iso-amylalcohol extraction as described previously (Suchodolski et al., 2004).

2.3. PCR amplification

Amplification of 16S rRNA genes was carried out separately for each sample using universal bacterial primers Univ-27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and Univ-1492R (5′-GGTTACCTTGTTACGACTT-3′) as described previously (Xenoulis et al., 2008).

2.4. Cloning of PCR amplicons and plasmid extractions

The purified PCR amplicons were ligated into plasmid vectors (pCR®4-TOPO, Invitrogen, Carlsbad, CA), and then transformed into competent One Shot DH5α *Escherichia coli* organisms (Invitrogen) as described previously (Xenoulis et al., 2008). Up to 48 colonies per sample were randomly picked.

2.5. Sequencing

Sequencing of the 16S rRNA gene inserts was performed using the BigDye Terminator Cycle Sequencing kit with an automated sequence analyzer (ABI PRISM 377 DNA Sequencer, Applied Biosystems, Perkin-Elmer Corporation, Foster City, CA) as described previously (Xenoulis et al., 2008). For grouping of clones, all clones were re-amplified from the 5′-terminal of the 16S rRNA gene using a single primer (Univ-27F; 5′-AGAGTTTGATCMTGGCTCAG-3′).

2.6. Analysis of DNA sequences

All sequences were manually analyzed using a software package (ChromasPro, Technelysium Pty Ltd., Eden Prairie, MN) to exclude sequences of bad quality. Sequences were edited to exclude the PCR primer binding sites, and scanned for possible chimeric artifacts (Chimera_Check available through the Ribosomal Database Project) (Cole et al., 2003). All obtained sequences were compared to existing sequences in NCBI and were aligned using the CLUSTAL-W program. A PHYLIP distance matrix was generated and used as the input file for the DOTUR software to determine operational taxonomical units (OTUs) (Schloss and Handelsman, 2005). An OTU was defined as sequences with less than 2% difference to each other based on the furthest-neighbor algorithm in DOTUR. The Unifrac distance metric was used to determine whether the bacterial communities of wild and captive birds were significantly different (Lozupone and Knight, 2005). The RDP Library Compare tool, based on a naïve Bayesian classifier, was used to classify the 16S rRNA gene sequences and to compare the clone libraries obtained from wild and captive birds (Wang et al., 2007).

The coverage of the clone library (i.e., the probability that any additional analyzed clone is different from any previously identified unique clone) was calculated using the formula $C_x = [1 - (N_x/n)] \times 100$, where N_x is the number of phylotypes represented by one clone and n is the total number of clones (Singleton et al., 2001). Information about species diversity within the bacterial communities

was obtained using the Shannon-Weaver diversity index (Ledder et al., 2007). Data were tested for normality using the Kolmogorov–Smirnov test. The Student's t-test was used to compare the coverage between the two groups. Fisher's exact tests were used to compare proportions of birds between groups (Prism5, GraphPad Software Inc., San Diego, CA). The RDP classifier was used to compare proportions of clones between groups. Significance level was set at $p < 0.05$ for all comparisons.

3. Results

A total of 632 clones were selected. Sequences of bad quality or inadequate length (104 sequences total) were excluded from analysis. Of the remaining 528 sequences, 10 (1.9%) were determined to be putative chimeras and were excluded from further analysis. A total of 518 sequences were subjected to final analysis. Of the 518 sequences, 280 were from captive birds and 238 from wild birds. The median coverage of the clone libraries was 99% for the wild bird group and 94% for the captive bird group. The difference in coverage between the 2 groups was not statistically significant ($p > 0.05$). A total of 49 unique OTUs were identified, 10 (20.4%) of which had 98% or less similarity to previously described sequences in NCBI. 36 OTUs were identified in captive birds and 15 OTUs were identified in wild birds. The mean ($\pm$SD) number of observed OTUs per sample was 2.6 ($\pm$0.7) in wild birds and 7.4 ($\pm$6.3) in captive birds. The mean Shannon-Weaver diversity index was significantly higher in captive than in wild birds (1.36 vs. 0.62; $p = 0.03$).

A dendrogram constructed using the Unifrac distance matrix (Fig. 1) illustrates clustering of the wild birds, indicating that the cloacal microbiota differs between wild and captive birds. In addition, there was a trend for clustering of the captive birds based on the species and/or aviary they came from.

The 16S rRNA gene sequences obtained in this study, regardless of the group, belonged to organisms from 4 bacterial phyla: Firmicutes (72.9%), Proteobacteria (14.9%), Actinobateria (12%), and Bacteroidetes (0.2%). In the captive bird group, representatives of all 4 phyla were present: Firmicutes (63.2%), Proteobacteria (22.9%), Actinobateria (13.6%), and Bacteroidetes (0.3%). In the wild bird group, sequences belonging to 3 phyla were identified: Firmicutes (84.5%), Proteobacteria (5.5%), and Actinobateria (10.0%).

The vast majority of sequences identified were classified in the phylum Firmicutes, and all 16 birds harbored sequences belonging to this phylum. However, Firmicutes were significantly more common in wild birds (84.5%) than in captive birds (63.2%; $p < 0.0001$). Conversely, Proteobacteria were found in 7 of 8 captive birds but only in 1 of 8 wild birds ($p = 0.01$), and were significantly more abundant in captive birds (22.9%) than in wild birds (5.5%; $p < 0.0001$).

The overall phylogenetic distribution of the sequences identified in captive and wild birds as well as their closest neighbor in GenBank is shown in Table 1. Nearly half of the sequences (48.2%) of the captive birds belonged to the order Lactobacillales and the majority of the sequences in this order (81.5%) belonged to the family Lactobacillaceae (genus Lactobacillus) (Table 1). In contrast, most sequences
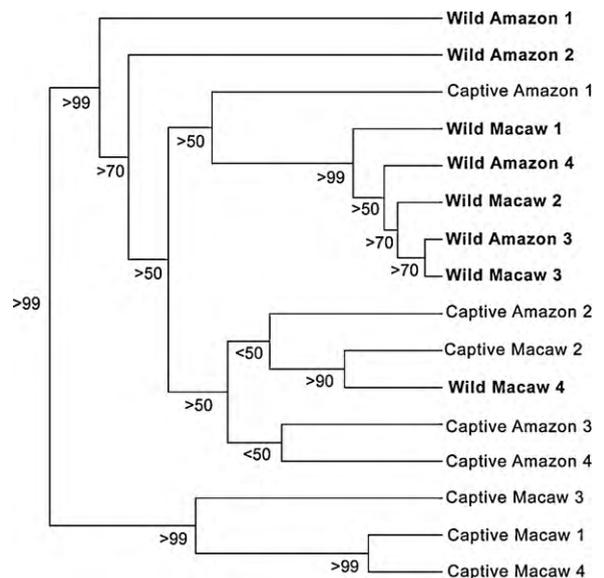

Fig. 1. Clustering of the cloacal microbiota in wild and captive birds. The dendrogram was constructed using the Unifrac distance metric. Jackknife counts are based on 100 replicates. Wild Macaw 1, blue and yellow; Wild Macaw 2, blue and yellow; Wild Macaw 3, red and green; Wild Macaw 4, blue and yellow; Captive Macaw 1, blue and yellow; Captive Macaw 2, red and green; Captive Macaw 3, blue and yellow; Captive Macaw 4, blue and yellow.

(72.3%) from wild birds belonged to the order Bacillales and 89.5% of the sequences in this order belonged to the family Staphylococcaceae.

At the family level, the most profound differences involved the families Staphylococcaceae, Lactobacillaceae, and Enterobacteriaceae. Sequences belonging to the Staphylococcaceae family were found in 7 of 8 wild birds and in none of the captive birds ($p < 0.01$). In addition, most (64.7%) of the sequences in the wild bird group belonged to the Staphylococcaceae family as opposed to 0% in the captive birds ($p < 0.0001$). The vast majority of the sequences in this family could be further classified as Staphylococcus saprophyticus. Sequences belonging to the Lactobacillaceae family were found in 5 birds in each group, but these sequences were significantly more abundant in captive birds (39.3%) than in wild birds (11.3%; $p < 0.0001$). Sequences belonging to the family Enterobacteriaceae were found in 5 of 8 captive birds and in 1 of 7 wild birds ($p > 0.05$), and were significantly more abundant in captive birds (13.2%) than in wild birds (4.6%; $p = 0.0007$). All Enterobacteriaceae sequences from captive birds were further classified as Escherichia coli (E. coli), while in the wild birds they were classified as Pantoea spp.

Based on previously published Gram staining characteristics of the respective bacteria (Table 1), it was found that the cloacal microbiota of both captive and wild birds was mainly comprised of Gram-positive bacteria. However, captive birds had significantly more sequences from Gram-negative bacteria (20.7%) compared to wild birds (6.7%, $p < 0.0001$), and Gram-negative bacteria were identified in 7 of 8 captive birds and in only 1 wild bird ($p = 0.0101$).

Obtained sequences were uploaded to GenBank with the accession numbers FJ750878–FJ750926.

Table 1

Taxonomic distribution of obtained sequences based on whether the birds were wild or captive and based on species (BYM, blue-and-yellow macaw; RGM, red-and-green macaw). The total number of sequences for each group is shown in parentheses.

Closest known neighbor in NCBI			Number of sequences (%)				
Species	Similarity	Accession no.	Captive (280) n = 8	Wild (238) n = 8	Amazons (262) n = 8	BYM (194) n = 6	RGM (62) n = 2
Acidovorax facilis	99	EU024133	1 (0.4)	0	1 (0.4)	0	0
Aerosphaera taetra	99	EF111256	1 (0.4)	0	1 (0.4)	0	0
Agrobacterium tumefaciens	99	X67223	2 (0.7)	0	2 (0.8)	0	0
Arthrobacter protophormiae	97	X80745	0	3 (1.3)	3 (1.1)	0	0
Bifidobacterium longum	99	EF370999	2 (0.7)	0	2 (0.8)	0	0
Bifidobacterium pseudocatenulatum	100	D86187	1 (0.4)	0	1 (0.4)	0	0
Bifidobacterium pseudolongum	99	AY174106	8 (2.9)	0	8 (3.1)	0	0
Bradyrhizobium canariense	100	AB195986	1 (0.4)	0	1 (0.4)	0	0
Burkholderia cepacia	100	AM992536	13 (4.6)	0	13 (5.0)	0	0
Campylobacter jejuni	100	EU127547	1 (0.4)	0	1 (0.4)	0	0
Chryseobacterium indoltheticum	99	AY468448	1 (0.4)	0	1 (0.4)	0	0
Clostridium colicanis	99	AJ420008	1 (0.4)	0	1 (0.4)	0	0
Corynebacterium kroppenstedtii	99	Y10077	25 (8.9)	0	0	1 (0.5)	24 (38.7)
Curtobacterium citreum	99	AM410690	0	15 (6.3)	10 (3.8)	5 (2.6)	0
Enterococcus faecalis strain IJ-12	100	EU547777	7 (2.5)	0	0	4 (2.1)	3 (4.8)
Enterococcus faecium	99	EU547780	3 (1.1)	0	3 (1.1)	0	0
Escherichia coli	100	AY186041	37 (13.2)	0	2 (0.8)	33 (17.0)	2 (3.2)
Exiguobacterium acetylicum	99	DQ019167	0	18 (7.6)	18 (6.9)	0	0
Lactobacillus acidophilus	99	EU483105	13 (4.6)	0	13 (5.0)	0	0
Lactobacillus coleohominis	97	AB289060	5 (1.8)	1 (0.4)	3 (1.1)	3 (1.5)	0
Lactobacillus fermentum	99	EU688978	4 (1.4)	0	4 (1.5)	0	0
Lactobacillus jensenii	94	AF243143	0	1 (0.4)	1 (0.4)	0	0
Lactobacillus johnsonii	99	EU511940	2 (0.7)	0	2 (0.8)	0	0
Lactobacillus kimchiyll	99	EU678893	0 (0.0)	2 (0.8)	2 (0.2)	0	0
Lactobacillus psittaci	98	AJ272391	60 (21.4)	21 (8.8)	23 (8.8)	53 (27.3)	5 (8.1)
Lactobacillus reuteri	99	EU626022	2 (0.7)	0	2 (0.8)	0	0
Lactobacillus ruminis	100	AB326354	2 (0.7)	0	2 (0.2)	0	0
Lactobacillus saerimneri	100	AY255802	2 (0.7)	0	2 (0.2)	0	0
Lactobacillus satsumensis	92	AB154519	0	2 (0.8)	0	2 (1.0)	0
Lactobacillus vaginalis	98	AB158767	16 (5.7)	0	15 (5.7)	1 (0.5)	0
Lactococcus lactis	99	EU483103	0	2 (0.8)	0	2 (1.0)	0
Long segmented filamentous organism	99	X80834	2 (0.7)	0	2 (0.8)	0	0
Microbacterium foliorum	98	AJ249780	0	1 (0.4)	1 (0.4)	0	0
Pantoea agglomerans	99	AY924376	0	11 (4.6)	0	11 (5.7)	0
Pediococcus acidilactici	100	EU147316	4 (1.4)	0	4 (1.5)	0	0
Peptoniphilus indolicus	98	AY153431	16 (5.7)	0	0	14 (7.2)	2 (3.2)
Scardovia inopinata	92	D89332	0 (0.0)	5 (2.1)	0	5 (2.6)	0
Sphingobium herbicidovorans	99	EF065102	4 (1.4)	0	4 (1.5)	0	0
Sporosarcina ureae	98	EU308120	20 (7.1)	0	20 (7.6)	0	0
Staphylococcus saprophyticus	99	EU419944	0	138 (58.0)	56 (21.4)	56 (29.0)	26 (41.9)
Staphylococcus sciuri	100	EU095646	0	16 (6.7)	16 (6.1)	0	0
Stenotrophomonas maltophilia	99	AY445079	0	2 (0.8)	0	2 (1.0)	0
Streptococcus gallolyticus	100	EU483247	13 (4.6)	0	11 (4.2)	2 (1.0)	0
Trichococcus pasteurii	99	AM943044	1 (0.4)	0	1 (0.4)	0	0
Variovorax paradoxus	100	AF209469	2 (0.7)	0	2 (0.8)	0	0
Uncultured	99	EU133046	2 (0.7)	0	2 (0.8)	0	0
Uncultured	99	DQ803910	1 (0.4)	0	1 (0.4)	0	0
Uncultured	100	EF399533	2 (0.7)	0	2 (0.8)	0	0
Uncultured	100	EF540484	3 (1.1)	0	3 (1.1)	0	0

4. Discussion

The aim of the present study was to characterize and compare the cloacal bacterial microbiota of wild and captive parrots. Cloacal swabs are commonly used in clinical practice and are believed to at least partially represent the fecal microbiota (Flammer and Drewes, 1988). Cloacal swabbing was selected as the preferred method for collection of fecal material in this study, because it was not feasible to obtain naturally passed feces from wild parrots. However, due to the fact that the cloaca serves as a common waste compartment for the gastro-intestinal, reproductive, and urinary systems, it is likely that cloacal samples do not represent solely intestinal, but rather a combination of the gastrointestinal, reproductive, and urinary microbiota (Hadley, 2005).

The results of the Unifrac analysis indicate that the cloacal bacterial communities differ between wild and captive parrots. We also observed a trend for bacterial communities of the captive birds to cluster based on the species and/or aviary of origin. Because this clustering according to species was not as clearly evident in the wild birds, it is likely that differences in the cloacal microbiota within captive birds are due to different environmental

conditions rather than the species. However, further studies are needed to determine the effect of species on the intestinal microbiota in captive birds. Due to the small number of birds in each species this was not possible in the present study. In comparison to other animal species, we observed a low species richness in cloacal swabs. The relatively low species richness of the parrot cloacal microbiota found in this study is in agreement with previous studies using culture-based techniques (Dorrestein et al., 1985; Bangert et al., 1988). The reason for the significantly lower species diversity in the wild birds is unknown, but several factors (mainly environmental, dietary, and health factors) could be implicated.

Although the bacterial communities of wild and captive birds were dominated by *Firmicutes*, the two groups differed significantly in the relative proportions of their subgroups. *Lactobacillus* spp. accounted for the majority (62.1%) of *Firmicutes* in captive birds, while *Staphylococcus* spp. accounted for the majority (76.6%) of *Firmicutes* in wild birds. The finding that *Lactobacillus* spp. are the most commonly found bacterial group in the cloaca of captive parrots is in agreement with previous studies using culture-based techniques (Bangert et al., 1988). In the wild parrots, members of the genus *Staphylococcus* (mainly *S. saprophyticus*) were the predominant bacterial group and were found in 7 of the 8 wild parrots, while they were not identified in captive birds. Sequences belonging to *Enterobacteriaceae* were significantly more abundant in captive parrots. *E. coli*-like organisms were found exclusively in captive birds. *E. coli* was also the most commonly identified Gram-negative bacterium from cloacal swabs and feces of healthy captive psittacines in previous studies using culture-based methods, and was isolated from 31% to 40% (Bowman and Jacobson, 1980; Flammer and Drewes, 1988) and 5% to 14% (Graham and Graham, 1978; Bangert et al., 1988) of all birds tested, respectively. Differences in the prevalence of *E. coli* in cloacal samples among species have been reported, and captive Scarlet Macaws have a reported prevalence of 9–100% (Bowman and Jacobson, 1980; Flammer and Drewes, 1988). Sequences classified as *E. coli* were not identified in any of the 8 wild birds in the present study. Although the total number of birds tested in the present study was small, these findings suggest that *E. coli* may be common in the cloaca of apparently healthy captive psittacines, but may not be common in wild psittacines.

Streptococcus gallolyticus (previously classified as *S. bovis*) was identified in 3 of the captive birds and in none of the wild ones. *S. gallolyticus* has been reported to be a major pathogen in pigeons and chickens (Chadfield et al., 2007). Although members of the genus *Streptococcus* are believed to be part of the normal intestinal microbiota in psittacines (Bowman and Jacobson, 1980; Bangert et al., 1988), no other *Streptococcus* spp. were found in the present study. *Enterococcus faecalis* and *E. faecium* were identified only in captive parrots. *E. faecalis* has been associated with respiratory disease mainly in canaries (Dorrestein, 1997), but the clinical significance of these two bacterial species in parrots is uncertain.

The clinical significance of the differences in the cloacal microbiota between wild and captive parrots cannot be determined based on the results of the present study because only apparently healthy birds were included. The differences identified in the present study possibly reflect an effect of captivity on the microbiota. Factors that may be responsible for these differences include environment, diet, age, genotype, and antibiotic use. Recent evidence suggests that the diets fed in captivity differ substantially from those consumed by birds in the wild, and this might be a plausible explanation for the differences identified in the present study (Brightsmith et al., 2010). In addition, although antibiotics had not been used for several months prior to sampling, the possibility of a persistent effect on the cloacal microbiota of previously used antibiotics in the captive birds cannot be excluded.

5. Conclusion

In conclusion, this study has demonstrated that molecular tools are useful for the characterization of the cloacal microbiota of wild ranging parrots. The present study also suggests differences in the phylogenetic composition of the cloacal microbiota between apparently healthy wild and captive parrots of the same species. Further studies are warranted to determine the clinical significance of these findings and explore whether these differences play a role in the pathogenesis of diseases commonly seen in captive psittacine species.

References

Bangert, R.L., Cho, B.R., Widders, P.R., Stauber, E.H., Ward, A.C.S., 1988. A survey of aerobic bacteria and fungi in the feces of healthy psittacine birds. Avian Dis. 32, 46–52.

Bowman, T.A., Jacobson, E.R., 1980. Cloacal flora of clinically normal captive psittacine birds. J. Zoo Anim. Med. 11, 81–85.

Brightsmith, D.J., Taylor, J., Phillips, T.D., 2008. The roles of soil characteristics and toxin adsorption in avian geophagy. Biotropica 40, 766–774.

Brightsmith, D.J., McDonald, D., Matsafuji, D., Bailey, C.A., 2010. Nutritional content of the diets of free-living scarlet macaw chicks in southeastern Peru. J. Avian Med. Surg. 24, 9–23.

Chadfield, M.S., Christensen, J.P., Decostere, A., Christensen, H., Bisgaard, M., 2007. Geno- and phenotypic diversity of avian isolates of *Streptococcus gallolyticus* subsp. *gallolyticus* (*Streptococcus bovis*) and associated diagnostic problems. J. Clin. Microbiol. 45, 822–827.

Cole, J.R., Chai, B., Marsh, T.L., Farris, R.J., Wang, Q., Kulam, S.A., Chandra, S., McGarrell, D.M., Schmidt, T.M., Garrity, G.M., Tiedje, J.M., 2003. The Ribosomal Database Project (RDP-II): previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy. Nucleic Acids Res. 31, 442–443.

Dorrestein, G.M., Buitelaar, M.N., Vanderhage, M.H., Zwart, P., 1985. Evaluation of a bacteriological and mycological examination of psittacine birds. Avian Dis. 29, 951–962.

Dorrestein, G.M., 1997. Passerines. In: Altman, R., Clubb, S., Dorrestein, G. (Eds.), Avian Medicine and Surgery. Philadelphia, Saunders, pp. 867–885.

Eckburg, P.B., Bik, E.M., Bernstein, C.N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S.R., Nelson, K.E., Relman, D.A., 2005. Diversity of the human intestinal microbial flora. Science 308, 1635–1638.

Flammer, K., Drewes, L.A., 1988. Species-related differences in the incidence of gram-negative bacteria isolated from the cloaca of clinically normal psittacine birds. Avian Dis. 32, 79–83.

Graham, C.L., Graham, D.L., 1978. Occurrence of *Escherichia coli* in feces of psittacine birds. Avian Dis. 22, 717–720.

Hadley, T.L., 2005. Disorders of the psittacine gastrointestinal tract. Vet. Clin. Exot. Anim. 8, 329–349.

Ledder, R.G., Gilbert, P., Huws, S.A., Aarons, L., Ashley, M.P., Hull, P.S., Mcbain, A.J., 2007. Molecular analysis of the subgingival microbiota in health and disease. Appl. Environ. Microbiol. 73, 516–523.

Leser, T.D., Amenuvor, J.Z., Jensen, T.K., Lindecrona, R.H., Boye, M., Moller, K., 2002. Culture-independent analysis of gut bacteria: the pig gastrointestinal tract microbiota revisited. Appl. Environ. Microbiol. 68, 673–690.

Lozupone, C., Knight, R., 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. 71, 8228–8235.

Lu, J.R., Idris, U., Harmon, B., Hofacre, C., Maurer, J.J., Lee, M.D., 2003. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. Appl. Environ. Microbiol. 69, 6816–6824.

McKusick, C., 2001. Southwest birds of sacrifice. Arizona Archaeological Society. Phoenix 411–416.

Ritchie, L.E., Steiner, J.M., Suchodolski, J.S., 2008. Assessment of microbial diversity along the feline intestinal tract using 16S rRNA gene analysis. FEMS Microbiol. Ecol. 66, 590–598.

Schloss, P.D., Handelsman, J., 2005. Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. Appl. Environ. Microbiol. 71, 1501–1506.

Singleton, D.R., Furlong, M.A., Rathbun, S.L., Whitman, W.B., 2001. Quantitative comparisons of 16S rRNA gene sequence libraries from environmental samples. Appl. Environ. Microbiol. 67, 4374–4376.

Stevens, C.E., Hume, I.D., 1998. Contributions of microbes in vertebrate gastrointestinal tract to production and conservation of nutrients. Physiol. Rev. 78, 393–427.

Suchodolski, J.S., Ruaux, C.G., Steiner, J.M., Fetz, K., Williams, D.A., 2004. Application of molecular fingerprinting for qualitative assessment of small-intestinal bacterial diversity in dogs. J. Clin. Microbiol. 42, 4702–4708.

Suchodolski, J.S., Camancho, J., Steiner, J.M., 2008. Analysis of bacterial diversity in the canine duodenum, jejunum, ileum, and colon by comparative 16S rRNA gene analysis. FEMS Microbiol. Ecol. 66, 567–578.

Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73, 5261–5267.

Xenoulis, P.G., Palculict, B., Allenspach, K., Steiner, J.M., Van House, A.M., Suchodolski, J.S., 2008. Molecular-phylogenetic characterization of microbial communities imbalances in the small intestine of dogs with inflammatory bowel disease. FEMS Microbiol. Ecol. 66, 579–589.