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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 adrenocorticotropic 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), whit an age between 2–8 years. At the sampling time of their faces, 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 ≥0.1% in at least one group. *p* values in bold are those significantly different.

	MB		GS		
Phylum	**Mean (%)**	**std. dev. (%)**	**Mean (%)**	**PT std. dev. (%)**	***p* Values**
Firmicutes	55.247	13.880	55.695	14.042	0.937
Fusobacteria	16.599	9.218	11.296	6.569	0.106
Bacteroidetes	21.306	11.162	10.742	10.611	0.024
Actinobacteria	3.608	2.868	3.453	1.827	0.871
Proteobacteria	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 ≥0.1% in at least one group. *p* values in bold are those who are significantly different.

	MB		GS		
Class	**Mean (%)**	**std. dev. (%)**	**Mean (%)**	**std. dev. (%)**	***p* Values**
Actinobacteria	3.608	2.868	3.453	1.827	0.871
Bacteroidia	21.298	11.157	10.724	10.603	0.023
Clostridia	39.318	11.190	33.425	8.456	0.146
Bacilli	2.019	1.476	14.761	7.947	0.000
Erysipelotrichi	8.471	7.689	4.664	3.423	0.113
Negativicutes	5.439	3.881	2.846	1.723	0.037
Fusobacteria	16.599	9.218	11.296	6.569	0.106
Deltaproteobacteria	0.158	0.217	0.973	0.504	0.000
Gammaproteobacteria	0.161	0.169	0.825	0.681	0.008
Epsilonproteobacteria	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 ≥ 0.1% in at least one group. *p* values in bold are those significantly different.

	MB		GS		
Order	**Mean (%)**	**std. dev. (%)**	**Mean (%)**	**std. dev. (%)**	***p* Values**
Coriobacteriales	3.502	2.839	2.471	0.991	0.222
Actinomycetales	0.081	0.044	0.946	1.758	0.131
Bacteroidales	21.298	11.157	10.724	10.603	0.023
Clostridiales	39.302	11.187	33.404	8.453	0.146
Lactobacillales	1.182	1.403	13.530	7.905	0.000
Erysipelotrichales	8.471	7.689	4.664	3.423	0.113
Selenomonadales	5.439	3.881	2.846	1.723	0.037
Bacillales	0.837	0.635	1.231	0.682	0.153
Fusobacteriales	16.599	9.218	11.296	6.569	0.106
Desulfovibrionales	0.111	0.147	0.952	0.503	0.000
Enterobacteriales	0.028	0.035	0.395	0.289	0.001
Aeromonadales	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 ≥ 0.1% in at least one group. *p* values in bold are those who are significantly different.

	MB		GS		
Family	**Mean (%)**	**std. dev. (%)**	**Mean (%)**	**std. dev. (%)**	***p* Values**
Coriobacteriaceae	3.502	2.839	2.471	0.991	0.222
Microbacteriaceae	0.030	0.029	0.587	0.907	0.067
Micrococcaceae	0.002	0.002	0.117	0.377	0.333
Corynebacteriaceae	0.002	0.001	0.042	0.104	0.222
Prevotellaceae	11.113	9.461	6.368	6.892	0.160
Bacteroidaceae	10.010	7.919	3.891	5.036	0.027
Porphyromonadaceae	0.165	0.209	0.448	0.315	0.019
Clostridiaceae	15.448	7.344	14.324	4.907	0.651
Ruminococcaceae	8.874	2.640	6.695	2.128	0.031
Erysipelotrichaceae	8.471	7.689	4.664	3.423	0.113
Veillonellaceae	3.811	3.828	0.908	0.560	0.014
Lachnospiraceae	3.810	2.160	2.799	1.241	0.155
Eubacteriaceae	1.990	1.349	1.766	1.604	0.714
Acidaminococcaceae	1.628	2.117	1.938	1.537	0.675
Streptococcaceae	0.608	1.407	3.440	2.936	0.010
Paenibacillaceae	0.525	0.472	0.640	0.371	0.501
Lactobacillaceae	0.417	0.442	8.616	7.893	0.005
Bacillaceae	0.281	0.383	0.222	0.085	0.584
Peptostreptococcaceae	0.215	0.127	0.148	0.063	0.097
Aerococcaceae	0.132	0.182	0.640	0.344	0.000
Peptococcaceae	0.091	0.069	0.334	0.201	0.002
Enterococcaceae	0.015	0.035	0.661	0.368	0.000
Thermoactinomycetaceae	0.010	0.010	0.136	0.248	0.120
Leuconostocaceae	0.008	0.017	0.131	0.152	0.021
Clostridiales Family XII. Incertae Sedis	0.008	0.020	0.177	0.116	0.001
Listeriaceae	0.001	0.001	0.180	0.577	0.324
Fusobacteriaceae	16.599	9.218	11.296	6.569	0.106
Helicobacteraceae	0.124	0.217	0.003	0.002	0.056
Desulfohalobiaceae	0.110	0.147	0.950	0.503	0.000
Succinivibrionaceae	0.090	0.155	0.223	0.272	0.168
Enterobacteriaceae	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 ≥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 ≥ 0.1% in at least one group. p values in bold are those who are significantly different.

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

Table 5. *Cont.*

	MB		GS		
Genus	**Mean (%)**	**std. dev. (%)**	**Mean (%)**	**std. dev. (%)**	***p* Values**
Catenibacterium	1.503	2.894	0.680	0.972	0.333
Erysipelothrix	0.481	0.827	0.436	0.334	0.853
Holdemania	0.100	0.293	0.139	0.128	0.667
Fusobacterium	16.573	9.213	11.284	6.562	0.107
Streptococcus	0.600	1.406	3.403	2.930	0.011
Lactobacillus	0.417	0.442	8.611	7.888	0.005
Aerococcus	0.129	0.181	0.627	0.344	0.001
Enterococcus	0.014	0.035	0.651	0.362	0.000
Megamonas	2.671	3.011	0.432	0.503	0.015
Phascolarctobacterium	1.176	1.325	1.365	1.042	0.692
Selenomonas	1.125	0.903	0.463	0.387	0.023
Acidaminococcus	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 ≥0.5% in at least one group. *p* values in bold are those who are significantly different.

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

Table 6. *Cont.*

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

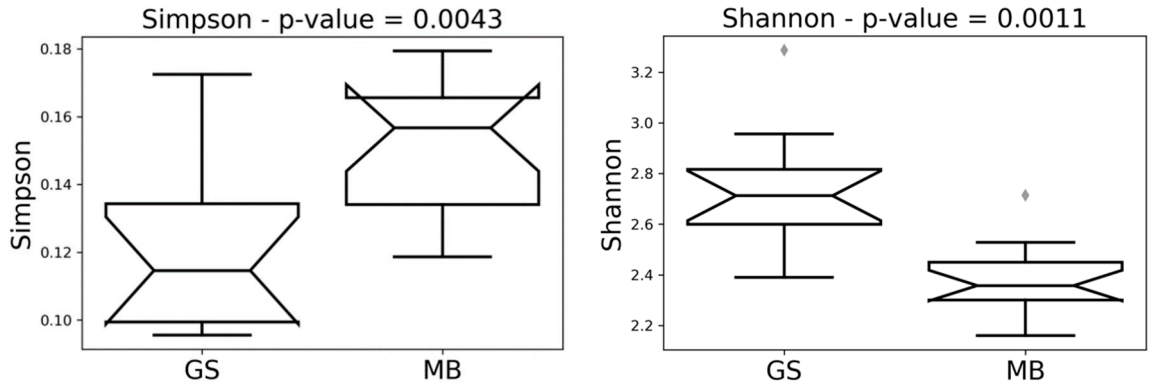

Figure 1. *Cont.*

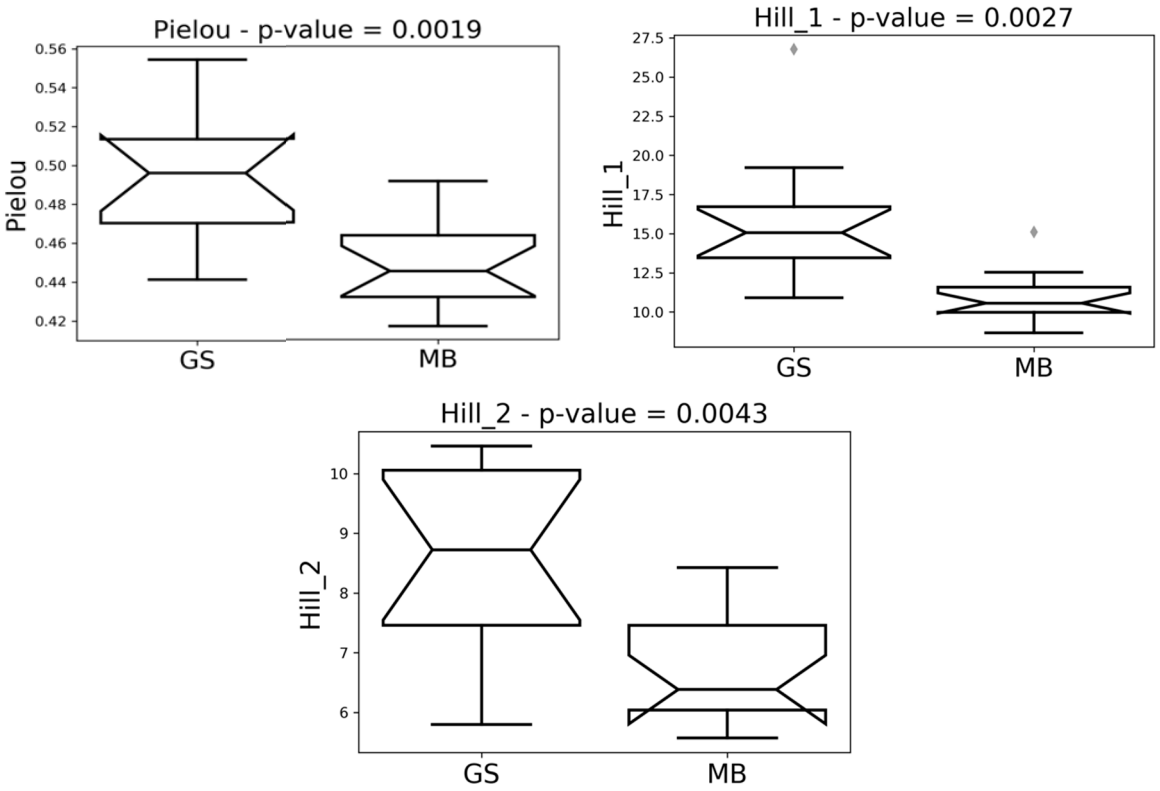

Figure 1. *p* values alpha diversity calculated by different indexes in the tested groups. GS = German Shepherd, MB = mixed breeds.

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

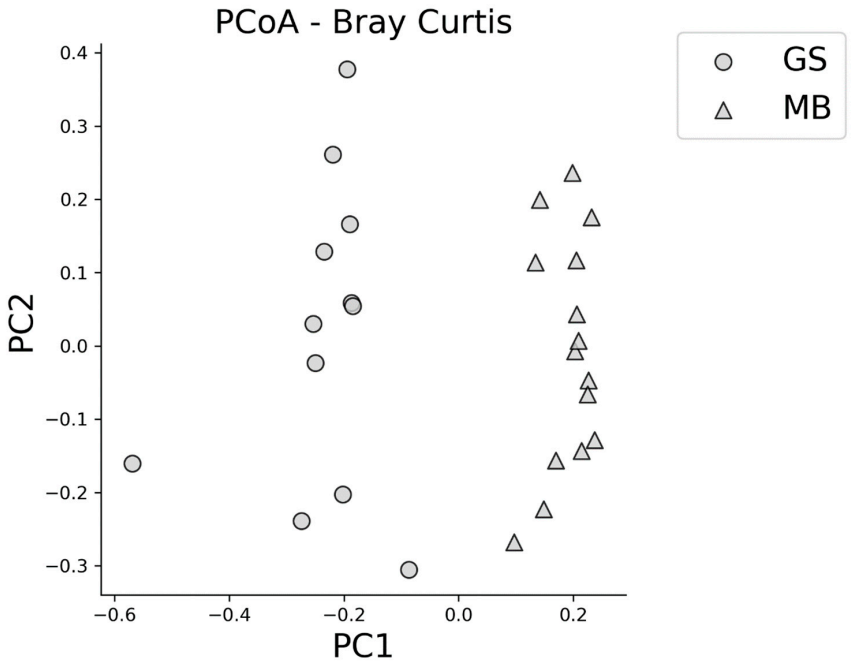

Figure 2. Beta diversity of the two study groups. Bray-Curtis beta diversity and Principal Coordinates Analysis (PCoA) the inter-sample variation highlighted a significant separation between the structural composition of the gut microbiota among study groups. (GM = German Shepherd, MB = mixed breeds).

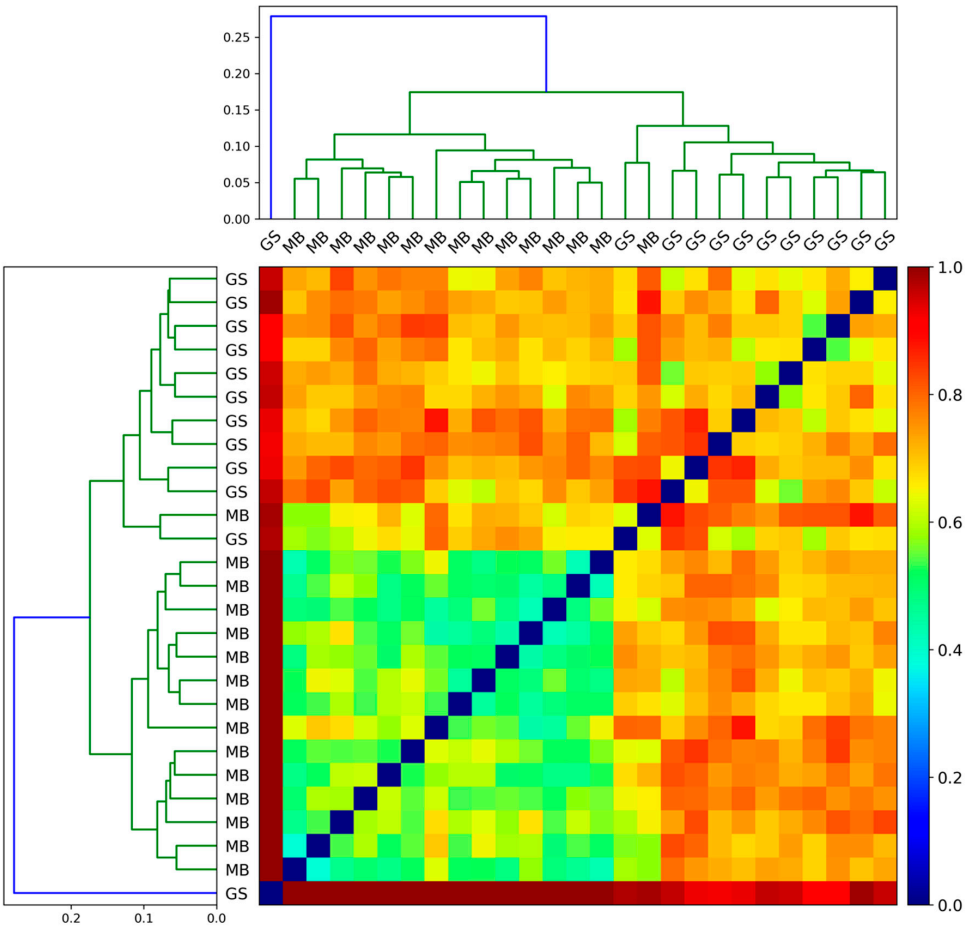

Figure 3. Heat-map diagram of the gut microbiota composition at genus level for two groups.

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, Pregnolato, Pylkkanen, Rasenick, Tonello, Tuszynski, 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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