




Article

Depression and Microbiome—Study on the Relation and Contiguity between Dogs and Humans

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Abstract: Behavioral studies demonstrate that not only humans, but all other animals including dogs, can suffer from depression. A quantitative molecular evaluation of fatty acids in human and animal platelets has already evidenced similarities between people suffering from depression and German Shepherds, suggesting that domestication has led dogs to be similar to humans. In order to verify whether humans and dogs suffering from similar pathologies also share similar microorganisms at the intestinal level, in this study the gut-microbiota composition of 12 German Shepherds was compared to that of 15 dogs belonging to mixed breeds which do not suffer from depression. Moreover, the relation between the microbiota of the German Shepherd's group and that of patients with depression has been investigated. The results indicate that the German Shepherd's gut-microbiota has a different composition compared to other dog breeds and is characterized by microbial groups identified in humans with depression, highlighting the existence of a “core” microbiota associated with depression.

Keywords: gut-microbiota-brain axis; dog depression; human depression; microbiome

1. Introduction

In recent years, several studies have shown that depression is not just a human disease but it has a molecular affinity within the animal kingdom [1,2]. Until now, research on depression have mainly been focused on the genetic, behavioral and neurological aspects of the mental illness. However, recent evidences from animal studies have shown that functions of the central nervous system (CNS) are influenced by the gut microbiota and this is also involved in the pathogenesis of mental diseases. This influence happens through inflammation, the hypothalamic-pituitary-adrenal (HPA) axis and by the production of neurotransmission precursors [3]. Such a network has an impact on physiological features affecting mammalian behavior. In the study conducted by Sudo and colleagues, it is showed germ-free (GF) mice had an abnormal functioning of the HPA axis, with higher adrenocorticotrophic hormone (ACTH) and corticosterone levels rather than specific pathogen-free (SPF) mice in response to restraint stress. These abnormal hormonal levels normalized after colonization with commensal bacteria [4]. Besides, GF had a lower level of brain-derived neurotrophic factor (BDNF) in the cortex and hippocampus [4]. Furthermore, several studies have found that administration of probiotics improves the health of the host by modulating anxiety phenotypes and stress hormones' response.

It has been observed that the administration of *Lactobacillus rhamnosus* (JB-1) and *Bacteroides fragilis* (NCTC 9343) reduces anxiety-like behavior [5,6]. Moreover, gut bacteria can produce neuroactive substances, such as precursors of monoamine neurotransmitters, which support the communication with the brain, affecting behavior, including anxiety, stress and depression [4,7]. One of the key roles in bidirectional gut-microbiota-brain communication is carried out by tryptophan, which impacts host serotonin and kynurenine levels, influencing both behaviors linked to serotonergic neurotransmission and immune system [8]. All these observations indicate that the gut microbiome and some behavioral patterns may be linked in mammals. In 2006, Cocchi [9] carried out a study on the similarity between the fatty acid profiles of platelets and neurons. Based on this similarity, they were able to identify different mood disorders (Major Depression and Bipolar Disorder) by creating an artificial neural network, whose crucial factors were three fatty acids (i.e., Palmitic Acid, Linoleic Acid, Arachidonic Acid) [10,11]. The same study was conducted taking into account different animal species and its most important evidence was the molecular similarity between two particular dog breeds (German Shepherd and Alaskan Malamute) and humans affected by Major Depression and Bipolar Disorder [2,12,13]. Considering the molecular similarity in fatty acids platelets between German Shepherds and humans suffering from depression and the studies on the gut-microbiota of the latter, the aim of this research was to analyze the gut-microbiota composition of German Shepherd in comparison to other canine mixed breeds not described as the suffering of depression disease.

2. Materials and Methods

2.1. Animals

Twenty-seven dogs were recruited from dog breeders (12 German Shepherds GS) and private owners (15 mixed breeds MB), with an age between 2–8 years. At the sampling time of their feces, all dogs were clinically healthy and had not received any medications that could have affected the gut microbiota in the 4 months before the sampling. Besides, all investigated dogs were fed with an industrial diet. The chemical composition of industrial feed was crude protein (18–21% of total content), crude fat (8–10%), crude fiber (3–5%), crude ash (7%) and 10% of moisture.

2.2. DNA Extraction from Stool Samples

A small sample of feces (i.e., 0.5–2 g) was collected from each of the 25 dogs into 2 mL sterile plastic tubes. The DNA was extracted from each sample using a bead-beating procedure as previously described De Cesare et al. 2017 [14].

2.3. Library Preparation and Sequencing

The libraries were prepared and then sequenced as described by De Cesare et al. 2019 [15]. All sequences were analyzed using MG-RAST (<http://metagenomics.anl.gov/>). After applying the quality control procedure, following the instructions of the MG-RAST manual, the taxonomic classification of the sequencing data was performed by applying the Best Hit Classification method and using the M5RNA database. All metagenomes deposited in MG-RAST are public under the project labelled as Prova Cani (<https://www.mg-rast.org/mgmain.html?mgpage=project&project=mgp86824>) and detailed in Table S1 (Supplement Material).

2.4. Statistical Analysis

The reads obtained from each sample were analyzed by using MG-RAST (<https://www.mg-rast.org/>) and the values of the relative frequency of abundance of each taxonomic level within each animal were compared using the *t*-test of Tukey-Kramer in the software Statistical Analysis of Metagenomic Profile (STAMP) v 2.0.9. *p* values < 0.05 were considered statistically significant. Alpha and beta diversity analysis were performed in Python 3.6.3. Alpha diversity was calculated using an in-house pipeline that computes the indices from the normalized read counts. Alpha diversities of different

animals were compared using the Student's *t*-test. *p* values were adjusted for multiple testing using the Benjamini-Hochberg procedure. Bray-Curtis beta diversity and Principal Coordinate analysis were computed using scikit-bio 0.4.2. Heat maps and complete linkage clustering dendrograms based on beta diversity were obtained with scipy 1.1.0.

3. Results

The most abundant phyla identified in the dog feces were represented by Firmicutes, Bacteroidetes and Fusobacteria (Table 1). The phylum Bacteroidetes was significantly higher in MB in comparison to GS, while Proteobacteria was significantly higher in GS rather than in MB (Table 1). The most abundant classes were Bacteroidia, Clostridia and Fusobacteria (Table 2). Among the classes with a relative mean frequency of abundance >0.1%, Bacilli, Deltaproteobacteria and Gammaproteobacteria were significantly higher in GS in comparison to MB. On the contrary, Bacteroidia and Negativicutes were significantly higher in MB rather than in GS (Table 2). The most abundant orders were Bacteroidales, Clostridiales, Lactobacillales and Fusobacteriales. The order Bacteroidales, as well as Selenomonadales, was significantly higher in MB than in GS, while Lactobacillales and Desulfovibrionales were significantly higher in GS in comparison to MB (Table 3).

Table 1. Mean relative frequency of abundance (%) and corresponding standard deviation (SD) (%) of the phyla identified in the caeca of mixed breeds (MB) and German Shepherd (GS) dogs. The species indicated are those showing a mean relative frequency of abundance $\geq 0.1\%$ in at least one group. *p* values in bold are those significantly different.

Phylum	MB		GS		<i>p</i> Values
	Mean (%)	std. dev. (%)	Mean (%)	PT std. dev. (%)	
<i>Firmicutes</i>	55.247	13.880	55.695	14.042	0.937
<i>Fusobacteria</i>	16.599	9.218	11.296	6.569	0.106
<i>Bacteroidetes</i>	21.306	11.162	10.742	10.611	0.024
<i>Actinobacteria</i>	3.608	2.868	3.453	1.827	0.871
<i>Proteobacteria</i>	0.540	0.293	1.916	0.756	0.000

Table 2. Mean relative frequency of abundance (%) and corresponding standard deviation (SD) (%) of the classes identified in the caeca of mixed breeds (MB) and German Shepherd (GS) dogs. The species indicated are those showing a mean relative frequency of abundance $\geq 0.1\%$ in at least one group. *p* values in bold are those who are significantly different.

Class	MB		GS		<i>p</i> Values
	Mean (%)	std. dev. (%)	Mean (%)	std. dev. (%)	
<i>Actinobacteria</i>	3.608	2.868	3.453	1.827	0.871
<i>Bacteroidia</i>	21.298	11.157	10.724	10.603	0.023
<i>Clostridia</i>	39.318	11.190	33.425	8.456	0.146
<i>Bacilli</i>	2.019	1.476	14.761	7.947	0.000
<i>Erysipelotrichi</i>	8.471	7.689	4.664	3.423	0.113
<i>Negativicutes</i>	5.439	3.881	2.846	1.723	0.037
<i>Fusobacteria</i>	16.599	9.218	11.296	6.569	0.106
<i>Deltaproteobacteria</i>	0.158	0.217	0.973	0.504	0.000
<i>Gammaproteobacteria</i>	0.161	0.169	0.825	0.681	0.008
<i>Epsilonproteobacteria</i>	0.161	0.225	0.022	0.016	0.037

The most abundant families in the tested feces were Prevotellaceae, Bacteroidaceae, Clostridiaceae and Fusobacteriaceae (Table 4). The abundance of the families Bacteroidaceae, Ruminococcaceae and Veillonellaceae were significantly higher in MB than in GS. On the contrary Microbacteriaceae, Streptococcaceae, Lactobacillaceae, Aerococcaceae and Enterococcaceae were significantly higher in GS in comparison to MB (Table 4).

Table 3. Mean relative frequency of abundance (%) and corresponding standard deviation (SD) (%) of the orders identified in the caeca of mixed breeds (MB) and German Shepherd (GS) dogs. The species indicated are those showing a mean relative frequency of abundance $\geq 0.1\%$ in at least one group. *p* values in bold are those significantly different.

Order	MB		GS		<i>p</i> Values
	Mean (%)	std. dev. (%)	Mean (%)	std. dev. (%)	
<i>Coriobacteriales</i>	3.502	2.839	2.471	0.991	0.222
<i>Actinomycetales</i>	0.081	0.044	0.946	1.758	0.131
<i>Bacteroidales</i>	21.298	11.157	10.724	10.603	0.023
<i>Clostridiales</i>	39.302	11.187	33.404	8.453	0.146
<i>Lactobacillales</i>	1.182	1.403	13.530	7.905	0.000
<i>Erysipelotrichales</i>	8.471	7.689	4.664	3.423	0.113
<i>Selenomonadales</i>	5.439	3.881	2.846	1.723	0.037
<i>Bacillales</i>	0.837	0.635	1.231	0.682	0.153
<i>Fusobacteriales</i>	16.599	9.218	11.296	6.569	0.106
<i>Desulfovibrionales</i>	0.111	0.147	0.952	0.503	0.000
<i>Enterobacteriales</i>	0.028	0.035	0.395	0.289	0.001
<i>Aeromonadales</i>	0.121	0.164	0.226	0.273	0.273

Table 4. Mean relative frequency of abundance (%) and corresponding standard deviation (SD) (%) of the families identified in the caeca of mixed breeds (MB) and German Shepherd (GS) dogs. The species indicated are those showing a mean relative frequency of abundance $\geq 0.1\%$ in at least one group. *p* values in bold are those who are significantly different.

Family	MB		GS		<i>p</i> Values
	Mean (%)	std. dev. (%)	Mean (%)	std. dev. (%)	
<i>Coriobacteriaceae</i>	3.502	2.839	2.471	0.991	0.222
<i>Microbacteriaceae</i>	0.030	0.029	0.587	0.907	0.067
<i>Micrococcaceae</i>	0.002	0.002	0.117	0.377	0.333
<i>Corynebacteriaceae</i>	0.002	0.001	0.042	0.104	0.222
<i>Prevotellaceae</i>	11.113	9.461	6.368	6.892	0.160
<i>Bacteroidaceae</i>	10.010	7.919	3.891	5.036	0.027
<i>Porphyromonadaceae</i>	0.165	0.209	0.448	0.315	0.019
<i>Clostridiaceae</i>	15.448	7.344	14.324	4.907	0.651
<i>Ruminococcaceae</i>	8.874	2.640	6.695	2.128	0.031
<i>Erysipelotrichaceae</i>	8.471	7.689	4.664	3.423	0.113
<i>Veillonellaceae</i>	3.811	3.828	0.908	0.560	0.014
<i>Lachnospiraceae</i>	3.810	2.160	2.799	1.241	0.155
<i>Eubacteriaceae</i>	1.990	1.349	1.766	1.604	0.714
<i>Acidaminococcaceae</i>	1.628	2.117	1.938	1.537	0.675
<i>Streptococcaceae</i>	0.608	1.407	3.440	2.936	0.010
<i>Paenibacillaceae</i>	0.525	0.472	0.640	0.371	0.501
<i>Lactobacillaceae</i>	0.417	0.442	8.616	7.893	0.005
<i>Bacillaceae</i>	0.281	0.383	0.222	0.085	0.584
<i>Peptostreptococcaceae</i>	0.215	0.127	0.148	0.063	0.097
<i>Aerococcaceae</i>	0.132	0.182	0.640	0.344	0.000
<i>Peptococcaceae</i>	0.091	0.069	0.334	0.201	0.002
<i>Enterococcaceae</i>	0.015	0.035	0.661	0.368	0.000
<i>Thermoactinomycetaceae</i>	0.010	0.010	0.136	0.248	0.120
<i>Leuconostocaceae</i>	0.008	0.017	0.131	0.152	0.021
<i>Clostridiales Family XII. Incertae Sedis</i>	0.008	0.020	0.177	0.116	0.001
<i>Listeriaceae</i>	0.001	0.001	0.180	0.577	0.324
<i>Fusobacteriaceae</i>	16.599	9.218	11.296	6.569	0.106
<i>Helicobacteraceae</i>	0.124	0.217	0.003	0.002	0.056
<i>Desulfohalobiaceae</i>	0.110	0.147	0.950	0.503	0.000
<i>Succinivibrionaceae</i>	0.090	0.155	0.223	0.272	0.168
<i>Enterobacteriaceae</i>	0.028	0.035	0.395	0.289	0.001

The most abundant genera identified in the tested samples were *Prevotella*, *Bacteroides*, *Clostridium* and *Fusobacterium*. *Bacteroides*, *Megamonas* and *Selenomonas* were significantly higher in MB in comparison to GS, while *Desulfonauticus*, *Streptococcus*, *Lactobacillus*, *Aerococcus* and *Enterococcus*, were significantly higher in GS compared to MB (Table 5). The most abundant species were *Clostridium bifermentans*, *Fusobacterium mortiferum*, *Lactobacillus murinus*, *Prevotella copri* and *Blautia* sp. Ser8. In particular, the species *Lactobacillus murinus* and *Blautia* sp. Ser8 were significantly higher in GS in comparison to MB, while *Clostridium bifermentans*, *Fusobacterium mortiferum* and *Prevotella copri* were significantly higher in MB rather than in GS (Table 6). The species with a p value $\geq 0.01\%$ were uncultured bacteria, *Bacteroides plebeius*, *Fusobacterium equinum* and *Clostridium scindens*. Among all, the uncultured bacteria were significantly higher in GS in comparison to MB, while *Bacteroides uniformis* was significantly lower in GS rather than in MB (Table 6).

Table 5. Mean relative frequency of abundance (%) and corresponding standard deviation (SD) (%) of the genera identified in the caeca of mixed breeds (MB) and German Shepherd (GS) dogs. The species indicated are those showing a mean relative frequency of abundance $\geq 0.1\%$ in at least one group. p values in bold are those who are significantly different.

Genus	MB		GS		p Values
	Mean (%)	std. dev. (%)	Mean (%)	std. dev. (%)	
<i>Microbacterium</i>	0.008	0.019	0.497	0.788	0.064
<i>Anaerobiospirillum</i>	0.090	0.155	0.223	0.272	0.168
<i>Paenibacillus</i>	0.521	0.471	0.634	0.374	0.512
<i>Bacillus</i>	0.200	0.390	0.160	0.088	0.718
<i>Thermoactinomyces</i>	0.009	0.010	0.133	0.246	0.124
<i>Prevotella</i>	11.078	9.463	6.317	6.814	0.157
<i>Bacteroides</i>	10.010	7.919	3.891	5.036	0.027
<i>Parabacteroides</i>	0.080	0.199	0.119	0.138	0.570
<i>Porphyromonas</i>	0.046	0.049	0.157	0.118	0.011
<i>Barnesiella</i>	0.023	0.033	0.118	0.111	0.017
<i>Helicobacter</i>	0.124	0.217	0.003	0.002	0.056
<i>Clostridium</i>	15.007	7.362	13.934	4.942	0.668
<i>Blautia</i>	6.746	3.580	5.307	2.441	0.245
<i>Ruminococcus</i>	5.411	2.917	3.892	0.998	0.086
<i>Faecalibacterium</i>	3.290	3.013	2.432	2.158	0.415
<i>Eubacterium</i>	1.987	1.350	1.753	1.586	0.699
<i>Hespellia</i>	1.141	0.728	0.778	0.265	0.101
<i>Robinsoniella</i>	0.519	0.513	0.442	0.501	0.707
<i>Coproccoccus</i>	0.514	0.846	0.222	0.625	0.331
<i>Roseburia</i>	0.479	0.527	0.133	0.127	0.031
<i>Butyrivibrio</i>	0.369	0.485	0.369	0.132	0.999
<i>Lachnospira</i>	0.246	0.517	0.098	0.119	0.316
<i>Peptostreptococcus</i>	0.215	0.127	0.148	0.063	0.097
<i>Alkaliphilus</i>	0.201	0.427	0.093	0.097	0.372
<i>Syntrophococcus</i>	0.105	0.326	0.001	0.001	0.253
<i>Ethanoligenens</i>	0.099	0.139	0.231	0.220	0.101
<i>Butyricoccus</i>	0.096	0.063	0.122	0.064	0.337
<i>Sarcina</i>	0.082	0.305	0.162	0.364	0.569
<i>Peptococcus</i>	0.037	0.064	0.222	0.102	0.000
<i>Fusibacter</i>	0.008	0.020	0.177	0.116	0.001
<i>Collinsella</i>	2.176	1.793	1.329	0.544	0.113
<i>Slackia</i>	0.926	0.759	0.726	0.365	0.394
<i>Enterorhabdus</i>	0.233	0.197	0.222	0.068	0.845
<i>Atopobium</i>	0.140	0.115	0.130	0.065	0.797
<i>Desulfonauticus</i>	0.102	0.147	0.923	0.495	0.000
<i>Escherichia</i>	0.009	0.023	0.249	0.156	0.000

Table 5. Cont.

Genus	MB		GS		<i>p</i> Values
	Mean (%)	std. dev. (%)	Mean (%)	std. dev. (%)	
<i>Catenibacterium</i>	1.503	2.894	0.680	0.972	0.333
<i>Erysipelothrix</i>	0.481	0.827	0.436	0.334	0.853
<i>Holdemania</i>	0.100	0.293	0.139	0.128	0.667
<i>Fusobacterium</i>	16.573	9.213	11.284	6.562	0.107
<i>Streptococcus</i>	0.600	1.406	3.403	2.930	0.011
<i>Lactobacillus</i>	0.417	0.442	8.611	7.888	0.005
<i>Aerococcus</i>	0.129	0.181	0.627	0.344	0.001
<i>Enterococcus</i>	0.014	0.035	0.651	0.362	0.000
<i>Megamonas</i>	2.671	3.011	0.432	0.503	0.015
<i>Phascolarctobacterium</i>	1.176	1.325	1.365	1.042	0.692
<i>Selenomonas</i>	1.125	0.903	0.463	0.387	0.023
<i>Acidaminococcus</i>	0.452	0.848	0.572	0.529	0.668

Table 6. Mean relative frequency of abundance (%) and corresponding standard deviation (SD) (%) of the species identified in the caeca of mixed breeds (MB) and German Shepherd (GS) dogs. The species indicated are those showing a mean relative frequency of abundance $\geq 0.5\%$ in at least one group. *p* values in bold are those who are significantly different.

Species	MB		GS		<i>p</i> Values
	Mean (%)	std. dev. (%)	Mean (%)	std. dev. (%)	
<i>Phascolarctobacterium</i> sp. YIT 12067	1.176	1.325	1.365	1.042	0.692
<i>Acidaminococcus fermentans</i>	0.448	0.833	0.572	0.529	0.654
<i>Aerococcus viridans</i>	0.118	0.174	0.612	0.333	0.000
<i>Bacteroides plebeius</i>	2.619	2.635	0.370	0.456	0.007
<i>Bacteroides fragilis</i>	1.689	2.246	0.628	0.938	0.126
<i>Bacteroides stercoris</i>	0.984	0.883	0.741	1.515	0.643
<i>Bacteroides coprocola</i>	0.963	0.790	0.390	0.460	0.033
<i>Bacteroides vulgatus</i>	0.907	2.099	0.055	0.121	0.152
<i>Bacteroides uniformis</i>	0.785	0.844	0.184	0.204	0.021
<i>Bacteroides ovatus</i>	0.506	0.464	0.360	0.565	0.497
<i>Clostridium bifementans</i>	5.080	3.341	3.625	1.436	0.158
<i>Clostridium sordellii</i>	3.167	3.022	3.073	1.121	0.916
<i>Clostridium bartlettii</i>	1.769	1.213	0.904	0.366	0.022
<i>Clostridium scindens</i>	1.142	1.020	0.230	0.120	0.005
<i>Clostridium hiranonis</i>	0.979	1.494	0.329	0.980	0.203
<i>Clostridium perfringens</i>	0.226	0.370	1.738	1.664	0.012
<i>Clostridium aminobutyricum</i>	0.044	0.120	0.813	0.551	0.001
<i>Collinsella intestinalis</i>	1.840	1.534	1.121	0.482	0.116
<i>Slackia heliotrinireducens</i>	0.890	0.734	0.684	0.363	0.370
<i>Desulfonauticus autotrophicus</i>	0.101	0.147	0.923	0.495	0.000
<i>Clostridium ramosum</i>	1.684	2.043	0.499	0.608	0.055
<i>Catenibacterium mitsuokai</i>	1.503	2.894	0.680	0.972	0.333
<i>Eubacterium bifforme</i>	1.060	1.492	0.879	0.730	0.695
<i>Lactobacillus vitulinus</i>	0.823	1.583	0.348	0.688	0.326
<i>Clostridium spiroforme</i>	0.790	0.914	0.390	0.239	0.135
<i>Eubacterium cylindroides</i>	0.650	0.643	0.563	0.351	0.670
<i>Streptococcus pleomorphus</i>	0.620	0.956	0.481	0.417	0.630
<i>Eubacterium fissicatena</i>	1.079	1.098	0.397	0.176	0.037
<i>Fusobacterium nucleatum</i>	6.030	3.870	4.852	2.987	0.398
<i>Fusobacterium mortiferum</i>	2.156	1.684	1.167	0.892	0.072
<i>Fusobacterium varium</i>	2.109	1.455	1.945	1.408	0.779
<i>Fusobacterium ulcerans</i>	2.002	1.457	1.547	0.961	0.359
<i>Fusobacterium equinum</i>	1.698	1.015	0.702	0.539	0.005
<i>Fusobacterium perfoetens</i>	1.628	1.375	0.602	0.592	0.021
<i>Fusobacterium periodonticum</i>	0.794	0.851	0.418	0.612	0.211
<i>Hespellia porcina</i>	0.682	0.472	0.451	0.191	0.112
<i>Robinsoniella peoriensis</i>	0.519	0.513	0.442	0.501	0.707

Table 6. Cont.

Species	MB		GS		p Values
	Mean (%)	std. dev. (%)	Mean (%)	std. dev. (%)	
<i>Coprococcus comes</i>	0.509	0.845	0.219	0.624	0.332
<i>Lactobacillus murinus</i>	0.014	0.015	5.202	5.009	0.006
<i>Lactobacillus reuteri</i>	0.004	0.003	1.193	1.600	0.031
<i>Prevotella copri</i>	5.976	5.824	2.289	2.473	0.045
<i>Prevotella intermedia</i>	1.434	1.868	1.018	1.008	0.483
<i>Prevotella oris</i>	1.024	1.700	0.325	0.556	0.166
<i>Prevotella ruminicola</i>	0.863	1.073	0.479	0.449	0.240
<i>Prevotella falsenii</i>	0.658	0.888	0.457	0.473	0.474
<i>Prevotella nigrescens</i>	0.599	0.811	1.281	2.520	0.404
<i>Faecalibacterium prausnitzii</i>	3.290	3.013	2.432	2.158	0.415
<i>Ruminococcus gnavus</i>	2.838	3.070	0.955	0.322	0.038
<i>Ruminococcus</i> sp. 5_1_39BFAA	0.885	1.151	0.835	0.282	0.878
<i>Ruminococcus obeum</i>	0.815	0.997	0.649	0.309	0.565
<i>Ruminococcus torques</i>	0.542	0.663	0.566	0.163	0.897
<i>Ruminococcus gauvreauii</i>	0.254	0.148	0.687	0.252	0.000
<i>Streptococcus agalactiae</i>	0.247	0.633	1.630	2.194	0.065
<i>Blautia</i> sp. Ser8	6.484	3.417	5.052	2.480	0.236
<i>butyrate-producing bacterium</i> SM4/1	1.262	1.041	1.201	0.538	0.852
<i>Megamonas hypermegale</i>	2.671	3.011	0.432	0.503	0.015
<i>Selenomonas ruminantium</i>	0.957	0.804	0.449	0.391	0.050

p values of alpha diversity used in the study by different indexes in the tested groups at different taxonomic levels are reported in Table 7 and Figure 1. The Index of median values in the two groups is described in Table 8. According to Bray-Curtis beta diversity and Principal Coordinates Analysis (PCoA), the inter-sample variation highlighted a significant separation between the structural composition of the gut microbiota among the study groups (Figures 2 and 3).

Table 7. p values of alpha diversity calculated by different indexes in the tested groups at different taxonomic levels. p values in bold are those significantly different.

	Phylum	Class	Order	Family	Genus	Species
Simpson	0.139	0.326	0.231	0.003	0.004	0.142
Shannon	0.356	0.024	0.002	0.000	0.000	0.117
Pielou	0.174	0.156	0.015	0.000	0.001	0.931
Hill_1	0.356	0.023	0.003	0.000	0.002	0.068
Hill_2	0.121	0.270	0.194	0.002	0.004	0.476

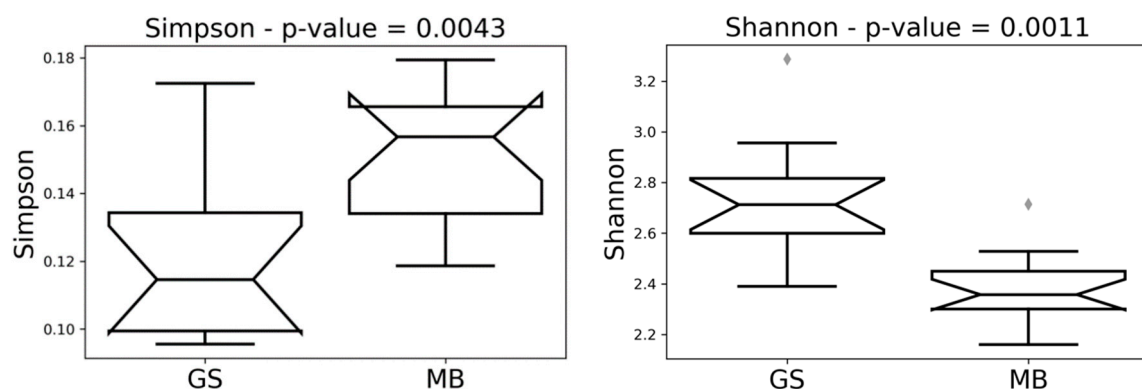


Figure 1. Cont.

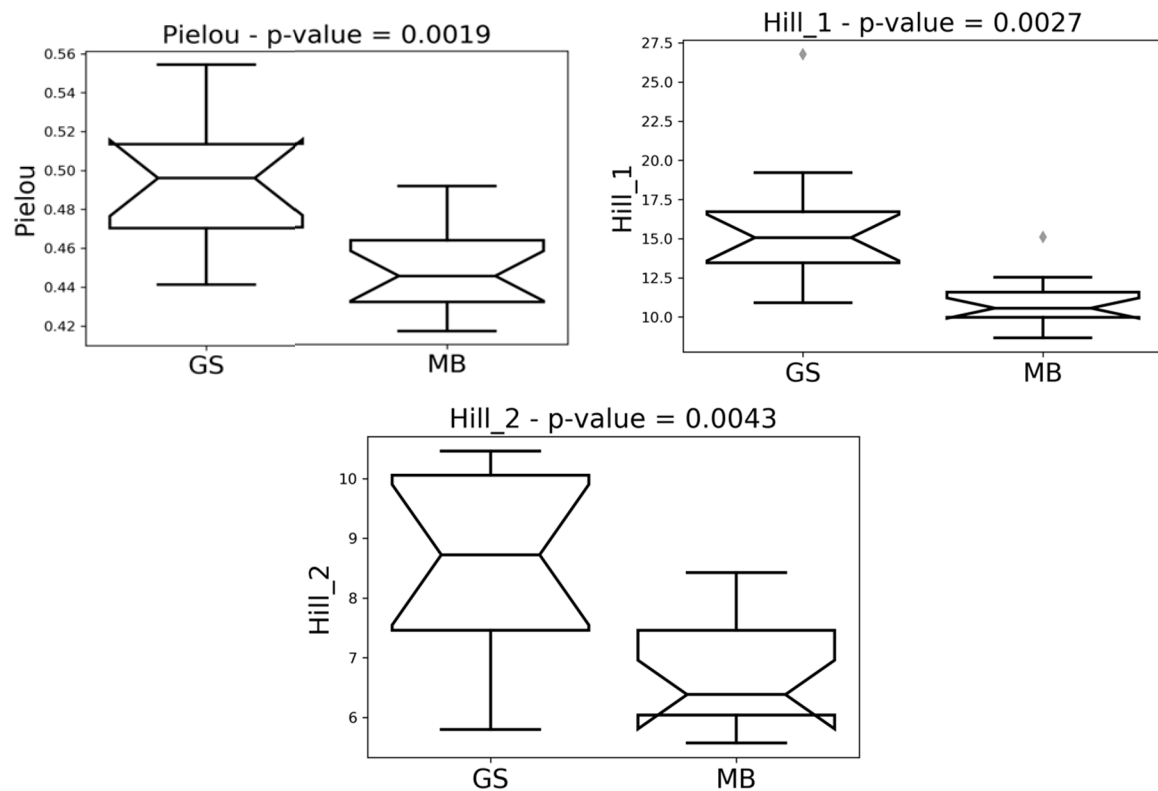


Figure 1. *p* values alpha diversity calculated by different indexes in the tested groups. GS = German Shepherd, MB = mixed breeds.

Table 8. Index of median values in two groups.

	Simpson	Shannon	Pielou	Hill_1	Hill_2
Phylum					
MB	0.446	1.010	0.420	2.792	2.382
GS	0.531	0.927	0.366	2.594	2.032
Class					
MB	0.296	1.467	0.486	4.365	3.454
GS	0.277	1.592	0.514	4.956	3.742
Order					
MB	0.296	1.482	0.388	4.434	3.456
GS	0.272	1.660	0.421	5.308	3.809
Family					
MB	0.171	2.090	0.462	8.148	6.021
GS	0.135	2.445	0.523	11.752	7.618
Genus					
MB	0.150	2.379	0.449	10.895	6.771
GS	0.122	2.736	0.496	15.835	8.511
Species					
MB	0.045	3.712	0.589	41.635	22.712
GS	0.065	3.881	0.590	50.536	20.544

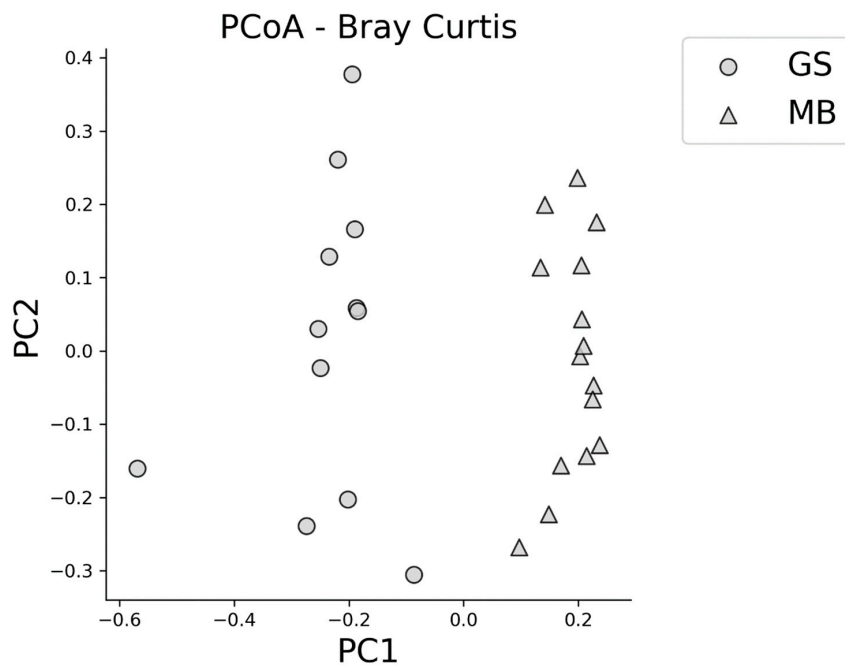


Figure 2. Beta diversity of the two study groups. Bray-Curtis beta diversity and Principal Coordinates Analysis (PCoA) the inter-sample variation highlighted a significant separation between the structural composition of the gut microbiota among study groups. (GM = German Shepherd, MB = mixed breeds).

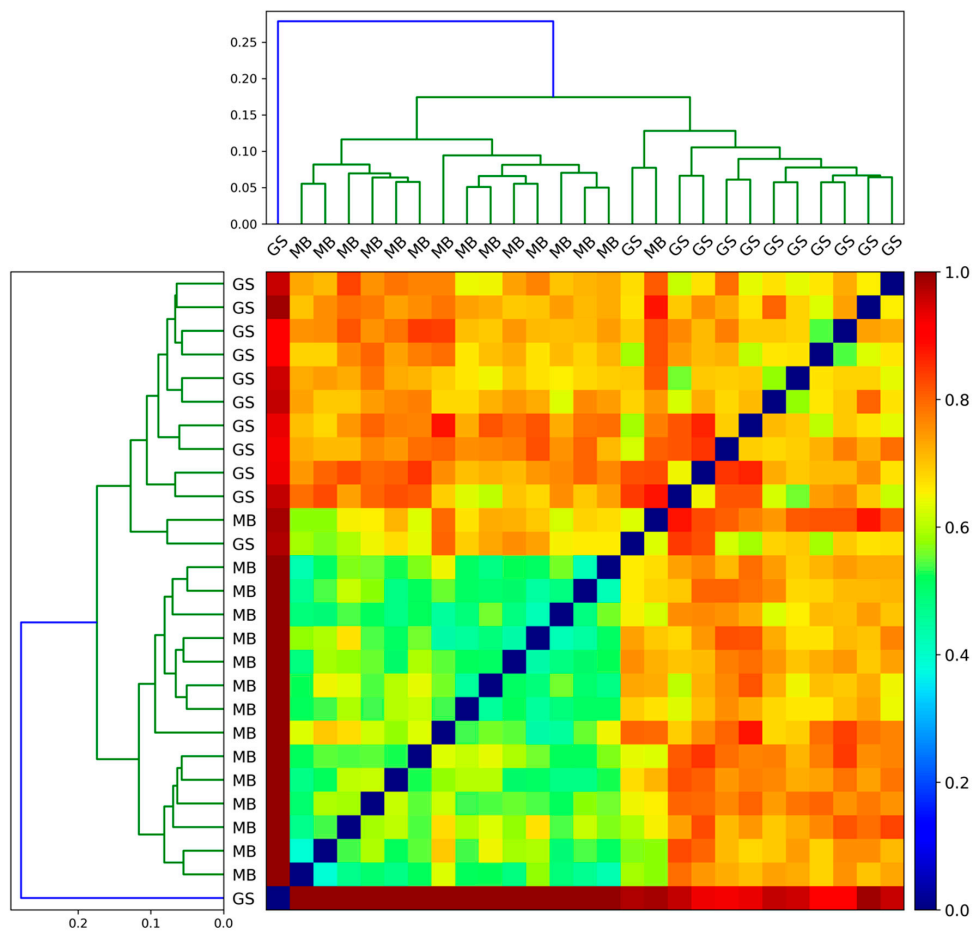


Figure 3. Heat-map diagram of the gut microbiota composition at genus level for two groups.

4. Discussion

Recently, gut-microbiota plays an important role in neuroscience research as a component of the microbiota-gut-brain axis [3]. Our current knowledge about the microbiota-depression relationship is mainly based on animal model studies because very few research has been conducted on human patients. Some findings on animal models cannot fit in with the real human condition, so it can be considered as one of the most intriguing and controversial topics [16].

In this study, the relationship between the microbiota of German Shepherds and other dog breeds was investigated for the first time. In addition to that, the microbiota of the German Shepherd group was compared with that of humans suffering from depression disorder [17]. The choice of German Shepherd is due to the results derived from the quantitative molecular evaluation of fatty acids in platelets of people with mood disorders and several animals (sheep, horse, donkey, dog, cat, bovine, rat, pig and guinea pig). This investigation was conducted using a complex mathematical function, as a Self-Organizing Map (SOM) and an index (B2). This allowed classifying the different depression disorders (major depression and bipolar depression) and identifying their molecular pathway. The data obtained from animals were put into the SOM, where human disorders were classified as pathological areas, bipolar depression area and healthy human area. The results showed that sheep, cats, bovines, horses and donkeys were distributed in the opposite area on the SOM than people with mood disorders, while, guinea pigs, rats and pigs occupied the area characterized by depressive subjects. Only two different breeds of dogs (German Shepherd and Alaskan Malamute) were collocated in the part of the map typical of humans affected by Major Depression (MD). This result was not found in any other animals [13].

In the literature, the human research that associates depression and microbiota composition is controversial. In 2015, Jiang and colleagues analyzed the gut microbiota composition of humans with a diagnosis of active major depressive disorder and responded-MDD [16]. This study evidenced that both groups have an increase in genera *Phascolarctobacterium*, *Roseburia* and *Parabacteroides* and a decrease in genera *Ruminococcus*, *Prevotella*, *Dialister* and *Faecalibacterium* [16]. The research held by Zheng et al. (2016) [18] and Valles-Colomer et al. (2019) [19] observed an increased relative abundance of *Lactobacillus* in depressed patients. Furthermore, Valles-Colomer et al. (2019) [19] found a higher prevalence of *Bacteroides* in people suffering from depression.

In this study, the gut microbiota structure of 27 dogs (12 German Shepherd and 15 mixed breeds) was analyzed. Moreover, we have compared the gut microbiota of our German Shepherds with the gut microbiota of depressed humans, previously analyzed by Jiang et al. [16]. The phylogenetic profiles of the canine gut microbiota observed in the cohort of this research were found to be aligned with those already reported in the literature for healthy dogs [20,21] but with a slightly higher abundance of Proteobacteria and a lower abundance of Bacteroidetes. According to our results, the gut microbiota of the GS group is characterized by higher fecal microbial diversity, estimated using the Shannon Index, rather than the MB group. Interestingly, gut microbiota diversity has been usually considered beneficial for human health but some studies have denied this statement [19,22]. The GS group was characterized by an enrichment of *Streptococcus* and *Lactobacillus* genera compared to the MB group. Bacteria belonging to the genus *Streptococcus* are known as serotonin producers, while those belonging to *Lactobacillus* are gamma-aminobutyric acid (GABA) producers. These molecules are recognized as neurotransmitters, able to regulate emotional behaviors [23–25]. Moreover, the GS group was characterized by a decrease in the *Bacteroides* genus, associated with metabolic diseases quite common in depression [26]. Furthermore, in the Firmicutes phylum, the abundance of the Ruminococcaceae family was significantly lower in the GS than in MB. Studies on mice reported a correlation between the Ruminococcaceae family and behavioral changes [27]. An overgrowth of the Gammaproteobacteria class, particularly Enterobacteriales and Enterobacteriaceae, was also detected in the GS group compared to MB. The Enterobacteriaceae includes enteric pathogens, which induce to inflammatory state and an increase of gut wall permeability, allowing bacteria to translocate

into mesenteric lymph nodes or in the systemic circulation [28]. This leads to an increase of plasma immunoglobulin A and/or M, which is common in depressed patients [29].

The results obtained in this research represent an important step for future studies on depression, as they demonstrate that the dog can be a good animal model for mood disorders in humans [30]. The similarities between humans and dogs are due to the coexistence of these two species for over 14 thousand years [31]. The canine species might be considered as an ideal model for the study of human depression also because of the structural and functional similarities found between the canine and the human gut microbiota. Thus, in this case, the studies about dogs provide a benefit both directly for dogs and for their potential to be generalized to humans [32].

5. Conclusions

The results of this research demonstrate that German Shepherd's gut microbiota have a different composition compared to other dog breeds and its microbiota is like that of depressed human beings. A comparative analysis on gut-microbiota of German Shepherd and humans affected by depression showed a close resemblance in the bacterial composition that led us to talk about bacterial geography. Moreover, this study confirms the molecular affinity between German Shepherd and humans suffering from depression. We consider it appropriate to deepen these aspects in the light of the recent document about consciousness in the animal kingdom ("*Cambridge Declaration on Consciousness*" in 2012 (<http://nonhumanrights.net/>), "*Document of Bologna*" in 2014 (The Document of Bologna was underwritten by Bernroider, Cocchi, Gabrielli, Globus, Malik, Mender, Mullis, Pessa, Pregnotato, Pylkkanen, Rasenick, Tonello, Tuszyński, Vitiello, Werneke, Zizzi in research institute for the quantitative and quantum dynamics of living organisms on June 20, 2014)). Future investigations about the interaction between gut microbiota and brain axis in animals and humans will clarify any doubts over this bidirectional communication.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2076-3417/10/2/573/s1>, Table S1. Metagenomes tested in this study and public available in MG RAST (<https://www.mg-rast.org/mgmain.html?mgpage=project&project=mgp86824>).

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used conceptualization, E.M. and M.C.; methodology, A.D.C. and G.M. (Gerardo Manfreda); software, G.M. (Gerardo Manfreda) and A.D.C.; validation, A.D.C. and G.M. (Giovanna Marliani); formal analysis, C.S.; investigation, M.C.; data curation, A.D.C. and E.M.; writing—original draft preparation, E.M. and G.M. (Giovanna Marliani); writing—review and editing, E.M. and A.D.C.; visualization, P.A.A.; supervision, M.C.; project administration, G.C. and M.C. All authors have read and agreed to the published version of the manuscript

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