Salamon *et al. BMC Biology* (2025) 23:352
https://doi.org/10.1186/s12915-025-02410-9

BMC Biology

RESEARCH

Open Access

Human-like associations between gut microbiome composition and inattention, hyperactivity, and impulsivity in dogs

Attila Salamon[1,2,3*], Attila Szabó[4,5], Tamás Felföldi[6], Soufiane Bel Rhali[3,6,7], Attila Andics[2,8], Ádám Miklósi[1,3], Enikő Kubinyi[2,3,9†] and Márta Gácsi[1,2,3†]

Abstract

Background Evidence suggests a link between attention-deficit/hyperactivity disorder (ADHD) and the gut microbiome in humans. Dogs are a valuable model for ADHD research, as their gut microbiome more closely resembles the human gut microbiome in composition and functional overlap compared to rodent models. This study investigated potential associations between dogs' ADHD-related traits and gut microbiome composition/diversity. We assessed inattention, Hyperactivity, impulsivity, and related functional impairments of 164 family dogs using the validated Dog ADHD and Functional Rating Scale, and analysed bacterial 16S rRNA gene sequences from their faecal samples to assess the gut microbiome composition.

Results Higher relative abundance of members of the family *Prevotellaceae* and genus *Prevotella* was associated with lower inattention and inattention-related functional impairment scores by both decision tree and Generalized Linear Model analyses.

Hyperactivity, impulsivity, and related functional impairments were found to be moderated by age, suggesting that these traits are predominantly age-related and only secondarily influenced by microbiome composition.

ADHD total score was negatively associated with *Erysipelotrichaceae* and positively associated with *Alloprevotella*.

Dogs with higher functional impairment scores had lower alpha-diversity in their gut microbiome, probably indicating reduced microbial health.

Conclusions This is the first study that found negative associations between inattention and the relative abundance of *Prevotellaceae* in dogs, which parallels findings in human studies. Our current correlational results in family dogs represent only the first step in gaining more insight into the interplay of gut microbiome and neurodevelopmental processes in non-human animals.

Keywords Gut-brain axis, Microbiota, Behavioural disorders, *Prevotellaceae*, *Firmicutes*

[†] Enikő Kubinyi and Márta Gácsi contributed equally to this work.

*Correspondence:
Attila Salamon
dr.attila.salamon@gmail.com; salamon.attila@ttk.elte.hu
Full list of author information is available at the end of the article

Background

Attention-deficit/hyperactivity disorder (ADHD) is a significant human mental health problem, which is characterized by developmentally impaired inattention, hyperactivity, and impulsivity symptoms [3, 62]. Inattention refers to a diminished capacity to maintain focus on tasks, coupled with distractibility, forgetfulness, disorganization, and a tendency to avoid activities demanding prolonged effort [3, 58]. Hyperactivity is marked by excessive motor activity, frequently evident through constant restlessness, fidgeting, and challenges in staying still or seated in situations where such composure is anticipated [3, 29]. Finally, impulsivity is identified by a proclivity to act on immediate urges or stimuli without adequate forethought and conscious judgement, distinguishing it from individuals with comparable levels of knowledge and capability [3, 5].

Dogs were demonstrated to naturally exhibit symptoms similar to human neurodevelopmental disorders [2, 84], including ADHD [11, 12]. Different questionnaires were developed to assess ADHD-related characteristics in family dogs (reviewed in [19]), but only a few recent studies attempted to assess the functional impairment effects related to dogs' inattention, hyperactivity, and impulsivity in their everyday life or a training environment [20, 21]. It is important to note that the first steps for human analogue diagnostics have already been taken for assessing ADHD-like behaviour in dogs [20, 31]. These studies found that inattention correlated negatively with age, positively with neutering status and negatively with training level [19, 20, 50]. Hyperactivity was negatively associated with age [20, 76], while impulsivity correlated negatively with age and training level [20, 76]. Csibra et al. [20] also found correlations between some functional impairment scores of the ADHD factors and age, sex, neutering status, and training level.

In humans, the aetiology and pathophysiology of ADHD include genetic (e.g. [26, 55]) and environmental (e.g. [55, 74]) risk factors, including the gut microbiome (e.g. [13, 14]). The gut microbiome can affect the development and function of the brain through the brain-gut-microbiome axis [52], which is a bidirectional communication network. Possible mechanisms by which the gut microbiome could affect brain development and function involve microbial metabolites, amino acid metabolites, immune factors, and neurotransmitters [9, 51, 52]. There is evidence that several neurotransmitters (some involved in ADHD symptoms) can be produced or their production can be influenced by gut microbiota. For example, some *Lactobacillus* produce acetylcholine; *Lactobacillus* and *Bifidobacterium* produce gamma-aminobutyric acid (GABA); *Bacillus*, *Serratia*, *Morganella*, and *Klebsiella* produce dopamine; *Escherichia*, *Bacillus*, and *Saccharomyces* produce noradrenaline; and *Candida*, *Streptococcus*, *Escherichia*, *Morganella*, *Hafnia*, and *Enterococcus* produce serotonin [17, 33, 56].

The gut microbiota composition of dogs is partly similar to that of humans [23, 37, 39], presumably because they live in the same environment and may also have a partially similar diet (in terms of higher starch/carbohydrate content), even though depending on the diet, certain bacterial taxa differ in both species [1, 23]. Interestingly, the dog gut microbiome more closely resembles the human gut microbiome in composition and functional overlap compared to rodent models [2, 23, 37]. Coelho et al. [23] showed on the phylum level that the mouse gut microbiome is composed of more *Firmicutes* and less *Actinobacteria* and *Proteobacteria* compared to the human and dog gut microbiomes. Alessandri et al. [1] reported that 23 genera were present in both dogs' and humans' gut microbiomes. From these, *Bacteroides* was the only one with a > 10% relative abundance in both species, but *Faecalibacterium*, *Blautia*, *Romboutsia*, *Clostridium* sensu stricto *1*, *Sutterella*, and *Escherichia-Shigella* were also present with > 1% relative abundance in at least one of the species. Ito et al. [39] found that *Blautia* and *Streptococcus* were among the top five dominant genera in both dog and human gut microbiomes, and further genera, such as *Bacteroides*, *Erysipelatoclostridium*, *Romboutsia*, and *Escherichia-Shigella*, were also present in both species in lower abundance. From these bacteria inhabiting the gut of both humans and dogs, some may produce similar neurotransmitters and/or metabolites, which may similarly influence the development and function of the brain in both species (e.g. [31, 42]).

Compared to typical controls, in ADHD patients, some microbial taxa were detected with higher relative abundance (e.g. families: *Peptostreptococcaceae*, *Bacteroidaceae*, *Moraxellaceae*; genera: *Fusobacterium*, *Intestinibacter*, *Desulfovibrio*) or lower relative abundance (e.g. families: *Lachnospiraceae*, *Ruminococcaceae*, *Prevotellaceae*; genera: *Coprococcus*, *Lachnoclostridium*, *Faecalibacterium*) (for reviews see [13, 14]). Some studies investigated ADHD factors separately and identified associations between the relative abundance of certain bacterial taxa and ADHD factors. Prehn-Kristensen et al. [63] found a positive correlation between *Bacteroides* and hyperactivity as well as impulsivity scores, but did not discuss these findings. Jiang et al. [40] showed a negative correlation between *Faecalibacterium* and the hyperactivity score and suggested that since *Faecalibacterium* has anti-inflammatory properties, its low relative abundance in ADHD patients may impact brain development through elevated levels of inflammatory cytokines. Szopinska-Tokov et al. [80] showed a trend of *Coprococcus*

Table 1 Previous studies examining dogs' gut microbiome communities from faeces. NR: not reported, BARF: bones and raw food

Study	*N*/sex/breed/age	Diet	Taxa	Most common (> 10% relative abundance)
Ericsson et al. [27]	16, all females lab beagles, 24–96 months	Purina Lab Diet 5006	Phyla	*Bacteroidetes* (33.43%) *Firmicutes* (33.38%) *Fusobacteria* (23.21%)
			Families	*Fusobacteriaceae* (23.20%) *Bacteroidaceae* (18.78%) *Ruminococcaceae* (13.23%)
			Genera	*Fusobacterium* (23.20%) *Bacteroides* (14.96%) *Faecalibacterium* (11.23%)
Hand et al. [34]	11, females (*N* = 7) males (*N* = 4), all miniature schnauzers, 14–136 months	dry food (*N* = 8), vet diet (*N* = 1), wet food (*N* = 1), trial diet BS0670 (*N* = 1)	Phyla	*Fusobacteria* (39.17%) *Bacteroidetes* (33.36%) *Firmicutes* (15.81%) *Proteobacteria* (11.31%)
			Genera	*Fusobacterium* (NR) *Bacteroides* (NR) *Cetobacterium* (NR)
Handl et al. [35]	12, females (*N* = 6) males (*N* = 6), 6 breeds and 5 mongrels, 7–122 months	NR	Phyla	*Firmicutes* (95.36%)
			Genera	*Clostridium* (22.73%) *Ruminococcus* (17.37%)
Kubinyi et al. [47]	29, females (*N* = 15) males (*N* = 14), 8 breeds and 12 mongrels, 36–156 months	Dry food (*N* = 8), cooked food (*N* = 1), BARF (*N* = 2), mixed diet (*N* = 18)	Phyla	*Bacteroidetes* (33.30%) *Firmicutes* (33.00%) *Fusobacteria* (24.40%)
			Genera	*Fusobacterium* (24.20%) *Bacteroides* (21.10%)
Alessandri et al. [1]	169, females (*N* = 108) males (*N* = 61), 51 breeds, 2–156 months	Dry food (*N* = 141), BARF (*N* = 28)	Phyla	*Bacteroidetes* (33.68%) *Fusobacteria* (25.53%) *Firmicutes* (23.56%)
			Genera	*Fusobacterium* (25.36%) *Prevotella 9* (13.86%) *Bacteroides* (13.43%)

2 being negatively associated with the inattention score, but did not interpret this finding.

Most studies did not find a difference in the gut microbiota diversity between ADHD patients and typical controls [13, 40, 65]. Notable exceptions include the study of Prehn-Kristensen et al. [63], who found that Shannon diversity of the gut microbiome was significantly lower in ADHD patients, and they also had different microbiota composition compared to typical controls. Fan et al. ([28], preprint) showed that the alpha-diversity estimators were all lower in predominantly inattentive children compared to control children.

There has been very little research on the relationship between ADHD and the microbiome in non-human species. In germ-free mice colonized with the faeces of ADHD patients (ADHD mice), alpha-diversity did not differ between control mice and ADHD mice, but unknown *Clostridiales* and 10 out of 17 genera from *Lachnospiraceae* were elevated in ADHD mice, and the families *Porphyromonadaceae*, *Eubacteriaceae*, *Christensenellaceae*, and *Ruminococcaceae* were less abundant in ADHD mice [82]. Interestingly, these findings do not seem to correspond with the results of human studies mentioned above. It was also found that the ADHD mice showed more anxiety in an open field test than control mice and that *Anaerostipes*, *Roseburia* (both from the *Lachnospiraceae* family), and *Ruminococcaceae UCG-004* (family *Ruminococcaceae*) all positively correlated with anxiety levels [82].

Dogs are an ideal model species for studying the phenomenon because, unlike mice, they spontaneously develop symptoms typical of human ADHD. For dogs, the validated Dog ADHD and Functionality Rating Scale (DAFRS), developed and validated by Csibra et al. [20, 21], offers a useful method for assessing their inattention, hyperactivity, impulsivity, and related functional impairments. However, in dogs, the association between the gut microbiota community and ADHD-related trait scores has not been studied yet.

In family dogs, several studies investigated the community composition of the gut (see Table 1). Most of these studies found the same three phyla (*Fusobacteria*, *Bacteroidetes*, and *Firmicutes*) and two genera (*Fusobacterium* and *Bacteroides*) with great variation in their relative

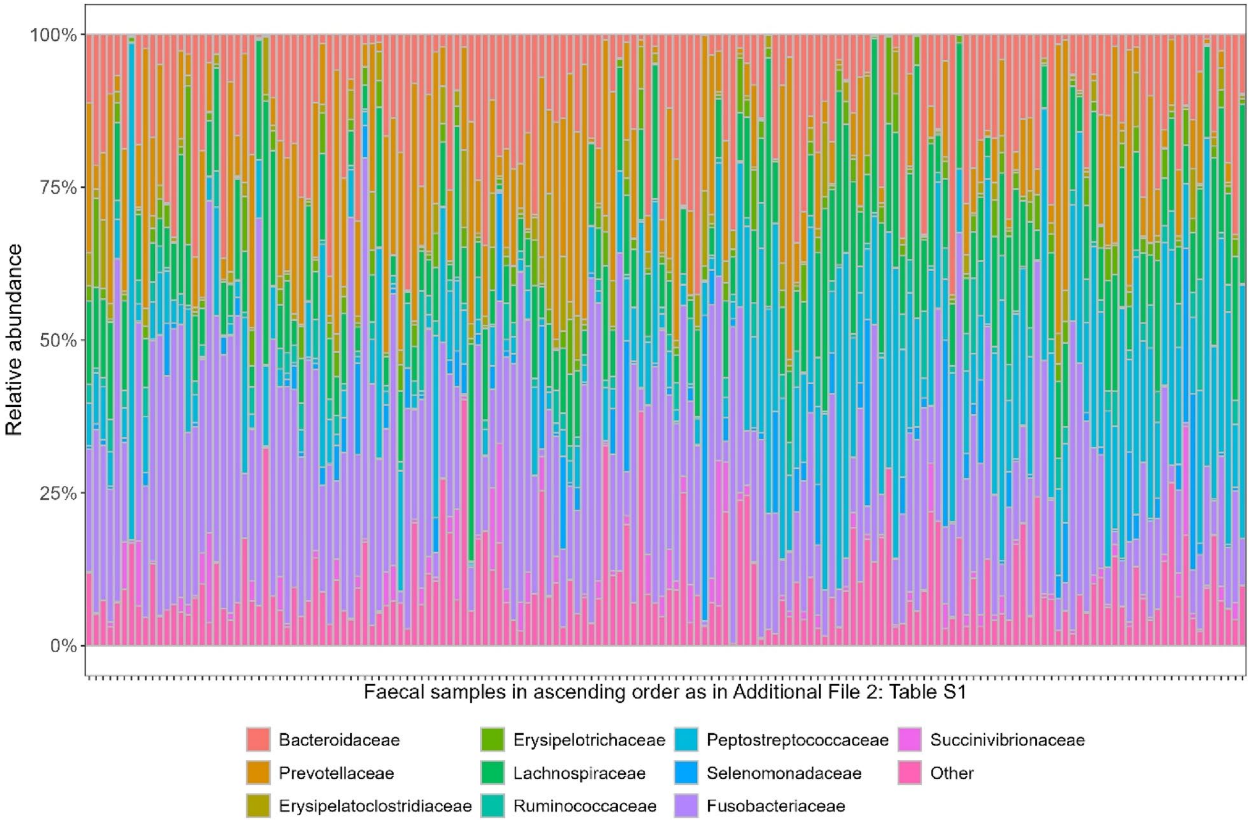

Fig. 1 Family-level gut microbiota composition in the faecal samples of 164 companion dogs. We visualized the 10 most abundant bacterial families based on Additional file 1: Table S1

abundances, except Handl et al. [35] who reported almost exclusively *Firmicutes* in dogs' faeces. The variation in the results was possibly due to a combination of the sample size, age, diet, breed, and molecular methods, and neither study could control for all of these variables.

As a first step to investigate such similarities and differences, this study aimed to examine the gut microbiome composition in a large, diverse sample of family dogs, and to identify associations between their ADHD traits and (i) the community composition of their gut microbiota and (ii) their microbial diversity. We assumed that if the functional links between microbiome composition/diversity and ADHD traits (inattention, hyperactivity, impulsivity) are based on evolutionarily relatively conserved but environment-modulated processes, then similar associations could be found in companion dogs to those reported in humans.

Results

Gut microbiome composition

A total of 7,059,096 High-quality bacterial 16S ribosomal ribonucleic acid (rRNA) gene sequences were obtained from the faecal samples (43,043 ± 13,889 reads per sample).

Three major phyla (represented by > 10% relative abundance of the total bacterial community on average) were identified in the dogs' faecal microbiome: *Firmicutes* (mean: 43.16%, standard deviation[SD] 20.50), *Bacteroidetes* (mean: 26.51%, SD 14.74) and *Fusobacteria* (mean: 24.04%, SD 15.20). Also, five major families (> 10% relative abundance on average; Fig. 1; Additional file 1: Table S1) and three main genera (> 10% relative abundance on average; Fig. 2; Additional file 1: Table S2) were identified.

Associations with ADHD scores

Decision tree analyses

In the case of inattention, on the family level, the first split in the decision tree model was based on the relative abundance of *Prevotellaceae*, yielding three groups ($F_{(2,161)} = 6.963$, $p = 0.045$; Fig. 3A; Additional file 1: Fig. S1A). Based on the relative abundance of *Prevotellaceae*, a further split was made based on *Lachnospiraceae* relative abundance ($F_{(1,80)} = 8.505$, $p = 0.041$; Fig. 3A).

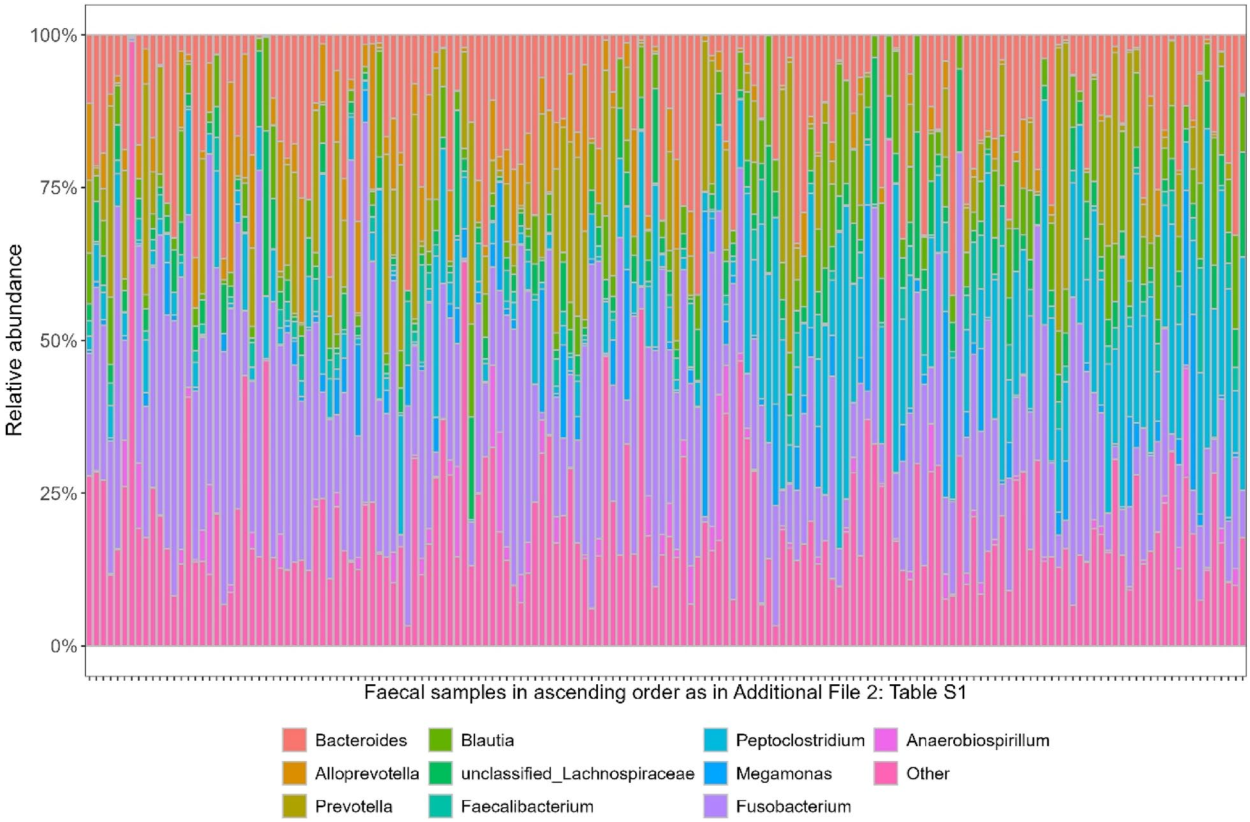

Fig. 2 Genus-level gut microbiota composition in the faecal samples of 164 companion dogs. We visualized the 10 most abundant bacterial genera based on Additional file 1: Table S2

On the genus level, the first split in the inattention score was based on the relative abundance of *Prevotella* ($F_{(1,162)}=15.679$, $\boldsymbol{p}=0.001$; Fig. 3B; Additional file 1: Fig. S1B). In the group with lower abundance of *Prevotella*, a further split was made based on *Streptococcus* relative abundance ($F_{(1,96)}=8.234$, $p=0.04$; Fig. 3B).

In the case of the inattention-related functional impairment, considering bacterial families, a split was made based on the relative abundance of *Prevotellaceae* ($F_{(1,162)}=12.486$, $p=0.005$; Fig. 4A; Additional file 1: Fig. S1C).

On the genus level, the first split in the inattention-related functional impairment score was based on the relative abundance of unclassified lineages of *Bacteroidales* ($F_{(1,162)}=11.666$, $p=0.006$; Fig. 4B; Additional file 1: Fig. S1D). In the group with the higher relative abundance of these lineages, a further split was made based on the relative abundance of unclassified lineages of *Succinivibrionaceae* ($F_{(1,113)}=10.119$, $p=0.009$; Fig. 4B).

As Hyperactivity, impulsivity, ADHD total, Hyperactivity-related functional impairment, and impulsivity-related functional impairment scores were split predominantly by age and only secondarily by microbiome, the results and decision trees related to these factors are presented in Additional file 1 (see Figures S2-S6, respectively). The correlation between age and the ADHD scores can also be found in Additional file 1: Table S3.

Generalized linear model analyses

The main findings of the decision trees were supported by the Generalized Linear Model (GzLM) results, and further associations were revealed. Despite the numerous significant associations found between ADHD scores and microbiome taxons in the models (see Additional file 1: Table S4 for family level and Additional file 1: Table S5 for genus level), we excluded several of them based on their High uncertainty due to their wide 95% Wald confidence intervals. We only present the results of highly certain associations (see Fig. 5). Microbial effects were stronger than demographic/other effects (age, weight, diet, training).

On the family level, inattention was affected by *Prevotellaceae* (Wald $\chi^2=7.462$, $p=0.006$), while on the genus level, it was affected by *Prevotella* (Wald $\chi^2=8.277$,

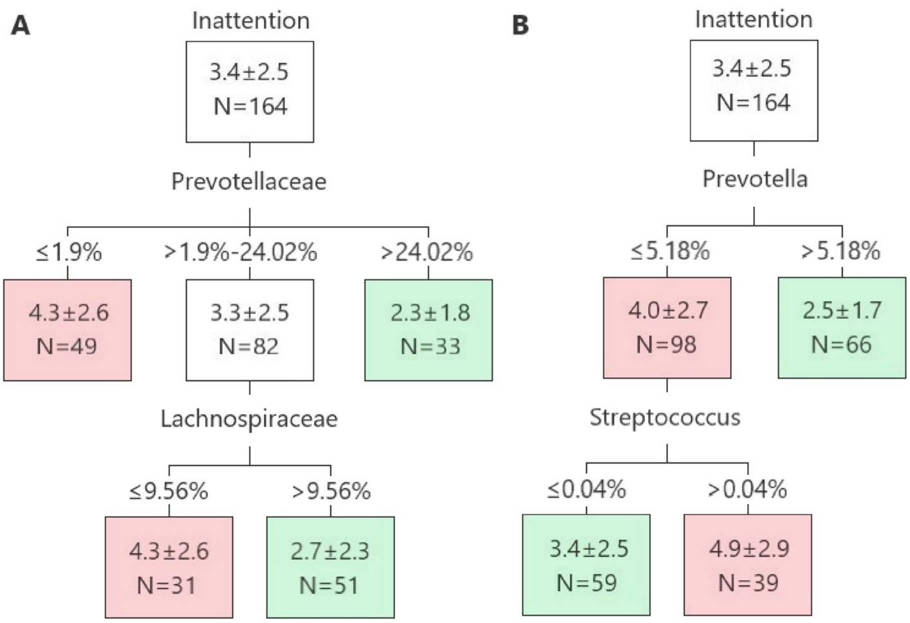

Fig. 3 Decision tree model for inattention about the relative abundance of bacterial families (**A**) and genera (**B**). Scores in the squares: inattention score mean ± SD. The percentage above the boxes shows the relative abundance of the taxa (family or genus) that the split is based on. Green shows lower, while red shows higher inattention score compared to the node that was split by the bacterial taxa

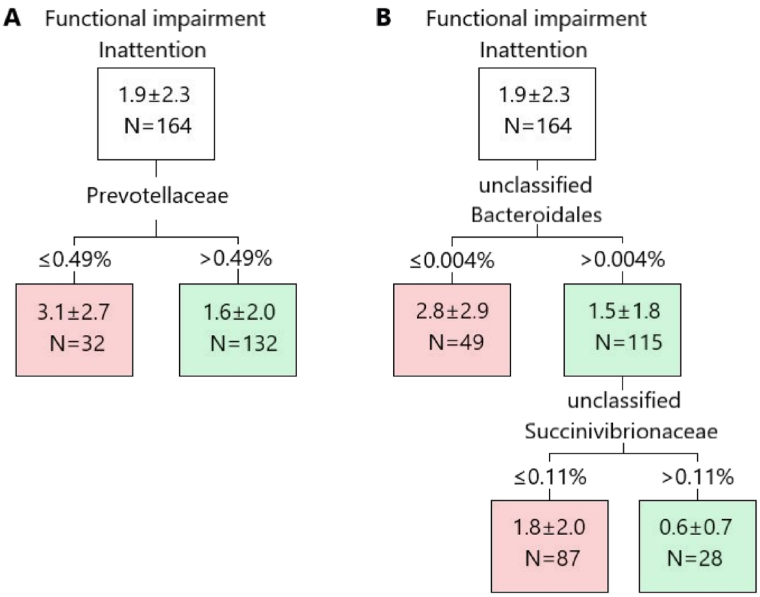

Fig. 4 Decision tree model for functional impairment of inattention about the relative abundance of bacterial families (**A**) and genera (**B**). Scores in the squares: mean functional impairment of inattention score ± SD. The percentage above the boxes shows the relative abundance of the taxa (family or genus) that the split is based on. Green shows lower, while red shows higher inattention-related functional impairment score compared to the node that was split by the bacterial taxa

$p = 0.004$). Dogs with higher inattention scores had lower relative abundance of *Prevotellaceae* and *Prevotella*.

On the family level, hyperactivity was affected by age (Wald $\chi^2 = 13.075$, $p < 0.001$), weight (Wald $\chi^2 = 8.957$, $p = 0.003$), while on the genus level, it was affected by the interaction of age and weight (Wald $\chi^2 = 18.912$, $p < 0.001$).

On the family level, impulsivity was affected by age (Wald $\chi^2 = 11.568$, $p < 0.001$), weight (Wald $\chi^2 = 5.601$, $p = 0.018$), and the interaction of age and training (Wald

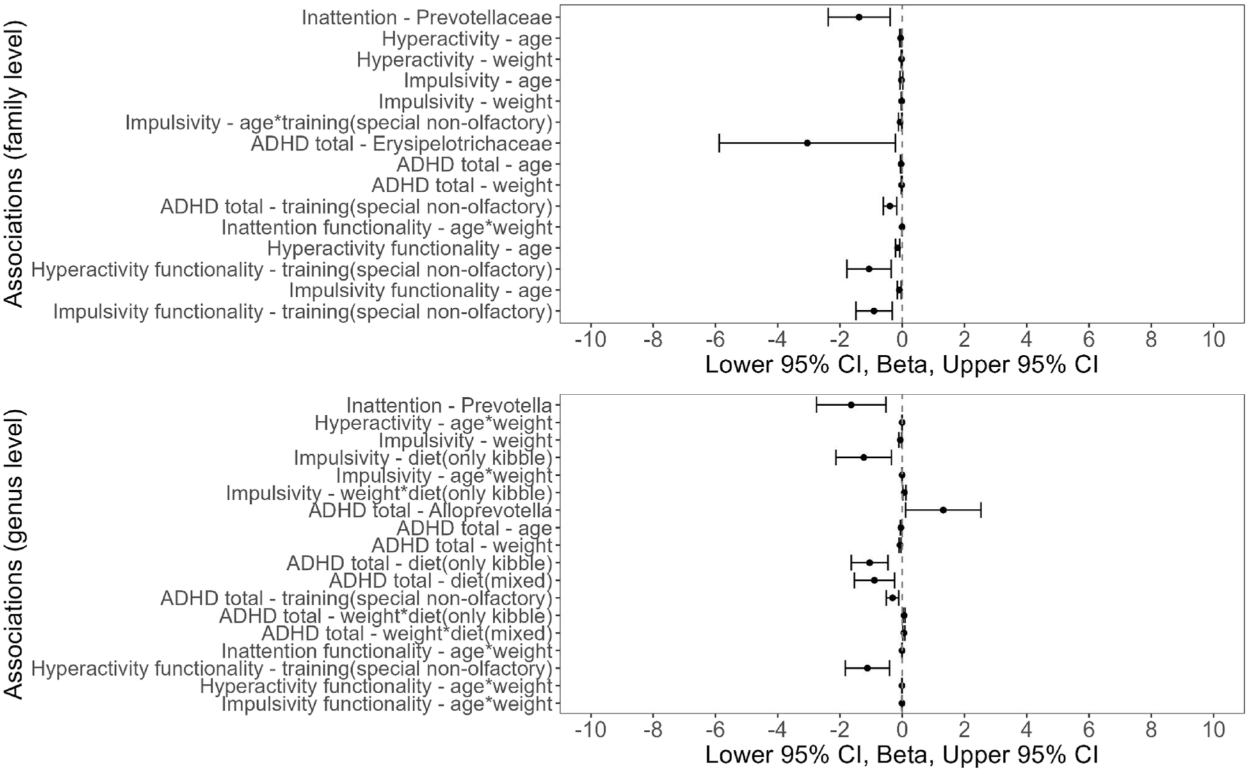

Fig. 5 Significant associations from both family- and genus-level Generalized Linear Models that Had High certainty based on the 95% Wald confidence interval

$\chi^2 = 10.279$, $p = 0.016$). For a given age (age*training), dogs with no training had higher impulsivity compared to dogs with special non-olfactory (Wald $\chi^2 = 5.533$, $p = 0.019$) and special olfactory training (Wald $\chi^2 = 3.911$, $p = 0.048$), but not with basic obedience training (Wald $\chi^2 = 0.049$, $p = 0.825$). On the genus level, impulsivity was affected by the interaction of age and weight (Wald $\chi^2 = 13.458$, $p < 0.001$), weight (Wald $\chi^2 = 4.849$, $p = 0.028$), diet (Wald $\chi^2 = 7.483$, $p = 0.024$), and the interaction of diet and weight (Wald $\chi^2 = 8.429$, $p = 0.015$). The effect of diet was driven by the higher impulsivity of raw only compared to kibble only feeding (Wald $\chi^2 = 7.363$, $p = 0.007$); the impulsivity of raw only and mixed feeding did not differ (Wald $\chi^2 = 2.977$, $p = 0.084$). However, for a given weight (diet*weight), raw only feeding was associated with lower impulsivity compared to kibble only feeding (Wald $\chi^2 = 8.026$, $p = 0.005$), but not mixed feeding (Wald $\chi^2 = 3.627$, $p = 0.057$).

On the family level, ADHD total was affected by age (Wald $\chi^2 = 12.288$, $p < 0.001$), weight (Wald $\chi^2 = 14.555$, $p < 0.001$), diet (Wald $\chi^2 = 14.332$, $p = 0.002$), the interaction of diet and weight (Wald $\chi^2 = 8.429$, $p = 0.015$), training (Wald $\chi^2 = 3.948$, $p = 0.047$), and *Erysipelotrichaceae* (Wald $\chi^2 = 4.459$, $p = 0.035$). Dogs with higher ADHD total score had lower relative abundance of *Erysipelotrichaceae*. The effect of training was driven by the higher ADHD total score of untrained dogs compared to dogs with special non-olfactory training (Wald $\chi^2 = 12.82$, $p < 0.001$); the ADHD total score did not differ between no training and basic obedience (Wald $\chi^2 = 0.476$, $p = 0.49$) or special olfactory training (Wald $\chi^2 = 3.828$, $p = 0.0501$). On the genus level, ADHD total was affected by age (Wald $\chi^2 = 20.295$, $p < 0.001$), weight (Wald $\chi^2 = 30.177$, $p < 0.001$), diet (Wald $\chi^2 = 12.24$, $p = 0.002$), the interaction of diet and weight (Wald $\chi^2 = 14.11$, $p < 0.001$), training (Wald $\chi^2 = 10.081$, $p = 0.018$), and *Alloprevotella* (Wald $\chi^2 = 4.558$, $p = 0.033$). Dogs with higher ADHD total scores had higher relative abundance of *Alloprevotella*. Raw only fed dogs had higher ADHD total score compared to kibble only (Wald $\chi^2 = 12.198$, $p < 0.001$) and mixed fed dogs (Wald $\chi^2 = 7.371$, $p = 0.007$). The effect of training was driven by the higher ADHD total score of untrained dogs compared to dogs with special non-olfactory training (Wald $\chi^2 = 9.611$, $p = 0.002$); the ADHD total score did not differ between no training and basic obedience (Wald $\chi^2 = 0.577$, $p = 0.447$) or special olfactory training (Wald $\chi^2 = 1.038$, $p = 0.308$). Interestingly for a given weight (diet*weight), raw only feeding was associated with lower ADHD total score compared to kibble only feeding

(Wald $\chi^2 = 13.982$, $p < 0.001$) and mixed feeding (Wald $\chi^2 = 10.455$, $p = 0.001$).

On the family level, inattention-related functional impairment was affected by the interaction of age and weight (Wald $\chi^2 = 9.178$, $p = 0.002$), while on the genus level, it was affected by the interaction of age and weight (Wald $\chi^2 = 12.486$, $p < 0.001$).

On the family level, hyperactivity-related functional impairment was affected by age (Wald $\chi^2 = 18.213$, $p < 0.001$) and training (Wald $\chi^2 = 11.991$, $p = 0.007$). The effect of training was driven by the higher hyperactivity-related functional impairment of untrained dogs compared to dogs with special non-olfactory training (Wald $\chi^2 = 8.6$, $p = 0.003$); hyperactivity-related functional impairment did not differ between dogs with no training and basic obedience (Wald $\chi^2 = 2.08$, $p = 0.149$) or special olfactory training (Wald $\chi^2 = 0.028$, $p = 0.867$). On the genus level, hyperactivity-related functional impairment was affected by the interaction of age and weight (Wald $\chi^2 = 21.153$, $p < 0.001$) and training (Wald $\chi^2 = 12.5$, $p = 0.006$). The effect of training was driven by the higher hyperactivity-related functional impairment of untrained dogs compared to dogs with special non-olfactory training (Wald $\chi^2 = 9.612$, $p = 0.002$); hyperactivity-related functional impairment did not differ between dogs with no training and basic obedience (Wald $\chi^2 = 2.53$, $p = 0.112$) or special olfactory training (Wald $\chi^2 = 0.002$, $p = 0.967$).

On the family level, impulsivity-related functional impairment was affected by age (Wald $\chi^2 = 10.32$, $p = 0.001$) and training (Wald $\chi^2 = 9.246$, $p = 0.026$). The effect of training was driven by the higher impulsivity-related functional impairment of untrained dogs compared to dogs with special non-olfactory training (Wald $\chi^2 = 9.157$, $p = 0.002$); impulsivity-related functional impairment did not differ between dogs with no training and basic obedience (Wald $\chi^2 = 1.03$, $p = 0.31$) or special olfactory training (Wald $\chi^2 = 1.861$, $p = 0.172$). On the genus level, impulsivity-related functional impairment was affected by the interaction of age and weight (Wald $\chi^2 = 18.613$, $p < 0.001$).

Alpha-diversity estimators

Decision tree analyses

The decision tree analysis did not split the inattention, hyperactivity, impulsivity, ADHD total, functional impairment of hyperactivity, and impulsivity scores based on the alpha-diversity estimators.

In the case of the inattention-related functional impairment score, a split was based on the number of observed species (sobs) ($F_{(1,162)} = 15.499$, $p = 0.001$; Fig. 6).

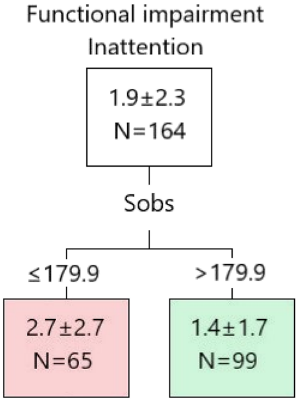

Fig. 6 Decision tree model for functional impairment of inattention about the number of observed species (sobs). Scores in the squares: mean functional impairment of inattention score ± SD. The number above the boxes shows the mean number of observed species that the split is based on. Green shows lower, while red shows higher inattention-related functional impairment score compared to the node that was split by sobs

Generalized linear model analyses

Sobs influenced inattention (Wald $\chi^2 = 4.924$, $p = 0.026$), impulsivity (Wald $\chi^2 = 3.93$, $p = 0.047$), ADHD total (Wald $\chi^2 = 5.114$, $p = 0.024$) and inattention-related functional impairment (Wald $\chi^2 = 7.065$, $p = 0.008$), but not hyperactivity, hyperactivity-related functional impairment and impulsity-related functional impairment. None of the ADHD factors and functional impairment scores were affected by the inverse Simpson.

Alpha-diversity of clusters

The cluster analysis of the ADHD factor scores yielded two clusters: a cluster containing subjects with lower mean ADHD scores (Inattention: 3, Hyperactivity: 3 and Impulsivity: 3), and another cluster with subjects with higher mean ADHD scores (Inattention: 5, Hyperactivity: 7 and Impulsivity: 10). There was no difference in the alpha-diversity of the bacterial community between the two clusters (sobs: $U = 3355$, $p = 0.251$; inverse Simpson: $U = 3252$, $p = 0.429$).

The cluster analysis of the functional impairment scores also divided the sample into two clusters: one containing subjects with lower mean functional impairment scores (Inattention: 1, Hyperactivity: 1, and Impulsivity: 1), and another cluster with subjects with higher mean functional impairment scores (Inattention: 5, Hyperactivity: 4, and Impulsivity: 5). The bacterial community was significantly richer in the cluster with lower functional impairment scores (sobs: $U = 1552$, $p = 0.012$; Fig. 7), while the inverse Simpson index did not differ between the two clusters ($U = 1858$, $p = 0.213$).

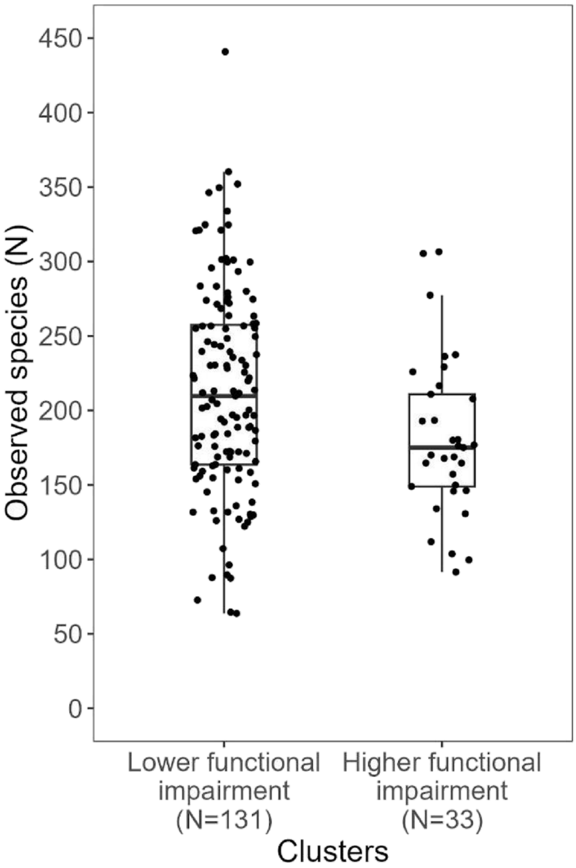

Fig. 7 Boxplots showing the number of observed bacterial species in the two clusters. The lower functional impairment cluster contains dogs with scores of Inattention: 1, Hyperactivity: 1, and Impulsivity: 1. The Higher functional impairment cluster contains dogs with scores of Inattention: 5, Hyperactivity: 4, and Impulsivity: 5

Discussion

This is the first study that associates microbiota with ADHD trait scores and their functional impairments in dogs. We identified microbiota parallels (e.g. *Prevotellaceae*) with human ADHD studies and the link between gut microbiota composition and functional impairment in dogs is particularly novel.

Our first aim was to investigate the gut microbiome community composition of a large sample of family dogs, and we found that the three most abundant phyla, *Firmicutes*, *Bacteroidetes*, and *Fusobacteria*, were the same as in previous studies [1, 27, 34, 47],however, differences were found in their relative abundance. The major bacterial families identified in our study, such as *Bacteroidaceae*, *Lachnospiraceae*, and *Prevotellaceae*, were in part similar to those found in certain studies in dogs (e.g. [1, 27]), and in the human gut as well [1]. However, Handl et al. [35] reported *Clostridiaceae* and *Ruminococcaceae* as the most abundant bacterial families in dog faeces. From the three main genera shown in this study, *Fusobacterium* and *Bacteroides* were in accord with previous research on dogs [1, 27, 34, 47], but in the study of Handl et al. [35] *Clostridium*, *Ruminococcus* and *Dorea* were the most abundant. The difference in the above results could be due to the different methodology used, as well as dogs' diet, sex, breed, or age (see Table 1).

We found that many of the bacterial taxa associated with ADHD factors were members of *Firmicutes*, even though we did not analyse phylum-level associations. This finding aligns with current literature showing an association between *Firmicutes* and several dog behaviours, especially behavioural problems (for a review, see [18]). A higher relative abundance of *Firmicutes* was noted in aggressive dogs (e.g. [24, 43]), fearful dogs [67], and dogs with anxiety (e.g. [60]), while a lower relative abundance of *Firmicutes* was found in dogs with a higher motivation score [24]. In the gut, *Firmicutes* have a role in dietary fibre breakdown, carbohydrate digestion, and short-chain fatty acid (SCFA) production, which can be an energy source for the gut microbiota and for the host. Its abundance has also been linked to glucose metabolism, fatty acid oxidation, synthesis and expenditure, gut permeability, and immune regulation [77]. Thus, especially the glucose metabolism and immune regulation, may be associated with the abovementioned dog behavioural problems. However, while these dog studies agree on the phylum level, the associations with such behaviours or behavioural problems show variability on the family and genus level.

Based on a large sample, our major aim was to investigate associations between bacterial community composition in the gut and ADHD traits in family dogs. Considering inattention, at the family level, a negative association was found with *Prevotellaceae* in both analyses and in the decision tree analysis, a secondary negative association was found with *Lachnospiraceae*, similar to that in predominantly inattentive children ([28], preprint). Further, Szopinska-Tokov et al. [80] observed in adult humans with ADHD a negative trend-like association between inattention and the relative abundance of *Coprococcus 2*, a genus within the *Lachnospiraceae* family. Members of *Prevotellaceae* and *Lachnospiraceae* are known to play a role in carbohydrate fermentation and degrading fibres, contributing to SCFA production, including butyrate, which is involved in maintaining intestinal homeostasis and has been linked to anti-inflammatory effects [15, 86], and acetate serving as an energy source for the host [15, 44]. SCFAs can also play a potential role in ADHD by modulating neurotransmission (increasing the rate-limiting enzymes in the synthesis of neurotransmitters) and controlling the transport of molecules in and out of the brain via influencing blood–brain barrier permeability [25].

At the genus level, we found partly similar associations compared to the family level. We found a negative association between inattention and *Prevotella* in both analyses, and in the decision tree analysis, a secondary negative association was found with *Streptococcus*. Previous studies did not detect such associations in dogs or humans, but Fan et al. ([28], preprint) reported negative associations between inattention and the relative abundance of several other bacterial genera, such as *Ruminococcus 2*, *Coprococcus 1*, and *Alistipes* in predominantly inattentive children. In another context, a positive association was observed between the relative abundance of *Prevotella* and working dogs' motivation score [24]. Based on our results, attentional deficits are associated with a lower relative abundance of *Prevotella*, similar to our findings with *Prevotellaceae* on the family level.

The positive association between inattention and the relative abundance of *Streptococcus* in dogs raises the possibility that ADHD symptoms in dogs, as in humans, can be associated with the dysregulation of various neurotransmitters [31, 32]. *Streptococcus* may produce serotonin through the transformation of tryptophan (e.g. [56]), which, for example, regulates mood, cognition, and social interactions in humans [6] by currently unclear mechanisms and is associated with impulsiveness, fear, and ADHD-like behaviour in dogs [31]. Further, some *Streptococcus* may produce GABA [75] that regulates mood and prevents inappropriate emotional and behavioural responses in humans [42] and have been shown to have a calming effect in dogs when orally administered [85]. However, this result needs to be taken with caution, since this was a secondary association and the relative abundance of *Streptococcus* was, on average, low in the samples.

Dogs' inattention score and its functional impairment score showed similar associations with the relative abundance of *Prevotellaceae*, which suggests similar associations for both scores. However, this cannot be stated at the genus level, as a negative association between inattention-related functional impairment and unclassified lineages of *Bacteroidales* and also a secondary negative association was found with unclassified lineages of *Succinivibrionaceae*. *Bacteroidales* includes many families and genera known for their role in polysaccharide degradation (e.g. [81]) and SCFA production (e.g. [15, 44]). Unclassified lineages of *Bacteroidales* may play a role similar to other well-characterized members of the order, such as *Prevotellaceae*, which have been shown to influence the gut-brain axis by regulating SCFA production and maintaining homeostasis (e.g. [15, 83]) as discussed above. The higher relative abundance of these unclassified taxa may indicate a more robust and diverse microbial community capable of producing beneficial metabolites that contribute to reduced functional impairment related to inattention in dogs. However, it is important to note that the mean relative abundance of these bacteria was low in the samples. The family *Succinivibrionaceae* includes strictly anaerobic and microaerobic bacteria that are involved in the production of succinate and acetate, major products during the fermentation of glucose and other carbohydrates [72, 79]. Reports on the effects of *Succinivibrionaceae* are scarce, which makes its negative association with inattention-related functional impairment difficult to interpret, although members of this family have been found in various faecal samples, such as that of chicken, cow, cats, dogs, and humans [16, 30, 72], some species having clinical importance, as being associated with diarrhoea and bacteraemia [72]. In contrast to the decision tree analysis, the more robust GzLMs did not find an association between bacterial taxa and inattention-related functional impairment.

The negative association between the ADHD total score and *Erysipelotriachaceae* have not been reported before in human ADHD studies. However, elevated levels were reported in aggressive dogs compared to control dogs [54]. In contrast, a recent study found a lower relative abundance of *Erysipelotrichaceae UCG-003* in a group of aggressive working dogs compared to non-aggressive ones [78]. Dogs fed with a kibble diet were also reported to have an increased abundance of *Erysipelotriachaceae*, and it was positively correlated with markers associated with carbohydrate digestion [7]. Of note, related to the diet, we found that only kibble fed dogs had lower impulsivity and ADHD total score than only raw fed dogs.

At the family level, the GzLM showed a positive association between the ADHD total score and *Alloprevotella*, which is in line with the finding of [65] reporting elevated levels of *Alloprevotella* in ADHD patients, even though its relative abundance did not differ significantly from that of healthy controls. Interestingly, a recent study in Thai pediatric patients found a positive association between the relative abundance of *Alloprevotella* and inattention (scored by the parent and the teacher) [57]. Since our ADHD total score is the sum of inattention, hyperactivity, and impulsivity, our identified microbial associations with this score should be considered in later studies examining gut microbiota communities of ADHD patients.

Age is a well-known mediating factor in both hyperactivity and impulsivity [20, 50, 76], with older dogs showing lower scores. In the decision tree analysis, hyperactivity, impulsivity, and their functional impairments were primarily associated with age and only secondarily with bacterial taxa, which were mostly unknown in terms of their function and/or association with ADHD traits in dogs or humans. The GzLM family-level analyses confirmed the effect of age for all these factors, while on the genus level, the interaction of age and body weight affected them. Still, in the case of impulsivity and impulsivity-related functional impairments, the findings are further complicated by the fact that in young dogs, it is harder to distinguish between impulsive individuals and those that are just impetuous due to their age. Therefore, these results need to be taken with caution, and further experiments should be conducted to elucidate the precise relationship between age and hyperactivity/impulsivity. Although we did not find a main effect of age for the diversity estimators, it is also known that the diversity and community structure of the gut microbiome changes with age, that is, decrease in bacterial diversity and/or decrease or increase in some bacterial taxa with age (e.g. [8, 10, 53, 90]) regardless of the impact of pathology. Of note, in humans, age-related inflammation and gut dysbiosis are associated with several diseases and health conditions, including neurodegenerative disorders [10].

Considering the analysis of dogs' gut microbial diversity, the GzLMs detected significant associations between the number of observed species (sobs) and inattention, impulsivity, ADHD total, and inattention-related functional impairment scores, which is in contrast to some human studies not reporting a difference in these estimators between typical people and adults/children with ADHD [14, 40, 65, 80]. In our alternative analysis, we found that the total number of observed species (sobs) was higher in dogs belonging to the cluster of a lower functional impairment score, indicating that dogs with a richer gut microbiome community are less likely to cause ADHD-related problems to the owners. These findings are in accord with other previous human studies [28, 63], preprint), although they found an association with different alpha-diversity estimators. Thus, these findings need to be clarified even in humans.

Limitations

Being the first attempt to reveal associations between the dog gut microbiome and some factors related to functionality, this study naturally carries some inevitable limitations. First, it is important to note that in contrast to most human studies where ADHD versus typical groups were compared, in dogs, we used a convenience sample and continuous ADHD scores, like in all previous studies [11, 12, 45]. Secondly, even though we confirmed several already known associations between ADHD factors and age or training level (e.g. [20]), we could not disentangle the interactions of all potential influential factors, such as diet or breed. We could not use breed in our analysis due to the diversity of the breeds and the different number of individuals in each breed. We used the body weight instead, which was found to have a strong correlation with breed, even though it is not a perfect alternative, since some breeds may have similar weights in general (e.g. [71]). Thus, replication experiments should be done to confirm the consistency of our findings, as well as further studies should focus on comparing various age groups, dog breeds, or dogs with markedly different diets. Finally, our results are limited to correlational associations, and we could not establish cause-and-effect relationships between ADHD factors/functional impairments and microbial taxa. So far, little is known about how exactly particular bacterial taxa may actually influence brain development and how these may be connected to ADHD in humans, and further studies on the function of these bacteria (e.g. metagenomic or metatranscriptomic sequencing) and on dogs (e.g. follow-up feeding experiments or probiotic/antibiotic interventions) may help gaining more insight about these mechanisms and clarify causality. Even so, in the future, these results may have potential therapeutic implications for modulating gut microbiota in managing ADHD-like behaviours in dogs, and may be useful to humans as well.

Conclusions

In family dogs, we have identified microbial taxa from *Firmicutes*, such as *Prevotellaceae*, that were associated with inattention and its related functional impairment, while hyperactivity, inattention, and their functional impairments were associated with age and microbiome interactions. This is the first study that examined such microbiological associations and also the first that identified parallels with human studies. Some evidence from previous studies in mice suggests ways in which these bacteria may affect behavioural profiles, but identifying and investigating those will require further studies in dogs. Thus, our current results in family dogs can be viewed as a first step in gaining deeper insight into these processes.

Methods

Subjects

In this study, we analysed the data of 164 dogs from 21 breeds (1 basset Hound, 1 Bavarian mountain Hound, 17 beagles, 4 Belgian Malinois, 22 border collies, 16 cocker spaniels, 5 short-haired German pointers, 1 wire-haired German pointer, 6 German shepherd dogs, 19 golden retrievers, 2 Groenendaels, 1 Hanover Hound, 9 Jack Russell terriers, 20 Labrador retrievers, 5 Parson Russell terriers, 1 petit basset griffon Vendéen, 1 small Münsterlander, 26 short-haired Hungarian vizslas, 3 wire-haired Hungarian vizslas, 2 Tervuerens, 3 Weimaraners). The sample contained 74 males (43 neutered) and 90 females

(66 neutered), with ages ranging from 0.5 to 16 years (mean: 5.1, SD: 4 years). Based on the owners' reports, dry food was the main diet of 87 dogs, 16 dogs were only fed with raw meat, while 61 dogs had a mixed diet (that could include dry food, raw meat, cooked meat, canned food, and vegetables). All subjects were reported to be healthy by their owners, based on their current behaviour, appetite, and general well-being. None of them were diagnosed with ADHD, and none of them were under medication (e.g. antidepressants, antibiotics, probiotics) during or in the preceding month of the sampling. Dogs received their regular ectoparasiticide treatment, and older individuals could get supplements and other (e.g. physiotherapy, acupuncture) treatments.

Sample collection and 16S rRNA gene amplicon sequencing

Faecal samples were collected shortly before or after the dogs had participated in an olfactory test [68–70]. Sample collection and microbiome analysis were conducted as given by Kubinyi et al. [47]. The samples were collected after spontaneous defecation and conserved at − 80 °C within 15 min. Gut microbiome composition was assessed using amplicon sequencing of the bacterial 16S rRNA gene. Briefly, total genomic deoxyribonucleic acid (DNA) extraction was performed using the QIAamp Power Faecal DNA kit (Qiagen, Venlo, Netherlands) following the instructions given by the manufacturer, while polymerase chain reaction (PCR) amplification of the target gene region was performed with primers B341F (5'-CCT ACG GGN GGC WGC AG –3' [36],) and 805NR (5'-GAC TAC NVG GGT ATC TAA TCC-3' [4],), as it was described in detail in Lange-Enyedi et al. [49]. DNA sequencing was performed on an Illumina MiSeq platform using MiSeq standard v2 chemistry as a service provided by the Genomics Core Facility RTSF, Michigan State University, East Lansing, MI, USA.

16S rRNA gene amplicon analysis

Bioinformatic analysis of the sequence reads was carried out with mothur v1.48 [73] using the MiSeq SOP (http://www.mothur.org/wiki/MiSeq_SOP downloaded on 8th January 2024). The deltaq parameter of the 'make.contigs' command was adjusted to 10 for additional quality filtering to eliminate sequencing errors. Primers were removed from the start and the end of the sequences, and singletons were also removed from the dataset, according to Kunin et al. [48]. For sequence alignment and taxonomic assignments, the ARB-SILVA SSU Ref NR 138 reference database [64] was used. Chimaeras were identified and removed using the mothur-implemented version of VSEARCH. Denoising was performed using mothur's pre.cluster command using the default algorithm [38] and applying the suggested 4-bp difference cutoff. Sequences were preclustered, and the operational taxonomic units (OTUs) were assigned at a 97% nucleotide sequence similarity level with the OptiClust algorithm [88]. Taxonomic assignment was carried out applying 1000 iterations and a minimum bootstrap confidence score of 80%. Reads of non-bacterial origin (e.g. Archaea, chloroplasts, mitochondria, Eukaryota, unknown) were removed from the dataset. For subsequent statistical analyses, reads were subsampled between samples to the read number of the sample with the lowest sequence count. Relative abundances were calculated from the OTUs, and taxa were filtered to retain only those with ≥ 0.01% mean relative abundance and 50% prevalence (present in at least 50% of the samples) on each taxonomic level. Raw sequence reads have been deposited in the NCBI Sequence Read Archive under BioProject ID PRJNA1297675.

Questionnaire for dog ADHD assessment

We used the DAFRS to assess ADHD-related traits of the tested dogs [20]. The DAFRS was developed in collaboration with a clinical expert and researcher who had extensive experience in ADHD and associated problems in humans. Further, the entire development process was evaluated and discussed with veterinarians specializing in dog behaviour, including a diplomat from the European College of Animal Welfare and Behavioural Medicine and a European Veterinary Specialist in Behavioural Medicine. Ethologists, clinicians, and researchers specializing in human ADHD as well as certified dog trainers contributed their knowledge during the questionnaire development too [20, 22]. The DAFRS questionnaire consists of 17 items, and the owners were asked to score How frequently they experienced the behaviours of their dogs mentioned in the questionnaire using a 4-point scale. The questionnaire assessed three factors: Inattention (6 items), Hyperactivity (4 items), and Impulsivity (7 items). The points given for the items were summed to get the factor scores. To calculate the ADHD total score, the three factor scores were weighted based on the number of items they contained, and the three weighted scores were summed. The internal consistency, test–retest reliability, interrater reliability, and convergent validity of the DAFRS is reported in Csibra et al. [20]. Briefly, the internal consistency was good for inattention and hyperactivity, while it was excellent for impulsivity. The test–retest analyses demonstrated excellent agreement between measurements for all subscales. The interrater reliability analyses showed fair (inattention) to moderate (hyperactivity and impulsivity) agreement between dog trainers and owners, similar to found in the case of humans.

Another three items summarized the functional impairment caused by the behaviours described in the

ADHD factors. The owners scored the functional impairment of inattention, Hyperactivity, and impulsivity on a 10-point scale answering the following question (one for each ADHD factor): to what extent does your dog's inattention/hyperactivity/impulsivity cause a problem for you in everyday life?

Statistical analysis

All statistical analyses were conducted in IBM SPSS v29. Figures were created in RStudio (v. 2022.7.1.554; [66]) using the packages 'ggplot2' [89], 'metafor' [87] and 'patchwork' [59]. As an exploratory approach, we carried out decision tree analysis, because they are ideal for analysing complex numeric and/or categorical data and detecting non-linear relationships (see description in [46]. We examined the associations between ADHD and functionality-related scores with Pearson correlations. Due to multicollinearity, we decided to create separate decision trees for each ADHD and functionality-related scores. In the decision tree analyses, we examined the relationship of the ADHD-related traits (inattention, hyperactivity, impulsivity, ADHD total, functional impairment of inattention, hyperactivity, and impulsivity with bacterial communities at two different taxonomic levels separately (families and genera and with alpha-diversity estimators (observed species—sobs and inverse Simpson,1/D. We chose to use the inverse Simpson because it is less affected by sequencing depth. Dog age, weight, diet (only raw, mixed, only kibble, training level (no training, basic obedience, special non-olfactory training, special olfactory training, and breed were also included in the decision tree analyses as independent variables due to their possible association with ADHD (e.g. [20]) and/or gut microbiome (e.g. [18, 61, 90]). In the decision tree analyses, we used the Chi-square Automatic Interaction Detector (CHAID) method [41], which uses an F test for continuous variables. We specified the minimum number of cases as 40 for parent nodes and 20 for child nodes, and cross-validated the decision trees using 10 folds.

We then did more robust analyses using GzLM. Separate models were done for each ADHD factor/functional impairment and taxonomy (families and genera), as well as for the alpha-diversity estimators. We controlled for dog age, weight, diet, and training level, and for their relevant interactions (age*weight, age*training level and diet*weight). Since our dependent variables contained positive values and zeros, Tweedie distribution with the log link function was employed. The final models, containing only significant factors, were reached with backwards elimination.

For the alpha-diversity estimators, we also used an alternative analysis. We performed two cluster analyses, one on the ADHD factors (inattention, hyperactivity, impulsivity) and another on the functional impairment factors (functional impairment of inattention, hyperactivity, and impulsivity). Then, Mann–Whitney tests were used to compare the estimators between the gained clusters.

Abbreviations

ADHD	Attention-deficit/hyperactivity disorder
CHAID	Chi-square Automatic Interaction Detector
DAFRS	Dog ADHD and Functionality Rating Scale
DNA	Deoxyribonucleic acid
GABA	Gamma-aminobutyric acid
GzLM	Generalized Linear Model
OTU	Operational taxonomic unit
PCR	Polymerase chain reaction
rRNA	Ribosomal ribonucleic acid
SCFA	Short-chain fatty acid
SD	Standard deviation
Sobs	Number of observed species

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12915-025-02410-9.

Additional file 1. Table S1: Relative abundance of microbial families. Table S2: Relative abundance of microbial genera. Table S3: The correlations between ADHD factors and their functional impairments. Figure S1: Decision tree models' first split of inattention and its functional impairment. Figure S2: Decision tree model of hyperactivity. Figure S3: Decision tree model of impulsivity. Figure S4: Decision tree model of ADHD total. Figure S5: Decision tree model of hyperactivity functional impairment. Figure S6: Decision tree model of impulsivity functional impairment. Table S4: Significant ADHD-microbiome associations on family level. Table S5: Significant ADHD-microbiome associations on genus level.

Additional file 2. Table S1: Family-level microbial composition of each sample. Table S2: Genus-level microbial composition of each sample. Table S3: Alpha-diversity estimator values of each sample. Table 4: Dog information of each sample.

Acknowledgements

The authors thank all the volunteers who participated in the experiments and provided data for this study. This research received funding from the HUN-REN-ELTE Comparative Ethology Research Group (01 031), the National Brain Programme 3.0 (NAP2022-I-3/2022), the National Research, Development and Innovation Office—NKFIH (Grant No. K132372), and the Hungarian Ethology Foundation (METAL). EK was also supported by the MTA-ELTE 'Lendület/Momentum' Companion Animal Research Group (Grant No. PH1404/21). AA was also supported by the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (Grant Agreement No. 950159).

Authors' contributions

MG conceived and designed the study, AS1 (Attila Salamon) and SBR carried out the data collection, SBR performed DNA extraction, EK and TF provided reagents and materials, AS1, AS2 (Attila Szabó) and MG analysed and interpreted the data, MG, EK and AA acquired funding, AS1 drafted the work and MG, ÁM, TF, AS2, AA and EK substantially revised it. All authors read and approved the final manuscript.

Funding

This research received funding from the HUN-REN-ELTE Comparative Ethology Research Group (01 031), the National Brain Programme 3.0 (NAP2022-I-3/2022), the National Research, Development and Innovation Office—NKFIH (Grant No. K132372), and the Hungarian Ethology Foundation (METAL). EK was also supported by the MTA-ELTE 'Lendület/Momentum' Companion

Animal Research Group (grant no. PH1404/21). AA was also supported by the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (Grant Agreement No. 950159).

Data availability

Raw sequence reads have been deposited in the NCBI Sequence Read Archive under BioProject ID PRJNA1297675. All data generated or analysed during this study are included in this published article and in an Excel table named „Additional file 2".

Declarations

Ethics approval and consent to participate

The data collection of the current study was connected to an olfactory test [68–70] for which ethical approvals were granted by the Animal Welfare Committee of Eötvös Loránd University (ELTE-AWC-020/2018 and ELTE-AWC-015/2023). A convenience sample of companion dog owners was recruited through social media and from the Family Dog Project database. They were asked to fill in a questionnaire, which was approved by the United Ethical Review Committee for Research in Psychology in Hungary (EPKEB,2023–04). An informed consent was also obtained from all owners.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]HUN-REN-ELTE Comparative Ethology Research Group, Budapest, Hungary. [2]NAP Canine Brain Research Group, ELTE Eötvös Loránd University, Budapest, Hungary. [3]Department of Ethology, ELTE Eötvös Loránd University, Budapest, Hungary. [4]Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Uppsala, Sweden. [5]Institute of Aquatic Ecology, HUN-REN Centre for Ecological Research, Budapest, Hungary. [6]Department of Microbiology, ELTE Eötvös Loránd University, Budapest, Hungary. [7]Doctoral School of Biology, ELTE Eötvös Loránd University, Budapest, Hungary. [8]Neuroethology of Communication Lab, Department of Ethology, Eötvös Loránd University, Budapest, Hungary. [9]MTA-ELTE Lendület 'Momentum' Companion Animal Research Group, Budapest, Hungary.

Received: 22 May 2025 Accepted: 4 September 2025

References

1. Alessandri G, Milani C, Mancabelli L, Mangifesta M, Lugli GA, Viappiani A, et al. Metagenomic dissection of the canine gut microbiota: insights into taxonomic, metabolic and nutritional features. Environ Microbiol. 2019;21:1331–43. https://doi.org/10.1111/1462-2920.14540.
2. Ambrosini YM, Borcherding D, Kanthasamy A, Kim HJ, Willette AA, Jergens A, et al. The gut-brain axis in neurodegenerative diseases and relevance of the canine model: a review. Front Aging Neurosci. 2019;11:130. https://doi.org/10.3389/fnagi.2019.00130.
3. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. Fifth Edition, Text Revision (DSM-5-TRTM). American Psychiatric Association; 2022.
4. Apprill A, McNally S, Parsons R, Weber L. Minor revision to V4 region SSU rRNA 806r gene primer greatly increases detection of SAR11 bacterioplankton. Aquat Microb Ecol. 2015;75:129–37. https://doi.org/10.3354/ame01753.
5. Bakhshani N-M. Impulsivity: a predisposition toward risky behaviors. Int J High Risk Behav Addict. 2014;3:e20428. https://doi.org/10.5812/ijhrba.20428.
6. Berger M, Gray JA, Roth BL. The expanded biology of serotonin. Annu Rev Med. 2009;60:355–66. https://doi.org/10.1146/annurev.med.60.042307.110802.
7. Bermingham EN, Maclean P, Thomas DG, Cave NJ, Young W. Key bacterial families (Clostridiaceae, Erysipelotrichaceae and Bacteroidaceae) are related to the digestion of protein and energy in dogs. PeerJ. 2017;5:e3019. https://doi.org/10.7717/peerj.3019.
8. Biagi E, Nylund L, Candela M, Ostan R, Bucci L, Pini E, et al. Through ageing, and beyond: gut microbiota and inflammatory status in seniors and centenarians. PLoS ONE. 2010;5:e10667. https://doi.org/10.1371/journal.pone.0010667.
9. Boonchooduang N, Louthrenoo O, Chattipakorn N, Chattipakorn SC. Possible links between gut–microbiota and attention-deficit/hyperactivity disorders in children and adolescents. Eur J Nutr. 2020;59:3391–403. https://doi.org/10.1007/s00394-020-02383-1.
10. Buford TW. (Dis)trust your gut: the gut microbiome in age-related inflammation, health, and disease. Microbiome. 2017;5:80. https://doi.org/10.1186/s40168-017-0296-0.
11. Bunford N, Csibra B, Gácsi M. Individual differences in response to ambiguous stimuli in a modified Go/no-go paradigm are associated with personality in family dogs. Sci Rep. 2019;9:11067. https://doi.org/10.1038/s41598-019-47510-z.
12. Bunford N, Csibra B, Peták C, Ferdinandy B, Miklósi Á, Gácsi M. Associations among behavioral inhibition and owner-rated attention, hyperactivity/impulsivity, and personality in the domestic dog (*Canis familiaris*). J Comp Psychol. 2019;133:233–43. https://doi.org/10.1037/com0000151.
13. Caputi V, Hill L, Figueiredo M, Popov J, Hartung E, Margolis KG, et al. Functional contribution of the intestinal microbiome in autism spectrum disorder, attention deficit hyperactivity disorder, and Rett syndrome: a systematic review of pediatric and adult studies. Front Neurosci. 2024;18:1341656. https://doi.org/10.3389/fnins.2024.1341656.
14. Cickovski T, Mathee K, Aguirre G, Tatke G, Hermida A, Narasimhan G, et al. Attention deficit hyperactivity disorder (ADHD) and the gut microbiome: an ecological perspective. PLoS ONE. 2023;18:e0273890. https://doi.org/10.1371/journal.pone.0273890.
15. Chen T, Long W, Zhang C, Liu S, Zhao L, Hamaker BR. Fiber-utilizing capacity varies in *Prevotella*-versus *Bacteroides*-dominated gut microbiota. Sci Rep. 2017;7:2594. https://doi.org/10.1038/s41598-017-02995-4.
16. Choi JY, Park JE, Choi SH, Kim JS, Lee JS, Lee JH, et al. *Succinivibrio faecicola* sp. nov., isolated from cow faeces. Int J Syst Evol Microbiol. 2022;72:005631. https://doi.org/10.1099/ijsem.0.005631.
17. Clarke G, Stilling RM, Kennedy PJ, Stanton C, Cryan JF, Dinan TG. Minireview: gut microbiota: the neglected endocrine organ. Mol Endocrinol. 2014;28:1221–38. https://doi.org/10.1210/me.2014-1108.
18. Crisante A, Newberry F, Clegg SR, Mitchell GL, Pike TW, Ratcliffe V, et al. A critical review of research concerning the gut microbiome in dogs and its relationship with behaviour. Appl Anim Behav Sci. 2025;19:106755. https://doi.org/10.1016/j.applanim.2025.106755.
19. Csibra B, Bunford N, Gácsi M. Evaluating ADHD assessment for dogs: a replication study. Animals. 2022;12:807. https://doi.org/10.3390/ani12070807.
20. Csibra B, Bunford N, Gácsi M. Development of a human-analogue, 3-symptom domain dog ADHD and functionality rating scale (DAFRS). Sci Rep. 2024;14:1808. https://doi.org/10.1038/s41598-024-51924-9.
21. Csibra B, Reicher V, Csepregi M, Kristóf K, Gácsi M. Towards an objective measurement tool for ADHD-like traits in family dogs: a comprehensive test battery. Animals. 2024;14:1841. https://doi.org/10.3390/ani14131841.
22. Csibra B, Bunford N, Gácsi M. Development of a human analogue ADHD diagnostic system for family dogs. Sci Rep. 2025;15:25671. https://doi.org/10.1038/s41598-025-09988-8.
23. Coelho LP, Kultima JR, Costea PI, Fournier C, Pan Y, Czarnecki-Maulden G, et al. Similarity of the dog and human gut microbiomes in gene content and response to diet. Microbiome. 2018;6:72. https://doi.org/10.1186/s40168-018-0450-3.
24. Craddock HA, Godneva A, Rothschild D, Motro Y, Grinstein D, Lotem-Michaeli Y, et al. Phenotypic correlates of the working dog microbiome. NPJ Biofilms Microbiomes. 2022;8:66. https://doi.org/10.1038/s41522-022-00329-5.
25. Dam SA, Mostert JC, Szopinska-Tokov JW, Bloemendaal M, Amato M, Arias-Vasquez A. The role of the gut-brain axis in attention-deficit/hyperactivity disorder. Gastroenterol Clin North Am. 2019;48:407–31. https://doi.org/10.1016/j.gtc.2019.05.001.
26. Demontis D, Walters RK, Martin J, Mattheisen M, Als TD, Agerbo E, et al. Discovery of the first genome-wide significant risk loci for attention deficit/hyperactivity disorder. Nat Genet. 2019;51:63–75. https://doi.org/10.1038/s41588-018-0269-7.

27. Ericsson AC, Personett AR, Grobman ME, Rindt H, Reinero CR. Composition and predicted metabolic capacity of upper and lower airway microbiota of healthy dogs in relation to the fecal microbiota. PLoS ONE. 2016;11:e0154646. https://doi.org/10.1371/journal.pone.0154646.
28. Fan C, Li S, Wang R, Fan X, Liang A, Qi K. SCFA producing bacteria shape the subtype of ADHD in children. Preprint; 2019. https://doi.org/10.21203/rs.2.16337/v1
29. Gawrilow C, Kühnhausen J, Schmid J, Stadler G. Hyperactivity and motoric activity in ADHD: characterization, assessment, and intervention. Front Psych. 2014;5:171. https://doi.org/10.3389/fpsyt.2014.00171.
30. Gilroy R, Ravi A, Getino M, Pursley I, Horton DL, Alikhan NF, et al. Extensive microbial diversity within the chicken gut microbiome revealed by metagenomics and culture. PeerJ. 2021;9:e10941. https://doi.org/10.7717/peerj.10941.
31. González-Martínez Á, Muñiz de Miguel S, Diéguez FJ. New advances in attention-deficit/hyperactivity disorder-like dogs. Animals (Basel). 2024;14:2067. https://doi.org/10.3390/ani14142067
32. González-Martínez Á, Muñiz de Miguel S, Graña N, Costas X, Diéguez FJ. Serotonin and dopamine blood levels in ADHD-like dogs. Animals (Basel). 2023;13:1037. https://doi.org/10.3390/ani13061037
33. Gorzelanna Z, Miszczak M. Through the intestines to the head? That is, how the gastrointestinal microbiota affects the behavior of companion animals. Pets. 2024;1:201–15. https://doi.org/10.3390/pets1030015.
34. Hand D, Wallis C, Colyer A, Penn CW. Pyrosequencing the canine faecal microbiota: breadth and depth of biodiversity. PLoS ONE. 2013;8:e53115. https://doi.org/10.1371/journal.pone.0053115.
35. Handl S, Dowd SE, Garcia-Mazcorro JF, Steiner JM, Suchodolski JS. Massive parallel 16S rRNA gene pyrosequencing reveals highly diverse fecal bacterial and fungal communities in healthy dogs and cats. FEMS Microbiol Ecol. 2011;76:301–10. https://doi.org/10.1111/j.1574-6941.2011.01058.x.
36. Herlemann DP, Labrenz M, Jürgens K, Bertilsson S, Waniek JJ, Andersson AF. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. ISME J. 2011;5:1571–9. https://doi.org/10.1038/ismej.2011.41.
37. Huang Z, Pan Z, Yang R, Bi Y, Xiong X. The canine gastrointestinal microbiota: early studies and research frontiers. Gut Microbes. 2020;11:635–54. https://doi.org/10.1080/19490976.2019.1704142.
38. Huse SM, Welch DM, Morrison HG, Sogin ML. Ironing out the wrinkles in the rare biosphere through improved OTU clustering. Environ Microbiol. 2010;12:1889–98. https://doi.org/10.1111/j.1462-2920.2010.02193.x.
39. Ito Y, Nagasawa M, Koyama K, Ito K, Kikusui T. Comparative analysis based on shared amplicon sequence variants reveals that cohabitation influences gut microbiota sharing between humans and dogs. Front Vet Sci. 2024;11:1417461. https://doi.org/10.3389/fvets.2024.1417461.
40. Jiang HY, Zhou YY, Zhou GL, Li YC, Yuan J, Li XH, et al. Gut microbiota profiles in treatment-naïve children with attention deficit hyperactivity disorder. Behav Brain Res. 2018;347:408–13. https://doi.org/10.1016/j.bbr.2018.03.036.
41. Kass GV. An exploratory technique for investigating large quantities of categorical data. J R Stat Soc, C: (Appl Stat). 1980;29:119–27. https://doi.org/10.2307/2986296
42. Kiełbik P, Witkowska-Piłaszewicz O. The relationship between canine behavioral disorders and gut microbiome and future therapeutic perspectives. Animals. 2024;14:2048. https://doi.org/10.3390/ani14142048.
43. Kirchoff NS, Udell MA, Sharpton TJ. The gut microbiome correlates with conspecific aggression in a small population of rescued dogs (*Canis familiaris*). PeerJ. 2019;7:e6103. https://doi.org/10.7717/peerj.6103.
44. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. Cell. 2016;165:1332–45. https://doi.org/10.1016/j.cell.2016.05.041.
45. Kovács T, Reicher V, Csibra B, Gácsi M. More inattentive dogs benefit from repetitive but not permissive training. Appl Anim Behav Sci. 2024;281:106449. https://doi.org/10.1016/j.applanim.2024.106449.
46. Kubinyi E, Turcsán B, Miklósi Á. Dog and owner demographic characteristics and dog personality trait associations. Behav Processes. 2009;81:392–401. https://doi.org/10.1016/j.beproc.2009.04.004.
47. Kubinyi E, Bel Rhali S, Sándor S, Szabó A, Felföldi T. Gut microbiome composition is associated with age and memory performance in pet dogs. Animals. 2020;10:1488. https://doi.org/10.3390/ani10091488.
48. Kunin V, Engelbrektson A, Ochman H, Hugenholtz P. Wrinkles in the rare biosphere: pyrosequencing errors can lead to artificial inflation of diversity estimates. Environ Microbiol. 2010;12:118–23. https://doi.org/10.1111/j.1462-2920.2009.02051.x.
49. Lange-Enyedi NT, Borsodi AK, Németh P, Czuppon G, Kovács I, Leél-Őssy S, et al. Habitat-related variability in the morphological and taxonomic diversity of microbial communities in two Hungarian epigenic karst caves. FEMS Microbiol Ecol. 2023;99:fiad161. https://doi.org/10.1093/femsec/fiad161.
50. Lit L, Schweitzer JB, Iosif AM, Oberbauer AM. Owner reports of attention, activity, and impulsivity in dogs: a replication study. Behav Brain Funct. 2010;6:1. https://doi.org/10.1186/1744-9081-6-1.
51. Lyte M. Probiotics function mechanistically as delivery vehicles for neuroactive compounds: microbial endocrinology in the design and use of probiotics. Bioessays. 2011;33:574–81. https://doi.org/10.1002/bies.201100024.
52. Martin CR, Osadchiy V, Kalani A, Mayer EA. The brain-gut-microbiome axis. Cell Mol Gastroenterol Hepatol. 2018;6:133–48. https://doi.org/10.1016/j.jcmgh.2018.04.003.
53. Mizukami K, Uchiyama J, Igarashi H, Murakami H, Osumi T, Shima A, Ishiahra G, Nasukawa T, Une Y, Sakaguchi M. Age-related analysis of the gut microbiome in a purebred dog colony. FEMS Microbiol Lett. 2019;366:fnz095. https://doi.org/10.1093/femsle/fnz095
54. Mondo E, Barone M, Soverini M, D'amico F, Cocchi M, Petrulli C, et al. Gut microbiome structure and adrenocortical activity in dogs with aggressive and phobic behavioral disorders. Heliyon. 2020;6:e03311. https://doi.org/10.1016/j.heliyon.2020.e03311.
55. Nigg JT, Sibley MH, Thapar A, Karalunas SL. Development of ADHD: etiology, heterogeneity, and early life course. Annu Rev Dev Psychol. 2020;2:559–83. https://doi.org/10.1146/annurev-devpsych-060320-093413.
56. O'Mahony SM, Clarke G, Borre YE, Dinan TG, Cryan JF. Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. Behav Brain Res. 2015;277:32–48. https://doi.org/10.1016/j.bbr.2014.07.027.
57. Panpetch J, Kiatrungrit K, Tuntipopipat S, Tangphatsornruang S, Mhuantong W, Chongviriyaphan N. Gut microbiota and clinical manifestations in Thai pediatric patients with attention-deficit hyperactivity disorder. J Pers Med. 2024;14:739. https://doi.org/10.3390/jpm14070739.
58. de la Peña IC, Pan MC, Thai CG, Alisso T. Attention-deficit/hyperactivity disorder predominantly inattentive subtype/presentation: research progress and translational studies. Brain Sci. 2020;10:292. https://doi.org/10.3390/brainsci10050292.
59. Pedersen TL. Package 'patchwork.' R package [http://CRAN.R-project.org/package= patchwork]; 2019.
60. Pellowe SD, Zhang A, Bignell DR, Peña-Castillo L, Walsh CJ. Gut microbiota composition is related to anxiety and aggression scores in companion dogs. Sci Rep. 2025;15:24336. https://doi.org/10.1038/s41598-025-06178-4.
61. Pereira AM, Clemente A. Dogs' microbiome from tip to toe. Top Companion Anim Med. 2021;45:100584. https://doi.org/10.1016/j.tcam.2021.100584.
62. Polanczyk GV, Willcutt EG, Salum GA, Kieling C, Rohde LA. ADHD prevalence estimates across three decades: an updated systematic review and meta-regression analysis. Int J Epidemiol. 2014;43(2):434–42. https://doi.org/10.1093/ije/dyt261.
63. Prehn-Kristensen A, Zimmermann A, Tittmann L, Lieb W, Schreiber S, Baving L, et al. Reduced microbiome alpha diversity in young patients with ADHD. PLoS ONE. 2018;13:e0200728. https://doi.org/10.1371/journal.pone.0200728.
64. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acid Res. 2012;41:D590–6. https://doi.org/10.1093/nar/gks1219.
65. Richarte Fernández V, Sanchez Mora CP, Corrales De la Cruz M, Fadeuilhe Grau C, Vilar Ribo L, Arribas Garcia L, Garcia Gea E, Rosales Ortiz SK, Soler Artigas M, Ribases Haro M, Ramos-Quiroga JA. Gut microbiota signature in treatment-naïve attention-deficit/hyperactivity disorder. Transl. Psychiatry 2021;11:382. https://doi.org/10.1038/s41398-021-01504-6
66. RStudio Team. RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA; 2022. http://www.rstudio.com/
67. Sacchettino L, Costanzo M, Veneruso I, D'Argenio V, Mayer M, Napolitano F, et al. Altered microbiome and metabolome profiling in fearful

companion dogs: an exploratory study. PLoS ONE. 2025;20:e0315374. https://doi.org/10.1371/journal.pone.0315374.
68. Salamon A, Baranya E, Zsiros LR, Miklósi Á, Csepregi M, Kubinyi E, et al. Success in the natural detection task is influenced by only a few factors generally believed to affect dogs' olfactory performance. Sci Rep. 2024;14:12351. https://doi.org/10.1038/s41598-024-62957-5.
69. Salamon A, Miklósi Á, Zsiros LR, Kovács T, Kubinyi E, Andics A, et al. Breed differences in olfactory performance of dogs. Sci Rep. 2025;15:2675. https://doi.org/10.1038/s41598-025-87136-y.
70. Salamon A, Lakatos B, Miklósi Á, Csibra B, Kubinyi E, Andics A, et al. Training level and personality affect border collies' olfactory performance in the natural detection task. Appl Anim Behav Sci. 2025;286:106625. https://doi.org/10.1016/j.applanim.2025.106625.
71. Salt C, Morris PJ, German AJ, Wilson D, Lund EM, Cole TJ, et al. Growth standard charts for monitoring bodyweight in dogs of different sizes. PLoS ONE. 2017;12:e0182064. https://doi.org/10.1371/journal.pone.0182064.
72. Santos EdeO, Thompson F. The family Succinivibrionaceae. In: Rosenberg E, DeLong EF, Lory S, editors. The Prokaryotes: Gammaproteobacteria. Berlin: Springer; 2014. p. 639–48. https://doi.org/10.1007/978-3-642-38922-1_368.
73. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol. 2009;75:7537–41. https://doi.org/10.1128/AEM.01541-09.
74. Sciberras E, Mulraney M, Silva D, Coghill D. Prenatal risk factors and the etiology of ADHD—review of existing evidence. Curr Psychiatry Rep. 2017;19:1. https://doi.org/10.1007/s11920-017-0753-2.
75. Strandwitz P. Neurotransmitter modulation by the gut microbiota. Brain Res. 2018;1693:128–33. https://doi.org/10.1016/j.brainres.2018.03.015.
76. Sulkama S, Puurunen J, Salonen M, Mikkola S, Hakanen E, Araujo C, et al. Canine hyperactivity, impulsivity, and inattention share similar demographic risk factors and behavioural comorbidities with human ADHD. Transl Psychiatry. 2021;11:501. https://doi.org/10.1038/s41398-021-01626-x.
77. Sun Y, Zhang S, Nie Q, He H, Tan H, Geng F, et al. Gut firmicutes: relationship with dietary fiber and role in host homeostasis. Crit Rev Food Sci Nutr. 2023;63:12073–88. https://doi.org/10.1080/10408398.2022.2098249.
78. Sun N, Xie L, Chao J, Xiu F, Zhai H, Zhou Y, et al. Study on the correlation between aggressive behavior and gut microbiota and serum serotonin (5-HT) in working dogs. Vet Sci. 2025;12:526. https://doi.org/10.3390/vetsci12060526.
79. Stackebrandt E, Hespell R. The family Succinivibrionaceae The Prokaryotes. 2006;3:419–29.
80. Szopinska-Tokov J, Dam S, Naaijen J, Konstanti P, Rommelse N, Belzer C, et al. Investigating the gut microbiota composition of individuals with attention-deficit/hyperactivity disorder and association with symptoms. Microorganisms. 2020;8:406. https://doi.org/10.3390/microorganisms8030406.
81. Tan H, Zhao J, Zhang H, Zhai Q, Chen W. Isolation of low-abundant Bacteroidales in the human intestine and the analysis of their differential utilization based on plant-derived polysaccharides. Front Microbiol. 2018;9:1319. https://doi.org/10.3389/fmicb.2018.01319.
82. Tengeler AC, Dam SA, Wiesmann M, Naaijen J, Van Bodegom M, Belzer C, et al. Gut microbiota from persons with attention-deficit/hyperactivity disorder affects the brain in mice. Microbiome. 2020;8:44. https://doi.org/10.1186/s40168-020-00816-x.
83. Tett A, Pasolli E, Masetti G, Ercolini D, Segata N. *Prevotella* diversity, niches and interactions with the human host. Nat Rev Microbiol. 2021;19:585–99. https://doi.org/10.1038/s41579-021-00559-y.
84. Topál J, Román V, Turcsán B. The dog (*Canis familiaris*) as a translational model of autism: it is high time we move from promise to reality. WIREs Cogn Sci. 2019;10:e1495. https://doi.org/10.1002/wcs.1495.
85. Uetake K, Okumoto A, Tani N, Goto A, Tanaka T. Calming effect of orally administered γ-aminobutyric acid in Shih Tzu dogs. Anim Sci J. 2012;83:796–8. https://doi.org/10.1111/j.1740-0929.2012.01024.x.
86. Vacca M, Celano G, Calabrese FM, Portincasa P, Gobbetti M, De Angelis M. The controversial role of human gut Lachnospiraceae. Microorganisms. 2020;8:573. https://doi.org/10.3390/microorganisms8040573.
87. Viechtbauer W. Conducting meta-analyses in R with the metafor package. J Stat Softw. 2010;36:1–48. https://doi.org/10.18637/jss.v036.i03.
88. Westcott SL, Schloss PD. Opticlust, an improved method for assigning amplicon-based sequence data to operational taxonomic units. mSphere. 2017;2:10–1128. https://doi.org/10.1128/mspheredirect.00073-17.
89. Wickham H. ggplot2: Elegant Graphics for Data Analysis. Springer; 2016.
90. You I, Kim MJ. Comparison of gut microbiota of 96 healthy dogs by individual traits: breed, age, and body condition score. Animals (Basel). 2021;11:2432. https://doi.org/10.3390/ani11082432.

Publisher's Note