Prevalence and breeding values of elbow dysplasia in the Estrela mountain dog

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ABSTRACT: The aims of this study were to determine the prevalence and heritability of elbow dysplasia in the Estrela mountain dog breed, to investigate genetic trends over the last 20 years (1990–2009) and to evaluate the association of individual records with breeding values. The elbows of 351 Estrela mountain dogs were examined using the flexed mediolateral radiographic view and evaluated using the International Elbow Working Group scoring system. Heritability and breeding values were estimated using a linear model. Elbow Dysplasia was found in 16.5% (59/351) of the dogs; males (27%, 34/127) were more affected than females (11%, 24/224) ($P < 0.05$). The heritability was very low (0.065) and the genetic trend showed a slight positive slope with an improvement in 2004 and 2005. The mean breeding values in elbow dysplasia grades were different but the overlap among grades was very pronounced. The prevalence and heritability of elbow dysplasia in the breed are thus low. Mass selection using individual phenotypes may not be effective. Elbow dysplasia genetic trends are similar to trends for hip dysplasia and passive hip laxity, so the use of selection against hip dysplasia may also result in genetic progress for elbow dysplasia.

Keywords: osteoarthritis; heritability; genetic trend; radiology; screening program

List of abbreviations

BV = breeding value, **ED** = elbow dysplasia, **EMD** = estrela mountain dog, **FCP** = fragmented medial coronoid process, h^2 = heritability, **IEWG** = International Elbow Working Group, **OCD** = osteochondritis dissecans, **SD** = standard deviation, **SE** = standard error, **UAP** = ununited anconeal process

Elbow dysplasia (ED) is a developmental genetic orthopaedic disorder. The term is used to describe several diseases of the joint, including elbow incongruity, ununited anconeal process (UAP), fragmented medial coronoid process (FCP) and osteochondritis dissecans (OCD) (Samoy et al. 2006). ED has been recognised as a major problem in medium and large-breed dogs for about 50 years (Hodgman 1963; Lewis et al. 2011). This developmental abnormality is considered to be polygenically inherited (Janutta and Distl 2008). However, the influence of one major gene has recently been proposed (Maki et al. 2004).

Elbow osteoarthritis is the most common debilitating condition in dogs affected with ED, with severe consequences on animal well-being. Moreover, an ideal treatment is not yet available (Hazewinkel 2007; Innes 2009; Lewis et al. 2011). Nevertheless, the number of animals with obvious clinical signs is low, even in animals with radiological signs of moderate to severe ED (Read et al. 1996). Active genetic control based on diagnostic tests for con-

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dition and selective breeding are the best tools to prevent their occurrence (Woolliams et al. 2011). Radiographic examination of the elbow joints is currently the only widely used method for screening dogs for ED (Beuing et al. 2000; Lewis et al. 2011; Lavrijsen et al. 2012). However, radiography has some limitations in the diagnosis of some primary ED lesions. To confirm a clinical diagnosis, other diagnostic techniques such as computed tomography, magnetic resonance imaging or arthroscopy may be recommended (Snaps et al. 1997; Griffon 2006; Tromblee et al. 2007).

The pattern of inheritance of ED is unclear as there is an important environmental influence, and ED is considered as a complex multifactorial disease (Maki et al. 2002; Hazewinkel 2007). Heritability is a biological parameter that is derived from the relationship between a dog's elbow phenotype and its genotype and it is helpful in quantifying the efficacy of a diagnostic test in terms of genetic change per generation (Silvestre et al. 2007).

Breeding value (BV) is a genetic parameter derived from the elbow quality of a dog's relatives and offspring as well as the weight of environmental factors. The estimated BV gives a more precise measure of a dog's genetic quality than individual records (Silvestre et al. 2007).

The Estrela mountain dog (EMD) is the most popular native Portuguese breed and about 500 animals have been registered annually in the past years. A previous radiographic elbow-screening program designed to study and to control the prevalence of ED in the EMD breed has never existed, so the prevalence of subclinical ED could be high.

The purposes of the study reported here were to determine the prevalence and heritability of ED in the EMD breed, to investigate genetic trends over the last 20 years and to evaluate the association of individual records with BV.

MATERIAL AND METHODS

Dogs and criteria for inclusion. Three hundred and fifty one EMDs (224 females, 127 males) were radiographically screened for ED at the Veterinary Teaching Hospital of University of Trás-os-Montes and Alto Douro between May 2002 and May 2011. All dogs were privately owned. The study was performed in collaboration with Portuguese EMD associations (Liga dos Criadores e Amigos do Cao da Serra da Estrela [LICRASE] and Associacao Portuguesa do Cao da Serra da Estrela [APCSE]), was advertised in EMD association journals, and all Portuguese EMD breeders and owners were invited to participate. The owners were asked about their animals' history of forelimb clinical lameness, their participation in dog shows and any titles that they had won. Show results from some animals were also complemented later with information obtained from the Portuguese Kennel Club. The dogs mainly came for hip dysplasia screening and none showed obvious signs of forelimb lameness.

The criteria for inclusion were: (1) that the EMD dogs were registered in the Portuguese Kennel Club; (2) that they were a minimum of 12 months old; (3) that they had normal health status and musculoskeletal development.

Radiographic technique and elbow scoring method. The dogs were sedated with intravenous administration of medetomidine 0.02 mg/kg (Domitor, Orion Pharma, Espoo, Finland), butorphanol 0.1 mg/kg (Torbugesic, Fort Dodge, Girona, Spain) and atropine sulfate 0.02 mg/kg (Atropina Injectavel, B. Braun, Barcarena, Portugal). The dogs were positioned on the radiographic table in right and left lateral recumbency with the lowermost limb being the one under examination. The elbows were placed directly on the cassette (no grid was used) in flexed position (about a 45° opening angle), the beam was collimated and centred on the medial humeral condyle, and the elbow mediolateral view was made. Radiographs were permanently marked to include the date of the examination, the identity of the dog and the identity of the owner.

Elbows were evaluated and classified according to the International Elbow Working Group (IEWG) criteria, into five grades as described by Fluckiger (2007): normal elbow joint, borderline, mild ED, moderate ED and severe ED. The presence of an obvious primary lesion such as UAP, FCP, OCD was evaluated as severe ED, without taking into consideration the severity of osteoarthritis. All the evaluations were made by three observers (SA-P, BC and MMG), and if no consensus between these observers was found, the final grade was the one attributed by two of the three.

Estimation of genetic parameters. The pedigree information used in the analysis was obtained from the Portuguese Kennel Club. The database included all known ancestors from the last four generations, the identification number of the dog, its parents, gender code and birth date.

The following linear model was assumed when estimating the variance components as well as the fixed and random effects for ED:

$$y_{ij} = \mu + \text{sex}_i + b_1 w_j + b_2 \text{age}_j + a_j + \varepsilon_{ijk}$$

where:
yij = ED score of animal j of sex i,
μ = overall mean
sex_i = fixed effect of the i^{th} sex class (male and female)
b_1 = fixed regression coefficient
w_j = weight of animal j
b_2 = fixed regression coefficient
age_j = age of animal j
a_j = random additive genetic effect of the j^{th} animal
e_{ij} = random residual effect. In matrix notation the model can be written as

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e}$$

where:
y = a vector of ED scores
b = a vector of fixed effects
a = a vector of animal effects and **e** is a residual vector
X, Z = incidence matrices relating the effects to the scores

The distributions of **a** and **ε** were assumed to be normal with zero mean and with Variance(**a**) = $\mathbf{A}\sigma_a^2$ and Variance(**ε**) = $\mathbf{I}\sigma_\varepsilon^2$. Covariance between **a** and **ε** was assumed to be zero. The additive genetic value (a_j) represents the average additive effects of genes an individual receives from both parents and is usually known as breeding value. Each parent contributes a sample half of its genes to its progeny. The breeding value of the progeny therefore is the sum of the transmitting abilities of both parents. Since the additive genetic value is a function of the genes transmitted from parents to progeny, it is the only component that can be selected and therefore the main component of interest (Silvestre et al. 2007). The variance components in the model were estimated using the restricted maximum likelihood method and ASREML software (Gilmour et al. 2000).

In the model, the IEWG outcome for ED was recoded in four numerical categories and was considered to be the mean value of both joints: normal = 1; borderline = 2; mild ED = 3; moderate ED = 4; severe ED = 5 (Swenson et al. 1997). The genetic trend was evaluated for the last 20 years (1990–2009 inclusive).

Statistical analysis. In the prevalence study the dogs were classified using the ED grade of their worst-affected elbow joint (Swenson et al. 1997). ED grades of normal and borderline were classified as unaffected and the remainder as dysplastic. The Chi-squared test was used to compare the ED grades between males and females, and between the right and left sides (Petrie and Watson, 1999). The one-sample Kolmogorov-Smirnov test and histograms were used to evaluate normal distribution of overall BVs and BVs of screened animals. ANOVA followed by the Bonferroni test was used to compare the mean BVs of animal ED groups. *P*-values < 0.05 were considered to be significant. Statistical calculations were performed using standard computer software (SPSS Version 12.0).

RESULTS

Sample and disease prevalence

The dogs' ages ranged from 12–168 months (mean ± SD, 29.1 ± 20.1 months) and their bodyweights ranged from 26–65 kg (mean ± SD, 40.7 ± 7.0 kg). Two hundred and seventy four (78.1%) dogs were graded as Normal, 19 (5.4%) as borderline, 33 (9.4%) as mild ED, 17 (4.8%) as moderate ED and 8 (2.3%) as severe ED, resulting in an ED prevalence of 16.5% (Table 1). Thirty five of the dysplastic dogs 35/58 (60.3%) had bilateral disease. ED prevalence differed between male and females, showing a prevalence of 27% and 11%, respectively

Table 1. Distribution of elbow quality in 351 Estrela mountain dogs using the International Elbow Working Group scoring system and the worse elbow score as reference

Sex	IEWG score					Total
	normal	borderline	mild ED	moderate ED	severe ED	
Female	191	9	18	4	2	224
Male	83	10	15	13	6	127
Total	274	19	33	17	8	351

IEWG = International Elbow Working Group; ED = elbow dysplasia

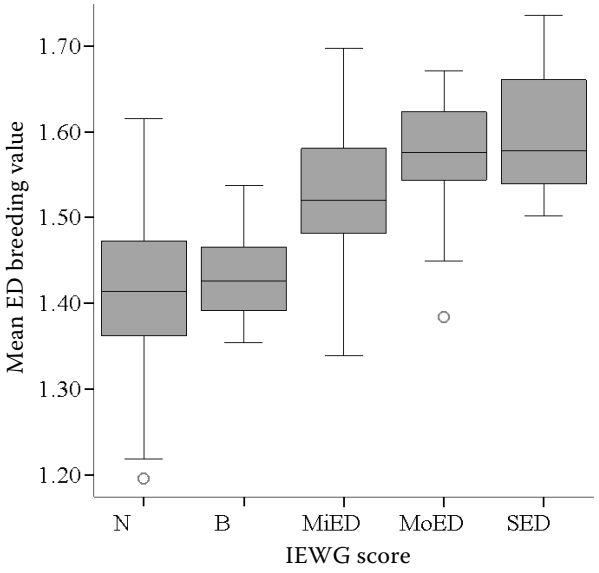

Figure 1. Box-and-whisker plot of the mean breeding values in International Elbow Working Group (IEWG) elbow dysplasia (ED) grades: N = normal; B = borderline; MiED = mild ED; MoED = moderate ED; SED = severe ED

($P < 0.05$). The left and right joint showed a similar distribution ($P > 0.05$).

Forelimb lameness information was only obtained in 304 animals: only one owner referred to a history of clinical lameness at a young age that wasn't diagnosed and when adult the dog become a show champion even with radiographic signs of severe ED; seven of 53 (13%) show champions had moderate or severe ED.

Genetic parameters

The pedigree information of 775 dogs with a close family relationship was used in the estimation of heritability (h^2) and BVs. The h^2 was low (mean ± SE, 0.065 ± 0.067) (Table 2). The BVs showed a normal distribution for the screened animals ($P > 0.05$), and a non-normal distribution in the overall BVs. The BVs estimates ranged from 1.20 to 2.74 (mean ± SE, 1.415 ± 0.003). Mean BVs were significantly different in some ED grades, but the overlap was evident among BVs of all grades (Table 3 and Figure 1). Over the last 20 years, the mean BVs per year of birth showed a slight worsening (positive slope in tendency line), with a nadir in 2003 and improvement in 2004 and 2005 (Figure 2).

Table 2. Estimates of additive genetic (σ_a^2), phenotypic (σ_p^2) and heritability (h^2) with standard errors (SE)

σ_a^2 ± SE	σ_p^2 ± SE	h^2 ± SE
0.0462 ± 0.048	0.71 ± 0.054	0.065 ± 0.067

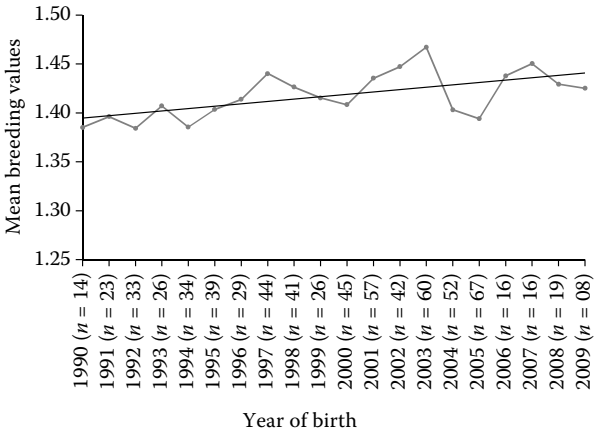

Figure 2. Mean breeding values of 691 Estrela mountain dogs by year of birth, born between 1990 to 2009 and tendency line; n = number of animals in each year

DISCUSSION

The low prevalence and severity of ED in the EMD breed found in the present study was not observed in other studies performed in large breed dogs (Swenson et al. 1997; Coopman et al. 2008). This fact was a positive surprise, as this ED screening program was the first to be ever used in the routine examination of the EMD breed. In initial ED screening programs, some breeds reached a prevalence of close to 60% (Swenson et al. 1997). As the majority of the participants were breeders, they may have rejected animals with clinical elbow problems at a young age. Older animals in the sample were a minority, only seven (2%) were older than 74 months and only one was affected with ED. Therefore, the influence of age-related osteoarthritis does not seem to be significant. Other environmental factors with influence on osteoarthritis, such as feeding or exercise levels, were not investigated. The higher prevalence of ED in males compared to females is in accordance with the majority of other studies (Swenson et al. 1997; Samoy et al. 2006). However, the fact that males are represented in small number (36%) in our sample could also have influenced the low prevalence. Some authors discuss the influence of genes on the sex chromosomes, which could affect, either by a direct or secondary sexual characteristic, or

Table 3. Mean breading values in elbow dysplasia grades

ED score	*n*	Mean ± SE	95 per cent CI for mean		Minimum	Maximum
			lower bound	upper bound		
Normal	274	1.411 ± 0.005[a]	1.402	1.421	1.196	1.615
Borderline	19	1.432 ± 0.011[a]	1.409	1.455	1.354	1.537
Mild ED	33	1.526 ± 0.015[b]	1.500	1.557	1.339	1.697
Moderate ED	17	1.569 ± 0.020[b]	1.527	1.610	1.384	1.670
Severe ED	8	1.599 ± 0.030[b]	1.529	1.670	1.502	1.736

Mean values with different superscripts in the same column are significantly different ($P < 0.05$)

ED = elbow dysplasia; *n* = number of cases; SE = standard error; CI = confidence interval

by different levels of genes, the penetration of the disease (Swenson et al. 1997). The low incidence of lameness associated with ED is in agreement with previous studies (Read et al. 1996). The fact that we saw dog show champions with severe ED was not expected and reinforces the subclinical character of the disease. The high level of inbreeding associated with show champions in the EMD breed could prove dangerous, as the intensive use of popular sires drifts the gene pool of a breed and introduces a disproportionately higher number of defective genes. The new IEWG protocol recommends more radiographic projections than the flexed mediolateral view, as this single view underestimates ED. In 12.5% of dogs ED is not associated with osteoarthritic changes (Lang et al. 1998; Fluckiger, 2007); therefore, our prevalence may be underestimated. However in several countries only one flexed lateral projection is used (Swenson et al. 1997).

The estimated heritability for the ED score in the present study, according to IEWG guidelines, was lower than all estimates reported for other breeds (Beuing et al. 2000; Janutta and Distl 2008). These heritability differences may be due to breed differences or to the method used for estimation (Janutta and Distl 2008). The small sample size in this study contributed to the high standard error (0.067). Mass selection against ED has been successfully used in prevalence reduction in some breeds (Swenson et al. 1997; Woolliams et al. 2011). However, the very low heritability for ED in the EMD breed based on the IEWG, and the overlap between BVs in ED grades are worrying, and it will not be easy to adopt an adequate response using phenotypic selection. Therefore, BV estimation can be used as an alternative procedure (Tellhelm 2007). The slight worsening of the ED genetic trend per year of birth (slight positive slope of tendency line) seems similar to the genetic trends in the same breed for passive hip laxity and hip dysplasia mentioned in previous studies (Ginja et al. 2008, 2009). These genetic trends are particularly similar in the years between 2003 and 2005 (years with a greater number of animals). In all three genetic trends the year of 2003 had the worst results and was followed by a sudden improvement in 2004 and 2005. Unfortunately, there is no data about genetic trend for HD and hip laxity in the breed after 2005, which would confirm the slight worsening of ED in this last period. The slight worsening until 2003 may be related to the absence of clinical signs in affected animals, the lack of knowledge of the disease, the absence of radiographic screening, the higher prevalence of moderate and severe ED in show champions and the absence of any kind of selection against the disease. The improvement in 2004 and 2005 could be due to the beginning of our collaboration with EMD breeder associations and the introduction of a voluntary ED screening programme and the worsening after 2006 to the reduced number of animals screened. When there exists a large genetic correlation between two traits, associated with a high and a low heritability for each trait, a potential increase in genetic progress is expected to occur in the trait with low heritability, when selecting for the other (Cachon et al. 2010; Lewis et al. 2011). Although showing differences between breeds, previous studies are suggestive of a positive genetic correlation between ED and hip dysplasia (Cachon et al. 2010; Lewis et al. 2011; Woolliams et al. 2011). Therefore, this positive genetic correlation can contribute towards explaining the improvement in BVs for ED in 2004 and 2005 as an indirect response to selection against HD, a disease with moderate heritability in the breed (Silvestre et al. 2007). For ED control within the EMD population, we recommend the

use of individual phenotypic records and the use for breeding of only those animals without ED or affected with mild ED. This selection strategy is not ideal and breeding dogs with radiographic assessments of mild ED may only be justified when its prevalence in the breed population is high (Read et al. 1996). The fact that animals with mild ED have mean BVs more similar to those of severe dysplastic animals than to normal animals also does not favour our selection strategy. However, considering ED in EMD as a particular situation that does not always lead to clinical signs, added to the very low heritability on the one hand and to the very high prevalence of hip dysplasia associated with some severe clinical problems on the other, and with the owner's priority being the control of hip dysplasia, the recommended strategy for ED control would be adequate. Based on these results we believe that the priority should be to get breeder collaboration in order to perform an extensive radiographic screening for elbow and hip dysplasia, direct the selection against hip dysplasia and monitor the genetic trends of ED and hip dysplasia in the breed. In the future, when the database of screened animals will become more complete, breeding selection using the BV would be recommendable.

The prevalence and severity of elbow dysplasia in the Estrela mountain dog is low. However, its prevalence would potentially be higher if a larger sample (preferably random) were used or more radiographic projections were taken. Most often, ED is associated with no or minimal clinical signs and owners do not feel the need for a control programme. With regard to the low heritability of ED in the EMD population and the possible genetic correlation between elbow and hip dysplasia, an improvement in elbow dysplasia in the breed could be achieved using selection against hip dysplasia. More studies should be carried out to clarify this correlation. In the future, breeding selection for ED using the BV estimation would be recommendable.

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