

BREEDING SHORT-MUZZLED DOGS

Criteria for the enforcement of Article 3.4. of the Animal Keepers Decree (*Besluit Houders van dieren*) – Breeding Companion Animals

Dr Marjan AE van Hagen

Department of Animals in Science and Society
and the Expertise Centre Genetics of Companion Animals

Commissioned by the Ministry of Agriculture, Nature and Food Quality

21 01 2019

NOTE: In the translation we have done our utmost to uphold the content of the original text in Dutch. However, for proper reading in some cases scientific English terms were used, instead of layman's language and where necessary a sentence was rephrased to increase readability.

Contents

Introduction.....	4
Part I: Brachycephaly – dysmorphology of the skull and muzzle	5
1. Morphology: skull and muzzle shape	5
2. Health and welfare risks for short-muzzled dogs.....	7
2.1. Brachycephalic Ocular syndrome (BOS)	8
2.3. Brachycephalic Obstructive Airway Syndrome (BOAS)	9
Part II: List of existing measuring methods	11
1. Skull conformation	11
1.1. post mortem	11
1.2. in vivo	12
2. Relative muzzle length	13
3. Nostril stenosis	14
3.1.: nares/cartilage ratio	
3.2.: The 'Liu degrees of nostril stenosis'	15
4. Presence of a nasal skin fold	16
5. Exposed sclera	17
6. Relative palpebral fissure length	17
7. Exercise tolerance tests	19
7.1. The 6-minute walk test	19
7.2. The 1000-metre walk test	19
8. Additional measurements	21
8.1. Breed-defining measurement protocols	21
8.2. Body condition score (excess weight)	21

Part III. Proposed criteria for the enforcement of Article 3.4. Breeding Companion Animals	23
1. Traffic-light system: Moving towards a healthy dog population	24
2. Widely endorsed movement of breeders, veterinary specialists and enforcement officers	24
I. Criteria verified by the Netherlands Food and Consumer Product Safety Authority (NVWA) and Inspectorate of the Dutch Society for the Protection of Animals (LID) inspectors	26
II. Additional criteria to check by primary veterinary surgeons and/or specialists	
A. Brachycephalic Ocular Syndrome	27
A.1. By a primary veterinary surgeon (or Ophthalmology specialist)	27
A.2. By a veterinary surgeon specialising in Ophthalmology	27
B. Brachycephalic Obstructive Airway Syndrome	29
B.1. By a primary veterinary surgeon (or ENT specialist)	29
B.2. Exercise tolerance test	30

Introduction

A number of changes in the Animal Keepers Decree entered into effect on 1 July 2014. Those changes were introduced in order to improve the welfare of animals involved in the commercial breeding of companion animals.

Article 3.4. of the Decree contains rules in respect of the breeding of companion animals. The general rule is that it is prohibited to breed companion animals in a manner that is detrimental to the welfare and health of the parent animal or its offspring. This means, among other things, ensuring, where possible, that when breeding dogs no serious abnormalities and diseases are passed on to or could be acquired by the offspring. In addition, where possible, no conformational features that may compromise the welfare and health of the animal must be passed on. The same applies to serious behavioural abnormalities. However, the Decree is limited to target requirements, making the standards open to interpretation and in need of further elaboration. To date, the sector has been slow in taking the action necessary to achieve this, making it difficult for the Inspectorate and judiciary to enforce the provisions laid down in the Article.

To enforce Article 3.4. it has to be brought to light that a breeder has made insufficient effort to prevent harmful conformational features, serious diseases or behavioural abnormalities from being passed on to offspring. A genetic predisposition to serious diseases or behavioural abnormalities is not always noticeable externally and this complicates enforcement based on prevention. In this project we have focused on clearly visible harmful conformational features of animals used for breeding related to brachycephaly which are passed on to offspring and represent a serious welfare risk.

The popularity of brachycephalic dogs (brachy = short and cephalic = relating to the head) is increasing throughout the world. Breeding selection has resulted in an ever increasing dysmorphism of dogs' skulls. Breeding dogs with this kind of serious skull and muzzle abnormalities results in physical and psychological distress and restrictions in their natural behaviour, which both infringes the animals' integrity and poses a great risk to their welfare. This is contrary to the provisions of Dutch legislation (the Animals Act (*Wet Dieren*)).¹ Breeding selection towards increasingly less extreme dysmorphism could remedy infringements of integrity and reduce welfare risks. The results of this project will therefore form an important prelude to the further extension of enforcement criteria for breeding dogs (and other animals) in terms of a wider range of health and welfare risks.

Composition of the project group

- Dr Hille Fieten, veterinary surgeon, Specialist in Internal Medicine for Companion Animals, coordinator of the Expertise Centre Genetics of Companion Animals, Faculty of Veterinary Medicine
- Dr Franck L.B. Meijboom, associate professor, Department of Animals in Science and Society (DWM), Faculty of Veterinary Medicine
- Dr Marjan AE van Hagen, veterinary surgeon, Animal Welfare Specialist, Ethics & Law, Associate Professor and staff member of the Animal Behaviour Clinic, DWM, Faculty of Veterinary Medicine

Part I:

Brachycephaly – dysmorphology of the skull and muzzle

1. Morphology: skull and muzzle shape

A dog's skull is anatomically shaped by the cranial vault (superior), the skull base (inferior) and the facial skeleton. The cranial vault comprises various bony parts, connected by skull sutures. The various skull bones in young animals are still open (fontanelle) to ease their passage through the birth canal. The muzzle is where the respiratory tract begins. The muzzle is connected to the throat, the inner ear and what are known as sinus cavities, cavities filled with air and coated with mucous membranes in the facial skeleton bone. The channel through which tears flow from the eyes (lacrimal pathway) also ends in the nose.

Dogs are probably the world's most morphologically diverse mammalian species, not least as far as the shape of the skulls is concerned, which varies remarkably.ⁱⁱ

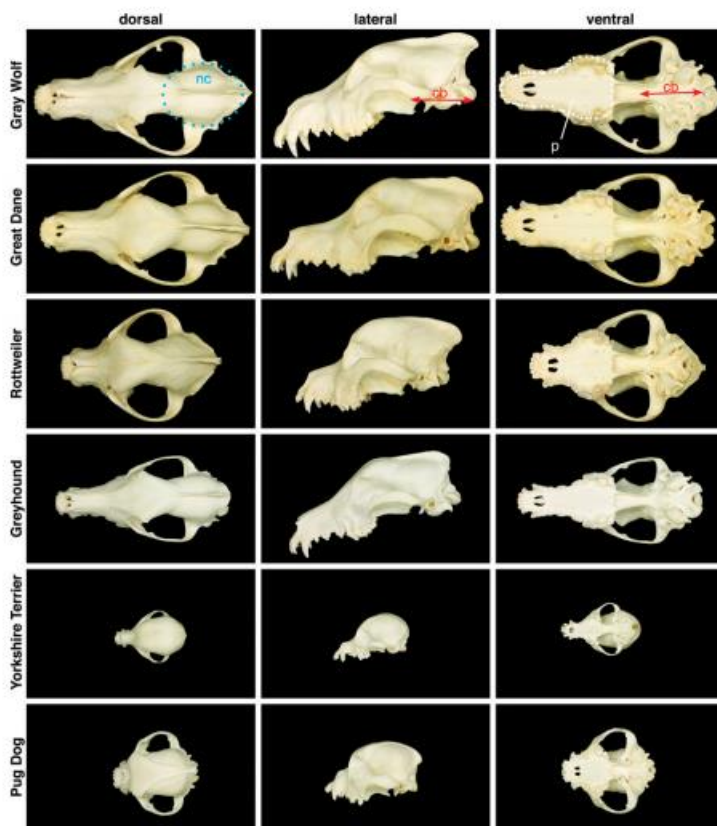


Figure 1: Collection of images showing the diversity in the shape of canine skulls. Source: The Genetics of Canine Skull Shape Variation. Jeffrey J. Schoenebeck and Elaine A. Ostrander GENETICS, 2013

In humans, brachycephaly is defined as a developmental disorder affecting the face and mandible and maxilla including, inter alia, facial retrusion (flattening of the facial skeleton), proximodistal shortness of the nose and widening of the hard palate. Various genetic backgrounds lie behind the developmental disorder. By comparing the genetic information of (374) dogs (various breeds and cross-breeds) with measurements of their skulls, American researchers were able to pinpoint DNA variations that are associated with different head shapes. One variation – found to disrupt the activity of a gene called SMOC2 – was strongly linked to the length of the dog's face. ⁱⁱⁱ

The appearance of brachycephalic dogs is characterised by a short, convex skull shape with a marked fold (stop) between the cranium and the nasal bridge.¹ In the past, dogs with this developmental disorder were selected for breeding because certain dysmorphic characteristics benefited them when carrying out the work for which they were used. This was because the dysmorphia of the skull is often accompanied by an underbite, enabling the dog to breathe through its nose when it grips something with its teeth. During the last century, the breeding selection of dogs with this type of developmental disorder assumed such extreme proportions that the functionality of the dogs was impaired.

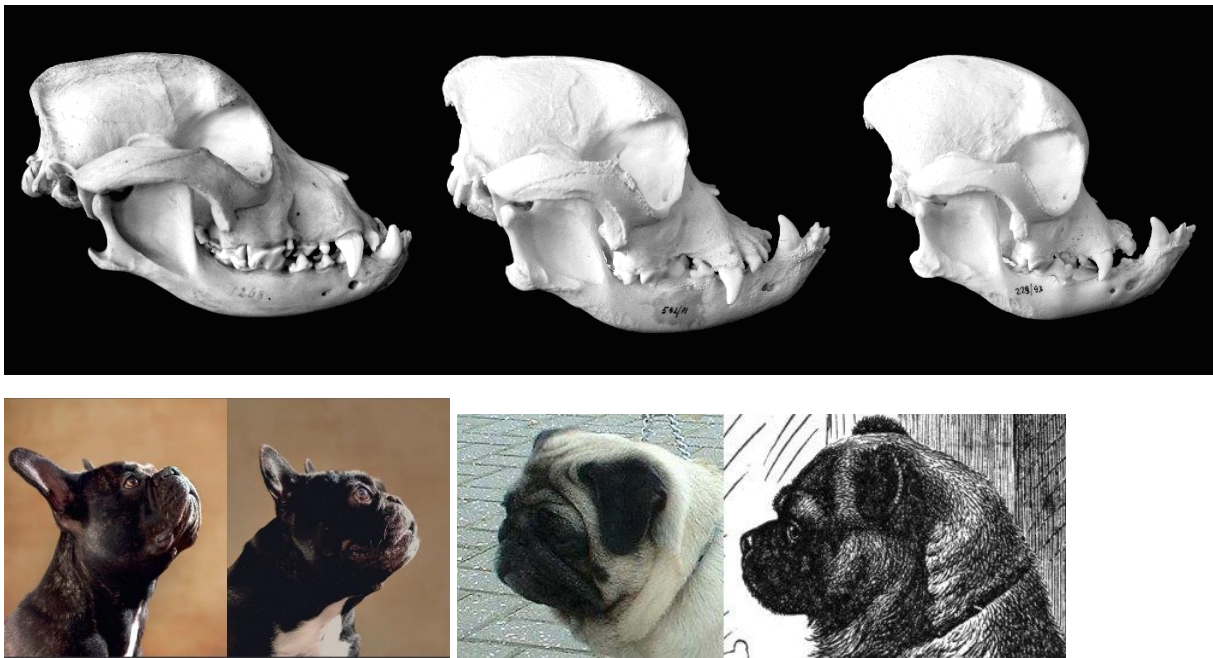


Figure 2: Top: 1. from right to left – brachycephalic skulls developing an increasingly extreme shape Bottom: 2. a brachycephalic French Bulldog (left) and an extremely brachycephalic dog (right), 3. On the left, an extremely brachycephalic Pug (left) and a (far less) brachycephalic ancestor (right).

¹ Source: <https://upload.wikimedia.org/wikipedia/commons/7/7b/Brachycephalie>

2. Health and welfare problems in short-muzzled dogs

As a dog's skull develops, there are four bony plates which meet in the upper middle of the head and are the last bony structures to fuse or ossify. Skull sutures usually close about 4-5 weeks after birth. There are cases where the opening is fairly slow to close and the process is not completed until the dog is six months old. In extreme cases the fontanelle remains open.

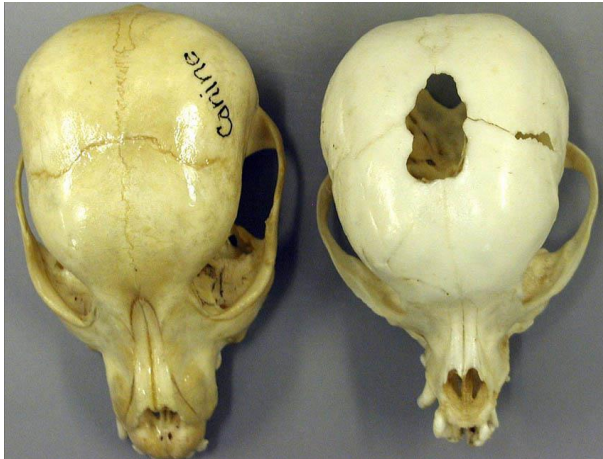


Figure 3. On the left is a normal dog skull and on the right a hydrocephalic skull with an open fontanelle.²

An open fontanelle is related to the development of water in the brain, or hydrocephalus ("hydro" (water) and "cephalus" (head)) – Fig. 3. Water in the brain occurs when the drainage system in the skull does not work properly, obstructing the absorption and elimination of cerebrospinal fluid from the brain and causing a build-up. The increased intracranial pressure means that the frontal and parietal bones do not close (Fig. 3.) In principle, an open fontanelle can occur in any breed of dog, but the condition is most commonly found in small, brachycephalic dog breeds such as the Maltese, Chihuahua, Boston Terrier and Pomeranian. An open fontanelle not only leaves the brain vulnerable to injury, but can also result in neurological abnormalities should water in the brain occur. Since the condition can be hereditary, a dog with an open fontanelle should not be used for breeding.

Not only do brachycephalic dogs (hereinafter called 'short-muzzled dogs') suffer from health and welfare problems related to the abnormal shape of their skulls, e.g. an open fontanelle and Chiari-like malformation – Syringomyelia – we have also observed other hereditary skeletal conditions within this population, such as patella luxation, in growing corkscrew tails and congenital malformations of vertebrae associated with neurological deficiencies.

In this project we have focused on a key group of harmful health and welfare problems related to the abnormal shape of skulls and muzzles, namely: Brachycephalic Ocular Syndrome (BOS) and Brachycephalic Obstructive Airway Syndrome (BOAS). The background to these harmful effects are described in more detail below.

² <http://vanat.cvm.umn.edu/vetAnomal/syNV/NV2.html>

2.2. Brachycephalic Ocular Syndrome

Brachycephalic Ocular Syndrome (BOS) is the collective name for a number of ocular abnormalities found in different combinations in short-muzzled dogs. Dogs afflicted with this syndrome may display a variety of ocular abnormalities, including^{iv}:

exophthalmus, abnormal protrusion of the eyes owing to a shallow orbit;

an oversized palpebral fissure, an excessively wide eyelid aperture relative to the size of the eyeball, and

lagophthalmus, the inability to close the eyelids completely.

Research shows that various conformational features of brachycephalic dogs constitute risk factors for, among other things, the occurrence of corneal damage, including corneal ulcers. Their protruding eyes make effective blinking impossible^v. Among other things, this causes drying of the eyes accompanied by loss of corneal sensation.^{vi} It is stated in a scientific publication on the impact of brachycephalic characteristics on the health of the cornea that dogs with **nasal folds** are nearly five times more likely to be affected by corneal ulcers than those without, and brachycephalic dogs with a **craniofacial ratio of < 0.5** are twenty times more likely to be affected than non-brachycephalic dogs. A 10% increase in **relative eyelid aperture width** more than tripled the ulcer risk. **Exposed sclera** was associated with nearly three times increased risk. The results demonstrate that artificially selecting for these facial characteristics greatly heightens the risk of corneal ulcers, and such selection should thus be discouraged to improve canine welfare.^{vii}

The abnormal shape of the head may be accompanied by other problems, including:

- **luxation of the eyeball** from the orbit: in brachycephalic dogs the eyeball is located in so shallow an orbit accompanied by an eyelid aperture that is so wide the eyeball can easily pop out of the orbit and end up in front of the eyelids (Stades et al., 2007). In some dogs this can happen when the skin of the head or neck is pulled, or when the dog becomes excited. Luxation of the eyeball is painful. The cornea dries quickly and can easily be damaged. Within a short time, the optic nerve will be damaged beyond repair. Even after it has been put back surgically or otherwise, the likelihood of permanent blindness in that eye is very high^{viii};
- **entropion of the medial lower eyelid**: inward rotation of the lower eyelid on the nose side, causing the hair on the surface of the eyelid to rub against the eye;
- **nasal fold trichiasis**: hairs of nasal skin folds come into direct contact (which can be constant) with the cornea;
- **distichiasis**: hairs similar to eyelashes which are abnormally positioned (i.e. on the free margin of the eyelid) and can therefore rub against the cornea;
- **poor tear production and/or quality**, which means that the cornea is more prone to damage as bacteria and potential abrasive particles that come into contact with it are not washed away;
- **pigment keratitis or exposure keratopathy**: pigmentation of the cornea as a result of long-term damage to the corneas through dryness; and
- **epiphora**: tear overflow (which can result in skin irritation in the nasal fold)

The underdeveloped muzzle is out of proportion with the normally developed scalp, resulting in deep nasal folds. In addition to the nasal fold trichiasis risk, the animals are also at high risk of developing dermatitis, inflammation of the skin in the nasal fold caused by rubbing and microbial overgrowth (bacteria and yeast), promoted by the elevated humidity and build-up of secretions in this environment. Skin fold dermatitis gives rise to an unpleasant odour and causes the dog discomfort; in some cases it can trigger self-injury through rubbing and/or scratching.

Daily cleaning of the folds is widely recommended as standard care for dogs of this type, but is often not enough to manage the problem. French Bulldogs have a nasal fold on the dorsal surface of the muzzle, which is so close the tip of the nose that it is completely impossible to clean this deep fold (verbal expert opinion).

2.3. Brachycephalic Obstructive Airway Syndrome

Canine brachycephalic obstructive airway syndrome (BOAS) is associated with a collection of respiratory conditions affecting short-muzzled dogs. They can occur in an individual dog all at the same time, but this will not necessarily always be the case. The various conditions include:

- **Stenosis and narrowed nostrils**
- **Excessively long and/or thickened palatum molle** (soft palate)
- **Enlarged tonsils**
- **Dysmorphism of the larynx** or an abnormally shaped larynx
- **Trachea hypoplasia** or an abnormally narrow windpipe

The clinical signs of BOAS present as a result of obstruction of the upper airways caused by anatomical abnormalities which narrow the airways and increase airway resistance. The abnormally short muzzle means that the mouth is unable to accommodate either the soft palate or the tongue. The tongue and the soft palate are too large and too long relative to the muzzle. Consequently, short-muzzled dogs will often be seen with their tongues hanging out and most make a snoring noise when breathing. This snoring noise is produced by the excessively long soft palate, which is suspended in the pharynx and caused to vibrate by the flow of air. The snoring can range from a mild form that occurs only when the dog exerts itself to a permanent noise audible even when the dog is resting. The increased resistance in the airways leads to an increased risk of secondary laryngeal collapse.



Figure 4. A scan of a canine skull of a normal length (left) and one of a short-muzzled dog; the air-filled frontal sinus and nasal cavity in the normal skull are coloured black. Source:<http://brachycephalia.com/>

Dogs at rest mainly breathe through their noses. Since dogs have no sweat glands, panting is their only means of expelling excess body heat. Breathing problems in dogs with BOAS therefore result not only in breathlessness, but also compromised thermoregulation because of their inability to expel body heat effectively. Complaints range from mild snoring to severe breathlessness (respiratory distress), exercise intolerance, loss of consciousness and death through overheating (heat stroke).

The impact of BOAS is not limited to the respiratory system, but can also lead to secondary problems in the oesophagus and stomach. Excessive salivation (hypersalivation) and retching can occur. The latter is caused by the negative pressure during inspiration which, as a secondary problem, can cause a widening of the opening where the oesophagus passes through the diaphragm (hiatus hernia), causing a reflux of gastric acid and, potentially, inflammation of the oesophagus. (gastro-oesophageal reflux and oesophagitis).

The main risk factors for BOAS are: a flat muzzle, thick neck and excess weight.^{ix} Short-muzzled dogs are generally at very high risk of developing BOAS.^x With a mild form of BOAS the dog's welfare is reduced owing to its difficulty in breathing and, with a severe form, limited mobility, poor appetite and reduced playfulness will seriously diminish its quality of life. Research shows that the risk of BOAS increases as the short-muzzle morphology becomes more exaggerated.^{xi} Careful selection of parent animals with far less extreme dysmorphic characteristics can gradually reduce the risk of BOS and BOAS in short-muzzled dogs. Although this has been known for decades, we can see barely any progress being made by breeders in this direction. Establishing standards to enable the enforcement of Article 3.4 will help to encourage breeders to start selecting parent animals with a greater emphasis on functionality.

Part II:

List of existing measuring methods

1. Skull shape measurements

1.1. Post mortem

In the past, craniofacial morphometric parameters were measured (**postmortem**) on a dry canine skull. ^{xii xiii} The mean skull length of the lion, **dog** and cat were found to be 39.7 ± 1.04 cm, **20.02 ± 1.4 cm** and 8.4 ± 1.5 cm respectively, with an average skull width of 28 ± 2.3 cm, **10.04 ± 0.5 cm** and 6.8 ± 1.4 cm, respectively. ^{xiv} For a long time, these were the only official measurement data for the average canine skull length.

Based on this postmortem measuring method, canine skulls are divided into dolicocephalic (long skull), mesocephalic and brachycephalic (short skull) skull shapes. Moreover, a ratio between the short, broad muzzle (*facial length*) and the *skull width* of 0.81 or greater is regarded as brachycephalic. However, German authors based their classification on the relationship between the *cranial length* and the *skull length*, where a skull length/cranial length ratio of 1.6 to 3.4 is deemed brachycephalic.³ (Figure 5).

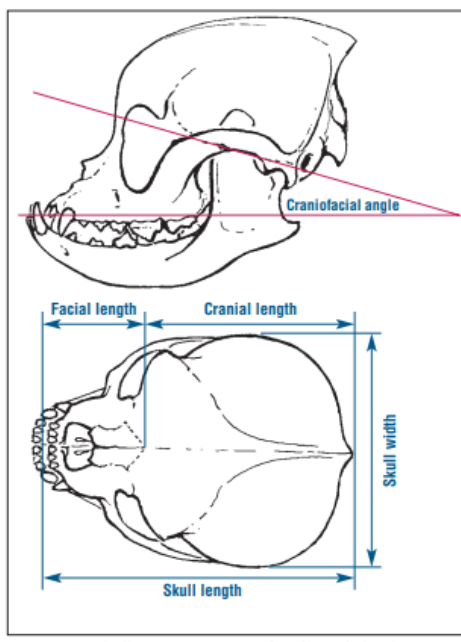


Figure 5. Post-mortem measuring methods used to define a brachycephalic (short skull) skull shape.

³ Brachycephalicsyndrome Compendium Koch, 2003

1.2. In vivo

In 2008, Sutter et al. examined measurements in vivo, i.e. on live animals, in relation to the classifications in dog breeds.^{xv} In total, thirteen conformational features were shown to be breed-defining. They are known as the '*Breed-defining measurement protocols*'. The following variables were defined in respect of the skull:

1. Snout length – SL
2. Cranial length – CL
3. Skull width – SW
4. Eye width – EW



Figure 6: Illustration of 'in vivo' measurements (French Bulldog): Left: snout length (SL) from P1 to P2, cranial length (CL) from P2 to P3, skull length P1 to P3, Right: Eye width (EW), Skull Width (SW)

A skull width of at least 80% of the length is a characteristic feature of brachycephalic dogs. A skull width of more than 80% of the length is therefore deemed extreme brachycephalic.⁴ This provides guidance for a standard that could be used to establish an enforcement criterion.

⁴ <https://www.petinsurancequotes.com/petinsurance/brachycephalic-skull.html>

2. Relative muzzle length

The craniofacial ratio (CFR), or relative muzzle length is calculated by dividing the muzzle length (cm) by the cranial length (cm). Relative muzzle length follows from:

$$\frac{\text{Muzzle length (A-B)}}{\text{cranial length (B-C)}}$$



Figure 7: Illustration of the determination of relative muzzle length by dividing the nose length from A to B and the cranial length from B to C. The relative muzzle length of the Cavalier King Charles Spaniel depicted is 0.27: nose length 28 mm/cranial length 102 mm.

This ratio proves a good gauge for measuring the severity of BOAS. (Fig. 7)



Figure 8.: This figure illustrates the relationship between the external craniofacial factor (CRF) and the corresponding internal anatomical structure of the upper airways



Fig 1. Diagram of how to measure (i) cranial length (A-B) and (ii) muzzle length (B-C). Measurements were taken using a soft measuring tape. Cranial length is defined as the distance (mm) from the occipital protuberance (A) to the stop (B). Muzzle length is defined as the distance (mm) from the dorsal tip of the nasal planum (C) to the stop (B). The precise locations of the nasal planum, stop, and occipital protuberance are determined through palpation as well as visually, but the lettering indicates their approximate locations on the photographs. This is demonstrated in (left-right) an extremely brachycephalic Pug (CFR = 0.08), a moderately brachycephalic Bulldog cross (CFR = 0.23) and a mildly brachycephalic Boxer (CFR = 0.35).

doi:10.1371/journal.pone.0137496.g001

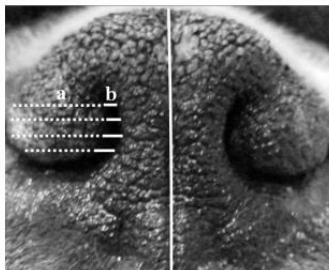
Figure 9. Relative muzzle length: muzzle length from A to B and cranial length from B to C. From left to right, the relative muzzle length is: 0.08, 0.23 and 0.35.

3. Nostril stenosis

Determining the width of the nostril as a measure of the severity of the restriction of airflow through the nose is another way of assessing the severity of the anatomical malformation of the airway. Two methods for quantifying the degree of narrowing of the external nares are described.

3.1. nares -cartilage ratio

With this measuring method, a calliper is used to take four measures of the width of the wing of the nostril – cartilage ('a'), along with the width of the adjacent airspace ('b'). xvi 1. The lowest measure of the lower edge of the nostril wing, 2. the highest measure of the upper edge of the nostril wing and 3. a further two measures of equal distance between the highest and lowest point. The calliper must be placed directly on the nostril so that the dog's head can be kept still to ensure that the measuring instrument does not injure the animal.



'a' (dotted lines) indicates the width of the *alae nasi* (nostril cartilage)
 'b' (solid lines) indicates the width of the adjacent external airway
 The central line indicates the philtrum, delineating the left and right sides of the nasal planum.

Figure 10.: Measuring the nostril using the four-part measuring method

The ratio of the mean nostril thickness to the mean nostril diameter (b/a) is referred to as the **nares ratio**. The greater the ratio, the greater the air opening.

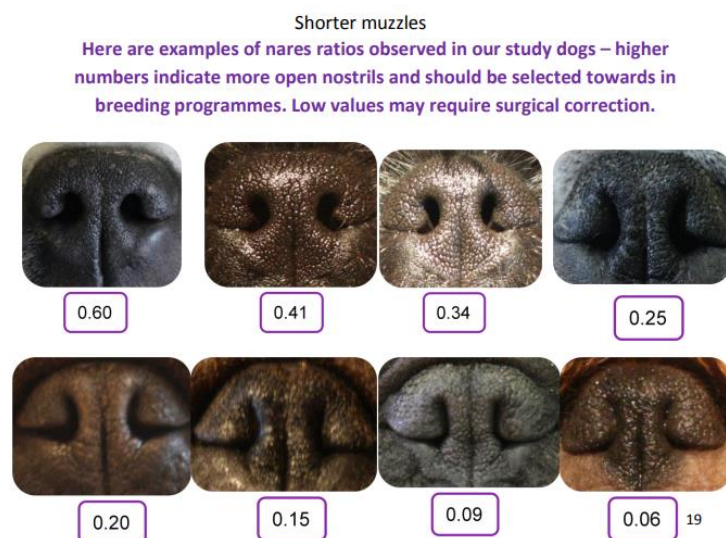


Figure 11. Examples of nostril air opening expressed as the 'nares ratio' ranging from a ratio of 0.06 (extremely nostril stenosis) to 0.60 – wide nostrils. Source: Building better brachycephalics, Packer et al. (2012) Animal Welfare, 21, 81-93 <https://www.ufaw.org.uk/downloads/welfare-downloads/building-better-brachycephalics-2013-report.pdf>

3.2. The 'Liu degrees of nostril stenosis

With this method, developed by Liu et al. (2016), the degree of nostril stenosis is divided into four grades, based on the extent to which the lateral structure makes contact with the medial septum.

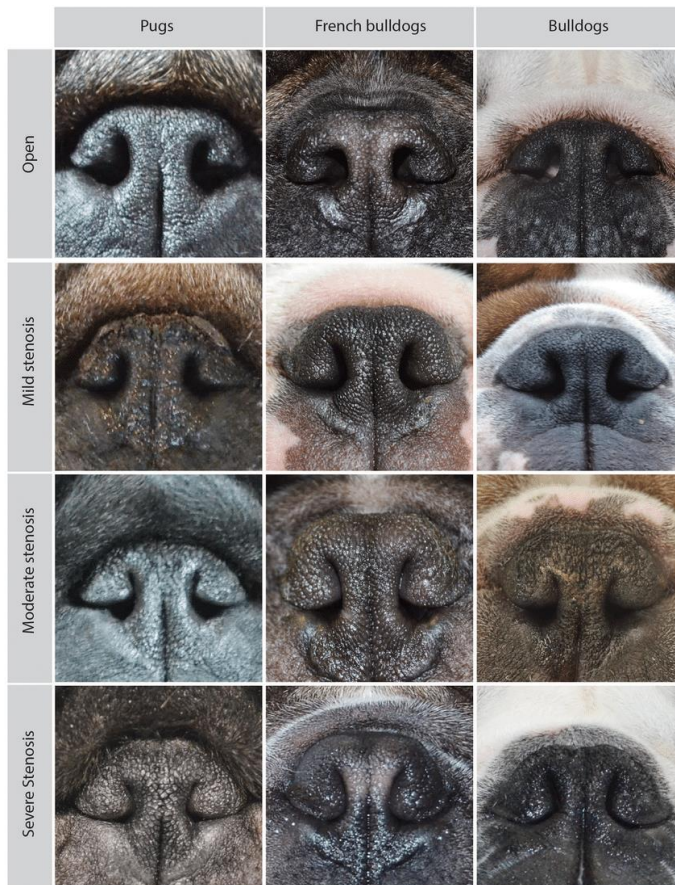


Figure 12. Grading the severity of nostril narrowing as in Liu, 2016. ⁵

Examples of different degrees of nostril stenosis in pugs, French bulldogs, and bulldogs.

The following descriptions were adapted from a published figure by the authors (Fig 1 in Liu *et al.* 2016):

“Open nostrils: nostrils are wide open; mildly stenotic nostrils: slightly narrowed nostrils where the lateral nostril wall does not touch the medial nostril wall. Immediately after the exercise tolerance test (ETT), the nostril wings should move dorsolaterally to open on inspiration; moderately stenotic nostrils: the lateral nostril wall touches the medial nostril wall at the dorsal part of the nostrils and the nostrils are only open at the bottom. Immediately after the ETT, the nostril wings are not able to move dorsolaterally and there may be nasal flaring (ie, muscle contraction around the nose trying to enlarge the nostrils; severely stenotic nostrils: nostrils are almost closed. The dog may switch to oral breathing from nasal breathing with stress or very gentle exercise such as playing.” (Liu *et al.* 2016).

<https://doi.org/10.1371/journal.pone.0181928.g001>

⁵

https://www.researchgate.net/publication/318842582_Conformational_risk_factors_of_brachycephalic_obstructive_airway_syndrome_B_OAS_in_pugs_French_bulldogs_and_bulldogs/figures

4. Presence of a nasal skin fold

Alongside the craniofacial ratio, the presence of a nasal fold proves to be a good gauge for measuring the severity of muzzle shortness and the accompanying risk of eye problems. The skin of mesocephalic or dolichocephalic dogs generally covers the facial bones without any folds being created. A nasal fold will usually be present in short-muzzled dogs. The nasal fold is described in the breed standards for certain short-muzzled dog breeds (Table 1).

Breed	Breed standard text referring to the presence of a nasal fold	Kennel Club 'Breed Watch' points of concern for special attention by judges
Pekingese	A slight wrinkle, preferably broken, may extend from the cheeks to the bridge of the nose in a wide inverted 'v'. This must never adversely affect or obscure eyes or nose. [49]	Heavy over nose wrinkle and prominent nasal folds
Bulldog	Over nose wrinkle, if present, whole or broken, must never adversely affect or obscure eyes or nose. Pinched nostrils and heavy over nose roll are unacceptable and should be heavily penalised. [50]	Heavy overnose wrinkle (roll); Excessive amounts of loose skin that impinge the eye (e.g. from nasal folds)
Pug	Wrinkles on forehead clearly defined without exaggeration. Eyes or nose never adversely affected or obscured by over nose wrinkle. [47]	Excessive nasal folds

doi:10.1371/journal.pone.0123827.t002

A nasal fold is defined as a discernible fold of skin on the dorsal surface of the muzzle that was present without manipulation of the skin, and could be easily grasped between vernier callipers

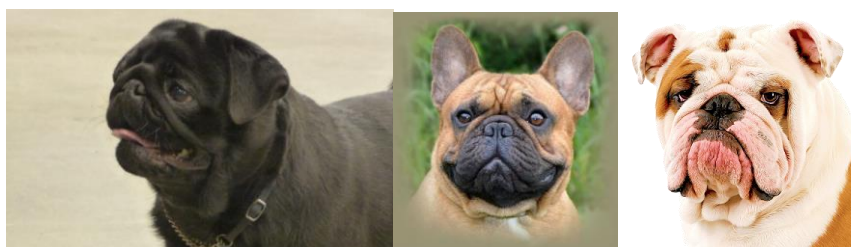


Table 12. Nasal folds in breed standards and nasal fold related statements from The Kennel Club (UK) 'Breed Watch' initiative. Source: Impact of Facial Conformation on Canine Health: Corneal Ulceration, Packer et al. Plos One, 2015

5. Exposed sclera

Exposed sclera is a guide used to establish the anatomical ratio between the orbit (skull) and eyeball size. There is relatively little variation in eyeball size between dog breeds [16], but in short-muzzled dogs the eye orbit is too flat and shallow to be able to accommodate the eyeball fully (and/or the palpebral fissure is too wide). This causes the eyes to protrude, exposing more white of the eye than would be visible with a healthy ratio.



Figure 13: Exposed sclera due to the protruding eyes (exophthalmus) in a pug. Source: <http://pug.at/>

Dogs are examined for the visibility of 'white of the eye' (sclera) when looking directly forwards. This is carried out by gaining the dog's attention (using a toy or treat, for example) and taking a photograph using a digital camera. The overall presence of visible sclera is recorded and further broken down into whether this was visible dorsally, ventrally or laterally to the iris of the eye. This translates to a score of 0-4 dependent on how many quadrants of sclera are visible (max. 4/4).

6. Relative palpebral fissure width

The *relative palpebral fissure width* (%) is an additional measure used to determine the disproportionate construction of the orbit in short-muzzled dogs. A soft tape measure (or callipers) are used to measure the unstretched palpebral fissure width (mm) in conscious dogs by pulling the tape measure taut between the outer and inner corners of the eye (from D-E).



Figure 14: Left: Quantitative determination of palpebral fissure width (D-E). Here, the palpebral fissure width is defined as the straight-line distance between the medial and lateral canthus. Right: Quantitative determination of cranial length (B-C)

$$\text{Relative palpebral fissure width} = (\text{palpebral fissure length (mm)} / \text{cranial length (mm)}) \times 100$$

Table 3. Breeds and relevant conformations of dogs affected by corneal ulcers.

Breed	Mean relative palpebral fissure width \pm SE	Mean craniofacial ratio \pm SE	Number of cases (n)	Percent of corneal ulcer cases (%)	Total breed population	Percent of breed affected (%)
Overall population mean	22.1 \pm 0.16	0.51 \pm 0.01				
Pekingese	34.18 \pm 0.53	0.12 \pm 0.01	2	6.5	3	66.7%
Pug	30.06 \pm 0.78	0.08 \pm 0.01	12	38.7	32	37.5%
Shih Tzu	28.53 \pm 0.59	0.20 \pm 0.01	4	12.9	13	30.8%
Bulldog	20.70 \pm 0.53	0.22 \pm 0.15	3	9.7	16	18.8%
Boston Terrier	26.78 \pm 1.13	0.15 \pm 0.01	1	3.2	6	16.7%
Pomeranian	28.77 \pm 0.84	0.43 \pm 0.04	1	3.2	6	16.7%
French Bulldog	23.59 \pm 0.85	0.19 \pm 0.13	2	6.5	13	15.4%
Cavalier King Charles Spaniel	26.99 \pm 0.51	0.40 \pm 0.01	3	9.7	26	11.5%
Staffordshire Bull Terrier	22.90 \pm 0.85	0.51 \pm 0.02	1	3.2	16	6.3%
Labrador Retriever	18.97 \pm 0.31	0.58 \pm 0.01	1	3.2	56	1.8%
Cross Breed	22.38 \pm 0.38	0.54 \pm 0.01	1	3.2	91	1.1%

Prevalences are also shown by breed.

doi:10.1371/journal.pone.0123827.t003

Unlike absolute palpebral fissure width, relative palpebral fissure width (relative to skull length) turns out to be highly relevant as regards the increased risk of corneal damage. Dogs with corneal ulcers had a wider palpebral fissure relative to the length of their skulls than dogs without corneal ulcers.

Relative palpebral fissure width was found to increase the risk of ulcers by 1.12. As an illustrative example, the mean relative palpebral fissure width for a Labrador Retriever was **19.0** and for a Pekingese was **34.2**, a difference of over 15%. This 5% increase equates to increased odds of 5.47 of corneal ulcers.

7. Exercise intolerance test

Since exercise intolerance and impaired recovery after exercise are major signs of BOAS, an exercise test, such as the 6-minute walk test^{xvii} or the timed 1000-metre walk test are used to assess BOAS severity.

7.1. The 6-minute walk test

In this test, dogs walk on a lead for six minutes at a reasonable pace (at least 5 km/hour) on an unimpeded pathway or hallway measuring approx. 25 meters. No other people or animals with the potential to distract the animal should be present during the walk test. Each dog was walked for six minutes, timed with a stopwatch.

In research settings where the 6-minute walk test is used heart rate, blood pressure (mean systemic arterial pressure, MAP) and blood oxygen levels are also measured (Swimmer, 2011). In that case the dogs' heart rates and blood pressures are measured before and after they have walked for six minutes. Heart rate is obtained by auscultation and MAP with the use of a blood pressure monitor. The blood oxygen level is determined with the aid of a pulse oximeter.

The average dog's normal blood pressure is approx. 133/76 mm Hg (systolic/diastolic). This normal value can vary slightly from breed to breed. At rest, a value of 160 mm Hg or higher is considered abnormal. When the animal is subject to stress, its blood pressure will rise and may exceed 160 mm Hg.

The equipment used to measure a dog's and/or a cat's blood pressure is generally the same as that used for humans. The inflatable armband is placed around the tail or a fore limb. It takes a few minutes to record the animal's blood pressure. The conclusion from this research was that the 6-minute walk test is simple to carry out and shows the difference between healthy dogs and dogs with an airway disorder.^{xviii}

The 6-minute walk test has also been used in research into pulmonary fibrosis in West Highland White Terriers to assess cardiopulmonary function.^{xix}

7.2. The timed 1000-meter walk test

In this fitness test, dogs cover 1000 meters in 12 minutes and be able to recover in 15 minutes. The test is conducted under the supervision of two veterinary surgeons. They establish the dogs' heart rate and temperature. This is done before the start of the test and five, ten and fifteen minutes after its completion. The breathing noises made by the dogs are also assessed at those points (*no noise is made, the dog is panting, or the panting is accompanied by a high-pitched sound*) If the temperature and heart rate drop and breathing returns to a *normal type* and frequency, the dog passes the walking test.

The rules are laid down in an agreement between the Dutch Kennel Club and two English Bulldog breed clubs.⁶⁸ Special days are organised during which a breed suitability inspection takes place. The Bulldogs are subjected to a fitness test on those days. (They are also tested for patellar luxation and receive a conformation score.)

⁶ The rules of the agreement and the breeding rules of the breed clubs apply to members of the breed club. EBCN, 2014

A Finnish study evaluated the severity of respiratory symptoms and anatomic components of BOAS in a group of prospectively recruited young adult English Bulldogs (n = 28) and investigated the correlations of the 6-minute walk test or the 1000-meter walk test with a veterinary assessment of BOAS severity. Based on veterinary clinical examination findings, the severity of the symptoms was scored using an ordinal 4-level scale for: *1. the presence or absence of upper respiratory noise at rest and 2. after exercise, 3. respiratory type at rest and 4. resting dyspnoea or cyanosis.*

English Bulldogs with more severe BOAS walked a shorter distance, more slowly and their recovery from exercise took longer than those with only mild symptoms of BOAS. Control dogs of different breeds (n = 10) performed the exercise tests significantly better (i.e. longer distance, faster time and recovery) than English Bulldogs. Increases in body temperature during exercise were significantly higher in English Bulldogs than in controls. The results of this study support the use of exercise tests for objective evaluation of the severity of BOAS in English Bulldogs.^{xx}

8. Additional measuring methods for determining skeletal shape

8.1. Breed-defining measurement protocols

In addition to skull measurements, in 2008 measurements of other parts of the body of live animals were described in relation to classification into dog breeds.^{xxi} These were the thirteen breed-defining metrics, known as 'Breed-defining measurement protocols, namely:

1. Snout length – SL
2. Cranial length – CL
3. Skull width – SW
4. Eye width – EW
5. Neck length (NL)
6. Neck girth
7. Chest girth
8. Body length - BL
9. Height of the withers
10. Height of the base of the tail
11. Fore limb circumference
12. Hind limb circumference

Researchers also identified a thick neck as a risk factor for the development of BOAS. What is known as '*relative neck girth*', which is established by dividing the circumference of the neck by the circumference of the chest (neck girth/chest girth), is a good indicator of a high BOAS risk. The circumference of the neck is measured in the centre of the distance between the two occipital bones and the point between the cranial borders of the right and left scapulae. The circumference of the chest is measured at the deepest point of the chest cavity.

Obesity is another important risk factor for the potential exacerbating effects.

8.2. Body condition score (overweight):

Among other things, overweight increases the risk of respiratory problems, heart disorders, and also joint and bone problems. Incidence of overweight and obesity in dogs exceeds 30%, and several breeds are predisposed to this heritable phenotype.^{xxii} Genetic predisposition to excess weight is demonstrated in, among other breeds, the Labrador Retriever, but to date insufficient research has been conducted within the short-muzzled dog population.^{xxiii} However, since short-muzzled purebred dogs are genetically predisposed to airway problems, resulting in breathlessness, they should never be allowed to become overweight.

Various scales have been developed to score excess weight objectively. The scoring system (1-9) promoted by the World Small Animal Veterinary Association is a frequently used scale.

Part III:

Proposed criteria for the enforcement of Article 3.4. Breeding Companion Animals

There are various scientifically substantiated measuring methods that can be used to quantify objectively the morphological characteristics of short-muzzled dogs related to the risks of BOS and BOAS developing (see the list in part II of this report). Choosing breeding dogs with mild dysmorphia can have a positive impact on the welfare risks faced by offspring. Unfortunately, few people involved in dog breeding today are making such choices.

Article 3.4. The purpose of the Breeding Companion Animals section of the Animal Keepers Decree is to ensure that no conformation features which could have a harmful effect on the welfare and health of the animal are passed on. The complexity of the pure-bred dog world, inhabited by hobby and commercial breeders and dealers, combined with standards that are open to interpretation has so far resulted in an inability to take adequate action when it comes to enforcing this Article. This inability to take adequate action can be remedied only through the adherence to enforcement criteria which are not only substantiated by science but are also monitored in the field by NVWA and LID inspectors for large groups of dogs.

To facilitate this transition to practice, the available measuring methods have been presented to a panel of veterinary specialists in the areas of ENT, ophthalmology, dermatology and animal welfare.

Membership of the advisory group:

- Dr Sylvia C. Djajadiningrat-Laanen – Veterinary Surgeon, Ophthalmology Specialist, University Clinic for Companion Animals, Faculty of Veterinary Medicine, Utrecht
- Dr Jeffrey de Gier – Veterinary Surgeon, Reproduction Specialist, Companion Animal sub-specialisation, University Clinic for Companion Animals, Faculty of Veterinary Medicine, Utrecht
- Dr Gert ter Haar – Veterinary Surgeon, Companion Animal Surgery Specialist (ENT), Specialist Animal Clinic, Utrecht
- Dr Paul J. J. Mandigers – Veterinary Surgeon, Neurology and Internal Medicine of Companion Animals Specialist, University Clinic for Companion Animals, Faculty of Veterinary Medicine, Utrecht
- Dr Yvette Schlotter – Veterinary Surgeon, Dermatology Specialist, University Clinic for Companion Animals, Faculty of Veterinary Medicine, Utrecht

The group was asked to identify the minimum number of criteria and the standard that could be used to ensure enforcement takes place in order to achieve the intended objective. Enforcement officers and specialists then entered into consultation. The outcome of that consultation is described in this section.

1. Traffic-light system: Moving towards a health dog population

In the workfield of dog breeding it is often stated that the **ideal standard**, which should be uphold concerning the animal health and welfare, is not suitable for certain short-muzzled (pure-bred) dog populations because hardly any of them would fall within the standard. While very worrisome in itself, this observation does require a practical approach. Hence the decision is made to opt for a transitional phase during which breeders are given the opportunity to shift – within two to three generations – in the direction of the minimum standard and a low-risk dog population by means of breed selection. Therefore the **TRAFFIC LIGHT principle** will apply, meaning the first (practicable) step of which is to set dogs with the **most extreme conformational features** as the limit and to continue to tolerate dogs with **less extreme conformational features** after which (in two generations' time, for example) the limits will be tightened so that ultimately all future breeding animals will meet or will have to meet the **ideal standard**.

In populations where virtually every dog turns out to exceed the limits of the standard, leaving little to no suitable dogs for breeding, a mandatory breeding programme using, for example, out-cross animals, could be a solution. Such a breeding programme would require not only an assessment of BOS and BOAS risks but also other desired health and behavioural characteristics.

2. Widely endorsed movement of breeders, veterinary specialists and enforcement officers

Given the circumstances in which enforcement officers sometimes have to work, a lot of measurements or investigations are impracticable for them to carry out. Enforcement will therefore have to be focused on criteria which the inspectors can determine themselves in situ. In cases of doubt or where the dog owner objects to findings made during an inspection, an assessment or 're-assessment' could be carried out by a designated and skilled primary veterinary surgeon and/or a veterinary surgeon specialising in ophthalmology and/or one specialising in internal medicine, surgery or ENT.

Centralised record-keeping is recommended to enable monitoring of the impact, positive or otherwise, of enforcement on the dogs. Keeping central records of all measurement results will show whether implementation of the enforcement and other criteria is having the desired effect on the dog population. Centralised record-keeping will provide an insight into the current state of affairs – baseline measurement – within different populations and also the speed at which breeding selection is bringing the risk level down to the ideal standard. This is a key evaluation step for the policy applied. The Expertise Centre Genetics of Companion Animals (ECCG) can analyse these data and, based on the findings, advise the NVWA and LID whether and when the standard should be adjusted from **red** to **amber** and **green**. In future, the database can be expanded to include additional measurements of other breed characteristics and/or hereditary disorders.

In addition to the minimum number of enforceable criteria, veterinary surgeons (specialists) can also perform supplementary measures that could help breeders to make better choices and select healthy breeding dogs at limited risk of acquiring BOAS or BOS.

The boundaries between green, amber and red are described in the tables in the following pages, accompanied by the score given when the limits of the standard are exceeded and breeding with the dog is not permitted.

This involves:

1. The criteria and standards for short-muzzled dogs with which NVWA and LID inspectors can arm themselves and use to select animals which are unhealthy and/or unsuitable ('high risk') for breeding and therefore need to be excluded.
2. Other, additional criteria which only a practitioner or specialist can verify and may be used as a supplement, for instance to supplement evidence in legal proceedings, but also with a view to careful breeding selection.
3. Elaboration of the action primary veterinary surgeons can take in accordance with the Royal Netherlands Veterinary Association Guideline *on Veterinary action in respect of welfare risks affecting dogs and cats with hereditary disorders and harmful breed characteristics* to assist with accurate breeding selection.

I. Criteria and standards for verification by NVWA and LID inspectors

It is contrary to Article 3.4 of the Keepers of Animals Decree, Breeding Companion Animals to use animals where there are indications of an increased risk of BOS and/or BOAS developing. The following table describes the criteria, including the interpretation thereof, on the basis of which you can verify whether a dog may be used for breeding (traffic light coding: green/amber/red). Breeders are in contravention and should not have used the dog for breeding where the limits of the standard for to use the animal for breeding have been exceeded.

	Criterion	Aim considering the risk of BOS and BOAS	Enforcement standard	Consequences of exceeding the limits of the standard
1	Abnormal breathing sound (stridor)	Absent	The dog makes loud sniffing, snoring or sawing sounds when at rest (not asleep) or a nasal, pharyngeal and/or laryngeal stridor can be heard when the dog is at rest	Exceeds the limits of the standard, irrespective of other criteria
2	Nostrils	Open nostrils	Mild to moderate stenosis = mild to moderate narrowing of the nostrils	Meets the standard for now, provided none of the limits of the other criteria is exceeded
			Severe stenosis = severe narrowing of the nostrils	Exceeds the limits of the standard, irrespective of other criteria
3	Relative muzzle length	Equal to or greater than 0.5 ¹	Greater than 0.3 but less than 0.5	Meets the standard for now, provided none of the limits of the other criteria is exceeded
			Less than or equal to 0.3	Exceeds the limits of the standard, irrespective of other criteria
4	Nasal fold	No nasal fold present	Nasal fold present but no visible contact between the hairs of the nasal fold and the conjunctiva or cornea of the eye, no wet nasal fold hairs adjacent to the cornea, no signs of ocular inflammation adjacent to the nasal fold	Meets the standard for now, provided any inflammation/dermatitis related to intertrigo is treated in accordance with the provisions of the Welfare Act and none of the limits of the other criteria is exceeded
			Nasal fold present and hairs from the nasal fold are in contact with or could come into contact with the cornea or conjunctiva (wet nasal fold hairs)	Exceeds the limits of the standard, irrespective of other criteria
5	Exposed sclera (white of the eye)	White of the eye not exposed or at most very slightly exposed near the lateral canthus (in the outermost corner of the eye) only	White of the eye exposed in two or more quadrants -> shallow orbit and/or an excessively wide eyelid aperture, with a poorly protected eyeball and increased risk of corneal ulcers developing	Exceeds the limits of the standard, irrespective of other criteria
6	Extent of eyelid closure when the eyelid reflex is elicited	Eyelid reflex present and eyelids close fully	Eyelids cannot be closed fully	Exceeds the limits of the standard, irrespective of other criteria

¹ **Signs of ocular irritation** include: tear overflow, brown discolouration of the fur surrounding the eyes, mucoid/mucopurulent ocular discharge, blinking, rubbing the eye, photo-sensitivity, red, swollen conjunctiva

≠ Short-muzzled dogs with a craniofacial ratio of <0.5 are twenty times more likely to develop corneal ulcers (a serious condition affecting the cornea) than those without shortened muzzles. (Source: Impact of facial conformation on canine health: corneal ulceration, Packer et al.) ²⁷

II. Additional criteria for testing by primary veterinary surgeons and/or specialist veterinary surgeons

To supplement the above criteria with a view to careful breeding selection where there are doubts regarding potential or current parent animals, or to be used as evidence in legal proceedings (objection/appeal).

A. Brachycephalic Ocular Syndrome (BOS):

A.1. By a primary veterinary surgeon (or veterinary surgeon, specialised in ophthalmology)

	Diagnosis	Aim	Enforcement standard	To be factored in or conclusive?
1	Tear production (Schirmer tear test)	13-25 mm in one minute (in the absence of corneal defects and/or sources or signs of eye irritation; in the presence thereof: see "9-12 mm of tear fluid in one minute")	9-12 mm in one minute (in the absence of corneal defects and/or sources or signs of eye irritation; in the presence thereof: see "less than 9 mm of tear fluid in one minute")	Meets the standard, provided none of the limits of the other criteria is exceeded
			Less than 9 mm in one minute and signs of keratoconjunctivitis sicca	Exceeds the limits of the standard, irrespective of other criteria
2	Trichiasis of the nasal fold (nasal fold hairs make contact with the margin of the eyelid, conjunctiva or eyeball; tell-tale sign: wet hairs)	No visible wet hairs in the nasal fold and no hairs visibly in contact with the eye lid margin, conjunctiva or eyeball (N.B. the nasal fold hair may not be trimmed)	Nasal fold hairs are in contact with the eyelid margin(s), conjunctiva or eyeball, but there are no visible signs of ocular inflammation adjacent to those hairs	To be factored in
			Nasal fold hairs are in contact with the eyelid margin(s), conjunctiva or eyeball and there are signs of ocular inflammation at the site of those hairs, such as detritus on the nasal fold hairs, oedema, pigmentation or defects of the cornea or corneal neovascularisation.	Exceeds the limits of the standard, irrespective of other criteria
3	Distichiasis (hair growth from the free margin of the eyelid)	No hair growth from the free margin of the eyelid	Hair growth from the free margin of the eyelid with no clinical signs of corneal irritation	To be factored in
			Hair growth from the free margin of the eyelid, with one or more of the following symptoms: stiff hairs; mucus plug surrounding the hairs; oedema and/or pigmentation and/or a defect of the adjacent cornea; corneal neovascularisation	Exceeds the limits of the standard, irrespective of other criteria
4	Ectopic cilia (hairs that grow through the conjunctiva from the eyelid next to the free margin of the eyelid)	No ectopic cilia	One or more ectopic cilia	Exceeds the limits of the standard, irrespective of other criteria
5	Pigmentation of the cornea	No corneal pigmentation	Pigmentation extends to or past the centre of the cornea	Exceeds the limits of the standard, irrespective of other criteria
6	Entropion of the medial lower eyelid (in the region of the nasal fold, often difficult to see)	No entropion of the medial lower eyelid	Entropion of the medial lower eyelid, but no signs of corneal irritation visible adjacent to the inverted part of the lower eyelid	To be factored in

			Entropion of the medial lower eyelid, including signs of irritation of the adjacent cornea, such as detritus on the hairs of the eyelid, oedema, pigmentation or defects of the cornea, or corneal neovascularisation	Exceeds the limits of the standard, irrespective of other criteria
7	Scars from surgical correction of medial entropion, entropion of the lower eyelid, nasal fold trichiasis, trichiasis of the upper eyelid, distichiasis or ectopic cilia	No scars	Scar or scars indisputably present	Exceeds the limits of the standard, irrespective of other criteria
8	Corneal ulcer (retention of fluorescein stain indicates the presence of a defect)	No corneal ulcer	Superficial corneal ulcer (epithelial)	To be factored in
			Deep corneal ulcer (stromal)	Exceeds the limits of the standard, irrespective of other criteria
9	Residual symptoms of luxated eyeball	No residual symptoms	Residual symptoms present (strabismus divergens and/or blindness owing to damage to the optic nerve)	Exceeds the limits of the standard, irrespective of other criteria

A.2. By a veterinary surgeon specialised in ophthalmology

	Diagnosis	Aim	Enforcement standard	To be factored in or conclusive?
1	Caruncle trichiasis	Only soft, short, hairs oriented towards the centre grow from the caruncle	Long hairs are growing from the caruncle and are in contact with the cornea and/or conjunctiva, but there are no signs of corneal irritation.	To be factored in
			Long hairs are growing from the caruncle and are in contact with the cornea and/or conjunctiva and there are signs of corneal irritation at the site of those hairs, such as tear overflow, detritus on the hairs, oedema, pigmentation or defects of the cornea or corneal neovascularisation	Exceeds the limits of the standard, irrespective of other criteria
2	Scars from a previous deep corneal ulcer	Not present	Present	To be factored in (as being of significant importance)
3	Corneal sensation (esthesiometry) ^{xxiv}	Normal	Reduced	To be factored in
4	Indications pointing to qualitative tear film deficiency (Bengal pink staining)	Bengal pink staining negative	Bengal pink staining positive	Meets the standard, provided none of the limits of the other criteria is exceeded

» The caruncula lacrimalis is the small, pink, globular nodule at the inner corner (the medial canthus) of the eye. It is made of skin covering sebaceous and sweat glands.

B. Brachycephalic Obstructive Airway Syndrome (BOAS): additional research

B.1. by a primary veterinary surgeon and/or a veterinary surgeon specialising in internal medicine, surgery or ENT

	Criterion	Efforts made in respect of the risk of BOAS	Enforcement standard	To be factored in or conclusive?
1	Relative neck girth	Equal to or < 0.7	0.7 – 0.75 > 0.75	To be factored in, meets the standard, provided none of the limits of the other criteria is exceeded
2	Exercise tolerance test, distance covered in 6 minutes when walking quickly	More than 500 metres in 6 minutes and heart rate and temperature recovery within 5 minutes	More than 500 metres in 6 minutes and heart rate and temperature recovery within 10 minutes	Meets the standard, provided none of the limits of the other criteria is exceeded
			Fewer than 500 metres in 6 minutes and heart rate recovery and/or no recovery within 10 minutes	Exceeds the limits of the standard, irrespective of other criteria

B.1. Exercise tolerance test based on a 6-minute walk and a 1000-meter walk

Walk test standard	6-minute walk	1000-meter walk
Minimum distance	> 500 (522.7 ± 52.4 m)	
Minimum duration	-	12 minutes
Heart beat rest value	Max. 120 beats/min (KRESS)	
Body temperature rest value	Max. 39 degrees	
Heart rate and body temperature recovery time	return to rest values within 15 minutes	
Stridor	No stridor at rest or when walking stridor (g-geluid) when walking? The dog makes loud sniffing, snoring or sawing sounds when at rest (not asleep) or nasal, pharyngeal and/or laryngeal stridor can be heard when at rest	
Nostrils	Mildly stenotic nostrils: the nostril wings move dorsolaterally to open on inspiration immediately after the start of the walk test. Moderately stenotic nostrils: in some cases the nostril wings do not move dorsolaterally immediately after the walk test, causing muscle contraction around the nose (nasal flaring) in an attempt to enlarge the nostrils. Severely stenotic nostrils: the dog may switch to oral breathing from nasal breathing during the test	

Article 1.3. Intrinsic value

1 The intrinsic value of the animal is recognised.

2. Recognition of intrinsic value as referred to in Subsection 1 is understood to mean recognition of the value that animals possess in their own right as sentient beings. In drawing up rules under or pursuant to this Act, and in taking decisions on the basis of these rules, due consideration shall be given to the impact of these rules or decisions on the intrinsic value of the animal, notwithstanding other legitimate interests. In all cases, any violation of the integrity or well-being of animals, beyond what is reasonably necessary, shall be avoided and the care reasonably required by the animals guaranteed.

3 For the purpose of Subsection 2, the care reasonably required by animals shall in any event include safeguarding the animals against:

- a. thirst, hunger and malnutrition;
- b. physical and physiological discomfort;
- c. pain, injury and diseases;
- d. fear, distress, and chronic stress;
- e. limitation of their natural behaviour; insofar as can be reasonably required.

ii The Genetics of Canine Skull Shape Variation. Jeffrey J. Schoenebeck and Elaine A. Ostrander

GENETICS 2013, 193, 2; 317-325

iii Canine Brachycephaly Is Associated with a Retrotransposon-Mediated Missplicing of SMOC2.

Marchant et al. Current Biology, 2017, 27; 1573-1584

iv Slatter's Fundamentals of Veterinary Ophthalmology. Maggs D, Miller P, Ofri R, Slatter D, 2008, 4th Ed. Elsevier Health Sciences: Edinburgh, UK

v A Retrospective Study of Ulcerative Keratitis in 32 Dogs. Kim JY, Won HJ, Jeong SW. International Journal of Applied Research in Veterinary Medicine, 2009, 7, 27-31

vi Corneal innervation in mesocephalic and brachycephalic dogs and cats: assessment using *in vivo* confocal microscopy. Christiane Kafarnik C et al. Veterinary Ophthalmology, 11, 2008

vii Impact of Facial Conformation on Canine Health: Corneal Ulceration. Packer RMA, Hendricks A, Burn CC (2015) PLoS ONE 10(5): e0123827. doi:10.1371/journal.pone.0123827

viii Prolapsus bulbi in small animals. A retrospective study of 36 cases. Fritsche, J., Spiess, B. M., Ruhli, M. B., and Bollinger, J., 1996 Tierarztl Prax. 24: 55-61

ix Conformational risk factors of brachycephalic obstructive airway syndrome (BOAS) in pugs, French bulldogs, and bulldogs, 2017, PLoS ONE 12(8):e0181928, DOI: 10.1371/journal.pone.0181928

x Surgical correction of brachycephalic syndrome in dogs: 62 cases (1991- 2004). Riecks TW, Birchard SJ and Stephens JA. Journal of the American Veterinary Medical Association, 2007, 230: 1324-1328 – Bronchial abnormalities found in a consecutive series of 40 brachycephalic dogs. De Lorenzi D, Bertonecello D and Drigo M. Journal of the American Veterinary Medical Association, 2009 235: 835-840 – Prevalence of gastrointestinal tract lesions in 73 brachycephalic dogs with upper respiratory

syndrome. Poncet CM, Dupre GP, Freiche VG, Estrada MM, Poubanne YA and Bouvy BM . Journal of Small Animal Practice, 2005,46: 273-279 32

- Results of surgical correction of abnormalities associated with brachycephalic airway obstruction syndrome in dogs in Australia. Torrez CV and Hunt GB. Journal of Small Animal Practice, 2006 .47: 150-154

- Nasopharyngeal turbinates in brachycephalic dogs and cats. Ginn JA, Kumar MSA, McKiernan BC and Powers BE. Journal of the American Animal Hospital Association, 2008, 44: 243-249 – The influence of phylogenic origin on the occurrence of brachycephalic airway obstruction syndrome in a large retrospective study. Njikam IN, Huault M, Pirson V and Detilleux J. The International Journal of Applied Research in Veterinary Medicine, 2009, 7: 138-143

xi Impact of Facial Conformation on Canine Health: Brachycephalic Obstructive Airway Syndrome. Packer RMA et al. I PLoS One. 2015; 10(10): e0137496. doi: 10.1371/journal.pone.0137496

xii Craniofacial angle in dolicho-, meso- and brachycephalic dogs: radiological determination and application. Annals of Anatomy- Anatomischer Anzeiger 175, 4, 1993;361-363

xiii Miller's Anatomy of the Dog: Saunders Evans HE, 1993

xiv Skull morphometric lion, dog and cat, Saber and Gummow. J. Vet. Anat. 2015

xv Morphometrics within dog breeds are highly reproducible and dispute Rensch's rule. Sutter NB et al. Mammalian Genome 19;713-723, 2008

xvi Building better brachycephalics, Packer et al. (2012) Animal Welfare, 21, 81-93
<https://www.ufaw.org.uk/downloads/welfare-downloads/building-better-brachycephalics-2013-report.pdf>

xvii Evaluation of the 6-minute walk test in dogs. Boddy KN et al. American Journal of Veterinary Research, 2004, Vol. 65, No. 3; 311-313, <https://doi.org/10.2460/ajvr.2004.65.311>

xviii Evaluation of the 6-minute walk test in pet dogs, Swimmer RA and. Rozanski EA. Journal of Veterinary Internal Medicine, 2011

xix Long-Term Outcome and Use of 6-Minute Walk Test in West Highland White Terriers with Idiopathic Pulmonary Fibrosis. Lilja Maula LIO et al. Journal of Veterinary Internal Medicine, 2014

xx Comparison of submaximal exercise test results and severity of brachycephalic obstructive airway syndrome in English bulldogs. Lilja-Maula LIO et al. The Veterinary Journal, 219, 2017: 22-26

xxi Morphometrics within dog breeds are highly reproducible and dispute Rensch's rule. Sutter NB et al, Mammalian Genome 19;713-723, 2008

xxii Dog obesity--the need for identifying predisposing genetic markers. Switonski M1, Mankowska M. Res Vet Sci. 2013 Dec;95(3):831-6. doi: 10.1016/j.rvsc.2013.08.015.

xxiii A deletion in the pro-opiomelanocortin (*POMC*) gene in Labrador retrievers is associated with increased appetite and risk of obesity. Davison L.J. et al. 2017 Mar-Apr; 31(2): 343–348

xxiv Absolute corneal sensitivity and corneal trigeminal nerve anatomy in normal dogs. Barrett PM, Scagliotti RH, Merideth RE. Progress in Veterinary & Comparative Ophthalmology, 1991; 1:245–254