The Influence of Phylogenic Origin on the Occurrence of Brachycephalic Airway Obstruction Syndrome in a Large Retrospective Study.

Ibrahim N. Njikam, DVM
Maxime Huault, DVM
Vincent Pirson, DVM
Johann Detilleux, DVM , MSc, PhD

Department of Animal Production,
Faculty of Veterinary Medicine
University of Liège,
Liège, 4000, Belgium.

KEY WORDS: BAOS, Phylogeny, Risk Factor, Brachycephaly

ABSTRACT

The objective of this retrospective study was to determine whether the occurrence of brachycephalic airway obstruction syndrome (BAOS) varried according to the phylogenic origins of dogs. The analysis of our hospital database showed that the frequency of BAOS was higher in modern than ancient breeds. This confirmed the findings that boxers are not as susceptible to BAOS as many other brachycephalic dog breeds belonging to the same phylogenic cluster.

INTRODUCTION

Patients with brachycephalic airway obstruction syndrome (BAOS) often present with noisy breathing, reduced tolerance to exercise and stress, respiratory distress, and, in severe cases, cyanosis and collapse.[7,8] The syndrome is frequently encountered in brachycephalic (BRA) breeds that have been selected for a shortened longitudinal axis of the skull. In these breeds, early ankylosis occurs in the cartilage of the base of the skull, leading to local chondrodysplasia.[11] Nares are often stenotic, and the soft palate is usually relatively long and thick compared to non-BRA breeds.[12] Many affected BRA dogs also have everted laryngeal saccules, hypoplastic trachea, and partial collapse of the left main bronchus.[1]

However, if BAOS is frequent in BRA dogs, not all BRA dogs present with it. Also, similarities between respiratory problems and BAOS were observed in mesocephalic breeds such as the Norwich and Norfolk Terriers.[11] These observations prompted us to hypothesize that characteristics other than the BRA condition may be considered as risk factors for BAOS. To test this hypothesis, BAOS frequency can be compared across BRA breeds, regardless of their phylogenic origins. The rationale behind this hypothesis proceeded from the particularities of the evolution and genetic structure of canine breeds that may be considered as genetic isolates[2,18] classified in four phylogenic clusters.[13] If BAOS frequency is different among BRA breeds with the same phylogenic origin (common ancestors), it

is credible that affected animals share some genetic characteristics in common, including the BRA characteristics, but not the ones associated with susceptibility to BAOS. If the frequency of BAOS is different across phylogenic groups, this suggests that BAOS susceptibility does not derive from the same ancestral characteristics.

Here, we analyzed routine clinical reports on canine BAOS with regards to each dog's phylogenic origin, brachycephalic condition, and breed characteristics after adjusting records for potential and reported confounding factors, such as age and sex.

MATERIALS AND METHODS

Data were collected on dogs presented between 2001 and 2006 to the Small Animal Clinic of the Veterinary Faculty of the University of Liège. Cases were defined as dogs suffering from BAOS as the main diagnosis. Under the supervision of their professor, veterinary residents identified cases on the basis of clinical symptoms. Koch et al.[11] provided a list of these, including laboured and constant open mouthed breathing, noisy breathing, snorting, excessive snoring, exercise and/or heat intolerance, general lack of energy, and pale or bluish tongue and gums due to a lack of oxygen. Additional information collected included the breed, sex, and age of the referred dog. Controls were dogs admitted to the hospital for diseases other than BAOS. Among all such potential dogs, we identified 15 random controls per case, matched on age at diagnosis (± 1 year) and sex (male, female).

Table 1. *Total number of dogs (n) referred at the clinics according to their phylogenic group (G) and breed.*

G1	n	G2	n	G3	n	G4	n
Chow-chow	10	Saint Bernard	17	Maltese bichon	204	Boxer	62
Malamute	7	Shetland	7	Dachshund	76	Mastiff	3
Shar-Pei	27	Barzoï	2	Whippet	11	Rottweiler	102
Husky	21	Colley	7	English Cocker	7	Newfoundland	20
Pekinese	10	Pug	31	Flat coat retriever	8	Bull terrier	26
Lhasa Apso	9	Greyhound	3	Golden retriever	100	French bulldog	39
Tibetan terrier	9	Belgian sheepdog	115	Cavalier King Charles	12	English bulldog	34
Shih Tzu	51			Basset hound	12	Pomeranian spitz	5
Afghan Hound	4			Chihuahua	11	German shepherd	138
Akita Inu	8			Doberman	34	Labrador Retriever	245
Irish Wolf-hound	15			West Highland White terrier	39	Bernese mountain dog	93
				Schnauzer	26		
				Doberman	34		
				Setter	12		
				Border Collie	52		
				American cocker spaniel	39		
				Beagle	38		
				Pointer	7		
				Great dane	29		
				Poodle	61		

Each of the brachycephalic dogs had a short, broad head with skull width to length ratio >80.3 They belonged to the Pug, Bos-

Table 2. *Number (N) and percentages (%) of cases and controls; odds ratio (OR) for BAOS between brachycephalic (BRA) vs. non-BRA dogs, and odds ratio for BAOS among phylogenic groups as defined in Table 1, before and after adjusting for the BRA condition. CI, confidence interval.*

Risk factor	Cases N (%)	Controls N (%)	Unadjusted OR (95% CI)	OR adjusted for BRA (95% CI)
BRA	39 (86.67)	96 (14.61)	37.98 (15.65-92.16)	n.a. a
G1	4 (8.89)	61 (9.28)	1.0 (ref)b	1.0 (ref)
G2	15 (33.33)	52 (7.91)	4.40 (1.37-14.07)	11.68 (3.25-42.03)
G3	6 (13.33)	288 (43.84)	0.32 (0.09-1.16)	8.70 (1.65-45.84)
G4	20 (44.44)	256 (38.96)	1.19 (0.39-3.61)	3.46 (1.09-11.00)

a group of reference, b not applicable

ton terrier, Pekingese, Boxer, Bulldog, Shih Tzu, Shar Pei, and King Charles spaniel breeds.

Dogs were classified in four phylogenic (PHYDO) clusters as shown in Table 1. The clusters are genetically distinct subpopulations created after analyses of patterns of allele frequencies in molecular markers.[13] The first one (G1) represents an ancient group of breeds with Asian and African origins, the second (G2) includes Shetland and Belgian sheepdogs, the third (G3) rallies modern breeds with hunting-associated behaviours, and the last group (G4) contains Mastiff-like breeds that share common physical characteristics.

We conducted a logistic regression analysis using the software program SAS. The potential risk factors for each case and control were the PHYDO groups (n = 4) and the BRA status (n = 2). The odds ratios (OR) are presented with their 95% confidence intervals. When differences were found across PHYDO groups, BAOS frequencies were also compared across breeds within each PHYDO groups.

RESULTS

During the study period, the residents examined 2207 dogs from around 80 breeds, of which 1.13% were affected with BAOS (n = 45). Control dogs were mostly presented for non-hereditary diseases (60%), hip dysplasia (6%), or inherited retinal diseases (2%), all presumably not directly related to BAOS. On average, dogs were diagnosed with BAOS at 3.5 years old (95% CI: 2.4-4.6), with an odds greater in males than females (OR = 2 with 95% CI: 1.05 - 3.9). Mean age at referral was 6.5, 6.4, 7.1, and 5.8 years in groups G1, G2, G3 and G4, respectively. After matching on age and sex, no difference (P>0.10) was found in age and sex repartition between PHYDO and BRA groups.

A total of 45 dogs presented with signs of BAOS, 39 of which were BRA. Results of the logistic regression are given in Table 2. The BRA dogs (19%) were the most likely to present with BAOS, with an OR of 38 (15.6-92.1). The percentages of BRA dogs were 60%, 37%, 1% and 24% within G1, G2, G3 and G4, respectively. The odds for occurrence of BAOS increased after adjustment for the BRA condition. It was significantly higher in G2 than G1 dogs, before and after adjustment for the BRA condition. For G3 and G4, ORs were significantly higher than in G1 only after they were adjusted for the BRA condition.

In Table 3, the prevalence of BAOS is shown for each BRA breed. The occurrence of BAOS was highest among pugs (60%), followed by the English (52 %) and the French bulldogs (35%). The prevalence was statistically different (P<0.05) among breeds within the PHYDO group G4, with no BAOS observed in the boxers and an average of 44% of BAOS in bulldogs.

DISCUSSION

Besides confirming the previously reported association between BRA condition and BAOS (Koch et al., 2003), this epidemiologic study revealed that membership in a PHYDO group may be an additional risk

factor for BAOS. This is shown by the increase in OR after adjustment for BRA for all ORs in Table 2. In the next sections, we will discuss the findings and weaknesses of the study design.

Influence of the Phylogenic Clusters

Our analysis suggests that BAOS is not an ancestral disease and could have originated from a founder of a specific subset of contemporary dog breeds. Indeed, differences in susceptibility to BAOS between PHYDO groups are accounted for, at least in part, by the differences between groups in the allelic frequency patterns of the microsatellite markers.13 Thus, the observation that whatever the BRA status of a dog, the risk of BAOS was the lowest in the G1 group should be linked to the genetic divergence between this and the other groups. The G1 group is distinct because it represents the most ancient descendants of the dog's wolf ancestor13, which suggests that BAOS appears relatively recently in the phylogeny of the domesticated dogs. Dogs from this group, like the Shih-Tzu, Shar-Pei, Lhassa Apso and Pekingese, also all originate from East Asia (China and Tibet), which is where early dogs migrated with nomadic human hunters to Africa and the Arctic.15 The risk of BAOS was greater in the other 3 clusters (G2, G3 and G4), which include more recent breeds, created primarily in Europe or North America in the past 200 years. In our particular sample, the effects of G2 and G3 on the risk of BAOS were confounded with the effects of breeds known to be susceptible to BAOS, i.e. the Pug and the Cavalier King Charles.12,17 Indeed, 37% of the dogs in G2 were pugs, of which 60% were diagnosed for BAOS. In G3, the overall risk of BAOS was high among BRA breeds, probably because only 4 dogs in this cluster were BRA, from which one Cavalier King Charles dog was diagnosed with BAOS (Table 3). The observation that Pugs and Cavalier King Charles were both susceptible to BAOS, but belong to different phylogenic clusters may be explained by the origins of the breeds. Indeed, it is believed the Cavalier King Charles was created in the 1800's from a cross between the pug and the old King Charles spaniel.10

We also found that (English and French) bulldogs and boxers, all brachycephalic and belonging to the same G4 cluster, differed in the frequencies of BAOS: The frequency of BAOS was around 44% in bulldogs and null in Boxer dogs. Similarly, Lorison[12] and Hendricks[9] reported BAOS was more common in English bulldogs than in boxers. This observation may narrow the search for risk factors other than BRA because these breeds belong to the same cluster. Thus, they have to share some characteristics, including BRA, but not the characteristics associated with susceptibility to BAOS. The breeds also relate in heritage and appearance to the Alaunt, a now extinct Molosser dog breed, of which the Tibetan Mastiff (G1

Table 3. *Frequency of BAOS in brachycephalic breeds belonging to the phylogenic groups defined in Table 1*

Phylogenic group	Brachycephalic breeds	Number of dogs	% BAOS intra-group
G1	Shih-Tzu	15	13.33
	Shar-Pei	15	6.67
	Lhassa Apso	4	0.00
	Pekinese	5	20.00
G2	Pug	25	60.00
G3	Cavalier King Charles	4	25.00
G4	Boxer	24	0.00
	English bulldog	23	52.17
	French bulldog	20	35.00

cluster) could be a living representative.[4] Furthermore, historical records point to the influence of the Brabanter Bullenbeisser, a descendant of the Tibetan Mastiff, and the Bulldog in the creation of the boxer breed.16

Weaknesses of the Study Design

The validity of our study rests in part on the assumption that the distribution of exposure (BRA and PHYDO) in the controls is representative of the Belgian population. However, clinical reports only include dogs referred for diagnosis and treatment. They do not represent a random sample of all Belgian dogs, and they may present most severe and complicated cases of BAOS.[6] However, there is no reason to believe that dogs from a specific breed were presented preferentially to the clinics to be treated for BAOS than for any other disease. Thus, we may assume that selection of controls suffered from biases similar to those that entered into the selection of BAOS cases. We may also assume no selection bias due to prior knowledge of clinicians of a potential association between PHYDO and BAOS. Furthermore, we randomly selected controls matched for age and sex.

We should also consider that differences between BAOS frequencies across breeds and PHYDO groups were due to effects not considered in the study. For example, symptoms associated with BAOS are exacerbated with excessive excitement or exercise, being overweight, or inflammation and oedema of the airway tissues.[11] Such effects were not reported, so controls could not be matched on them. Results of the study may also be biased by the breed definition. Indeed, dogs were assigned to a particular breed on the basis of their appearance and the owner's statement, but they could have been of mixed ancestry. Also, we assumed that dog breeds constitute homogenous entities, but the popularity of some dogs may have created isolated subsets of dogs within some breeds, as in the Portuguese livestock guarding dogs.[14]

The definition of BAOS was another potential of source of bias, as it was based on clinical symptoms with possibilities for misclassification.[5] For example, dogs with vocal fold granulomas, frequent in French Bulldogs, could have been misclassified as having BAOS because clinical symptoms are similar in both diseases. Barometric whole-body plethysmography findings that exactly characterize the respiratory variables in BAOS dogs[1] could be considered as a more precise tool. However, further clinical studies and analytic methods, such as reverse phenotyping and structural equation models, are necessary to confirm them as useful endophenotypes of BAOS.

ACKNOWLEDGEMENTS

We thank the service of small animal clinic for providing data and the EADGENE (European Animal Disease Genomics) Network for sponsoring.

REFERENCES

1. Bernaerts F, Talavera J, Leemans J, Hamaide A, Claeys S, Kirschvink , Clercx C. 2008. Description of original endoscopic findings and respiratory functional assessment using barometric whole-body plethysmography in dogs suffering from brachycephalic airway obstruction syndrome. The Vet. J. doi : 10.1016/j.tvjl.2008.09.009.
2. Calboli FCF, Sampson J , Fretwell N , Balding DJ. Population structure and inbreeding from pedigree analysis of purebred dogs . Genetics 2008; 179:593-601.
3. Evans HE. Arthrology, In : Miller's Anatomy of the dog. 3rd ed. Philadelphia : W.B. Saunders, 1993; 219 257.
4. Fleig D. Fighting dogs breeds. In: TFH Publications, ed. Neptune City NJ, USA. , 1993,
5. Galibert F, André C, Hitte C. Dog as mammalian genetic model. M S-Medicine Sciences 2004; 20, 761-766.
6. Grimes D, Schulz K. Bias and causal associations in observational research. The Lancet 2002; 359: 248-252.
7. Harvey CE. Inherited and congenital airway conditions. J. Small Animal Practice 1989; 30, 184-187.
8. Hendricks JC. Brachycephalic airway syndrome. Vet. clinics of North America : Small animal practice 1992; 22, 1145-1153.
9. Hendricks JC. Brachycephalic airway syndrome. In: King LG, ed. Textbook of Respiratory Disease in Dogs and Cats. 1st ed. Saint Louis: Saunders, 2004:310–318.
10. Kendall F, Montague S. Pugs in public. In: Stewart, Tabori and Chang, eds., U.S. Media Holdings, 1999, New York: 1-79.
11. Koch DA, Arnold S, Hubler M, Montavon PM. Brachycephalic syndrome in dogs. Comp. Continu-

ing Education for the Practicing Veterinarian 2003; 25, 48-55.

12. Lorinson D, Bright RM, White RAS. Brachycephalic airway obstruction syndrome - A review of 118 cases. Canine Practice 1997, 22, 18-21.
13. Parker HG, Kim LV, Sutter NB, Carlson S, Lorentzen TD, Malek TB, Johnson GS, DeFrance HB, Ostrander EA, Kruglyak L. Genetic structure of the purebred domestic dog. Science 2004; 304, 1160–1164.
14. Petrucci-Fonseca F, Pires AE, Ribeiro S, Almendra L, Clemente A, Collaço MT, Matos J, Simões F. The use of livestock guarding dogs for wolf conservation in Portugal. First results. Galemys 2000; 12,135-48.
15. Savolainen P, Zhang YP, Luo J, Lundeberg J, Leitner T. Genetic evidence for an East Asian origin of domestic dogs. Science 2002; 298, 1610-1613.
16. Somerfield E. The Boxer. Popular Dogs Publishing Company Ltd, 1997; London, United Kingdom.
17. Torrez CV, Hunt GB. Results of surgical correction of abnormalities associated with brachycephalic airway obstruction syndrome in dogs in Australia. Journal of Small Animal Practise 2006; 47, 150-154.
18. Vilà C, Savolainen P, Maldonado JE, Amorim IR, Rice JE, Honeycutt RL, Crandall KA, Lundeberg J, Wayne RK. Multiple and ancient origins of domestic dog. Science 1997; 276, 1687-1689.