Nontraumatic elbow joint luxation has been described as a congenital or hereditary disease in different breeds, including Miniature Poodle, Pekingese, Pug, and Pomeranian. According to Kene et al., three forms of nontraumatic elbow joint luxation can be distinguished in dogs: type I is characterized by a caudolateral dislocation of the radial head with little or no displacement of the ulna; type II is characterized by a marked rotational dislocation of the ulna and a lesser degree of displacement of the radial head; and type III is characterized by dislocation of the radial head and the ulna. Although no longitudinal studies have been published, the disorder is thought to develop postnatally. Subluxation of the radial head is suggested to be related to abnormal growth of the distal physis of the ulna. Although movement of the elbow joint is not painful, the range of motion of the joint may become limited. Several surgical interventions have been described, such as corrective osteotomies, transposition of the radial head and temporary transarticular fixation, and radial head resection, but severe degenerative joint disease caused by the malformation of the elbow joint makes the prognosis guarded.

The purpose of the study reported here was to study radiographic and genetic aspects of hereditary radial head subluxation in Bouviers des Flandres.

**Materials and Methods**

- **Dogs**—Two 10-month-old Bouviers des Flandres with clinical signs of bilateral forelimb lameness and bilateral deformity were evaluated at the Department of Clinical Sciences of Companion Animals (Temwichitr, Leegwater, Auriemma, Voorhout, Hazewinkel) and Biochemistry and Cell Biology (van’t Veld, Zijlstra), Faculty of Veterinary Medicine, Utrecht University, 3584 CM, Utrecht, The Netherlands. Dr. Temwichitr’s present address is Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand. Address correspondence to Dr. Hazewinkel (H.A.W.Hazewinkel@uu.nl).

**Objective**—To study radiographic and genetic aspects of hereditary radial head subluxation in Bouviers des Flandres.

**Animals**—26 related Bouviers des Flandres affected with bilateral subluxation of the radial head, 10 unaffected related dogs, and 29 unrelated Bouviers des Flandres with diagnoses of nonskeletal diseases.

**Procedures**—All dogs were radiographically studied, and their DNA was analyzed with a genome-wide screen of 1,536 single nucleotide polymorphisms. In addition, karyotyping was performed in an unaffected dam and its affected offspring.

**Results**—Both forelimbs of affected dogs were disproportionately short with caudolateral subluxation or luxation of the radial head. Angulation of the radial axis at the mid-diaphysis ranged from 9.3° to 30.3° (mean ± SD, 14.9 ± 6.1°), with an estimated age of onset from 0 to 4 months. Poorly defined medial coronoid processes and osteoarthritis of the elbow joint, cranial bowing of the olecranon, and disturbed growth in length of the ulna with sharply demarcated spurs were noticed on radiographs of affected dogs. Genealogical analysis indicated that most affected dogs were closely related, but the mode of inheritance was not clear. The DNA analysis found that 205 single nucleotide polymorphisms were monomorphic in the affected dogs. Conventional chromosome staining revealed no numerical chromosomal aberration.

**Conclusions and Clinical Relevance**—Congenital radial head luxation and subluxation in the studied Bouviers des Flandres were characterized by angulation of the radial axis leading to caudolateral subluxation of the radial head and insufficient growth of the distal portion of the ulna together with cranial bowing of the olecranon. (Am J Vet Res 2010;71:884–890)
of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University. In collaboration with the Dutch Bouvier des Flandres breeders club, a group of 43 dogs, consisting of 24 affected Bouviers des Flandres and 19 nonaffected relatives (34 dogs from The Netherlands and 9 dogs from Sweden), was also investigated. The dogs were 2 months to 11 years old, and all underwent complete orthopedic examination.8 The family relationship among affected dogs was investigated, and the associated pedigree comprised 31 affected dogs, of which 24 were clinically ascertained. All 7 affected dogs that were not examined by the authors had been euthanized because of their phenotype before the start of this study. The parents that were not examined have been champions in dog shows, either national or international! on the basis of their phenotype typical for Bouviers des Flandres. In addition, the DNA samples of 29 Bouviers des Flandres, unrelated to the aforementioned group and with a diagnosis of other nonskeletal diseases, were used as controls for molecular genetic investigation.

Radiographic study—Standard craniocaudal and mediolateral radiographs of the right antebrachium and a ventrodorsal view of the pelvis with the hind limbs extended were obtained from 26 (16 affected and 10 unaffected dogs) of the 43 Bouviers des Flandres. These radiographs were examined with regard to configuration, alignment, congruity, and subluxation of the elbow joint; evidence of sclerosis of the incisura trochlearis; deviation of the radial head; degree of curvature of the radius; relative length of the ulna; configuration of the distal ulnar and radial metaphyses; and presence of a core of cartilage in the distal radial and ulnar metaphyses.

The axis of the distal and the proximal parts of the radius was drawn on each craniocaudal view, according to Newton,9 and the angle between these axes was measured (in degrees) with a goniometer. Measurements were obtained in all 26 dogs, and mean ± SD values were determined. In addition, the distance between the point of angulation and the proximal metaphysis (a) and between the point of angulation and the distal metaphysis of the radius (b) was measured, and the values of a/b and a/(a+b), the latter expressed as percentage ± SD, were calculated. On the mediolateral view of the antebrachium, a straight line was drawn between the top of the olecranon process and the tip of ulnar styloid process; the configuration of the ulna and olecranon process was evaluated in terms of the proportion of this line lying outside the ulnar projection. Genotyping—Blood samples were collected from all affected dogs and the available unaffected relatives, and DNA was isolated according to Miller et al.9 The set of 1,536 SNPs was used; the SNPs were evenly spread along the dog genome, as described.10 The genotyping of the SNP set in the DNA from 24 affected and 48 unaffected dogs was performed with microarray analysis.9 Alleles were clustered and assigned by use of a dedicated program.9 Genotype frequencies of related and unrelated dogs, and also of affected and unaffected dogs, were compared.

Cytogenetic study—For chromosome analyses or karyotyping, blood was obtained from an unaffected bitch and 1 of its affected female offspring of a litter with 2 probands (Figure 2). Metaphase chromosomes were obtained from blood lymphocytes stimulated with 25 µg of concanavalin A/mL following standard procedures. Briefly, cells were cultured for 72 hours and treated with colchicine11 (0.4 µg/mL) for 60 minutes at the end of culture. Cells were swollen by immersion in 0.075M KCl (15 minutes at 37°C) and fixed in a mixture of methanol and glacial acetic acid (3:1). Chromosome preparations were made, and slides were conventionally stained with Giemsa stain.9 In well-spread metaphases, the number of chromosomes and the presence of the X chromosome were determined by use of a microscope equipped with imaging software.9

Statistical analysis—Differences in the angle between the axes and the value a/(a+b) in affected and unaffected dogs were evaluated with a Student t test.9 A value of P < 0.05 was considered significant.

Results

Physical features—None of the breeders noted abnormal alignment of the forelimbs when the pups were born. In affected dogs, both forelimbs had valgus deviation of the feet at the age of investigation (Figure 1). The elbow joints of affected dogs were wider at the level of the radial head than those of unaffected dogs. In most affected dogs, the configuration of the elbow joint was abnormal with a decreased range of motion; the antebrachium was abnormal, and the forelimbs were short, relative to the hind limbs. Affected dogs had lameness, difficulty of movement, and limited function of the elbow joint. With the exception of these abnormalities, the dogs had a normal appearance without deformity of the face or skull. No evidence of ocular disease or other abnormalities was found in any of the affected dogs.

Radiographic study—All 16 affected dogs had malalignment, incongruity, or abnormal configuration of the elbow joint (Figures 2 and 3), whereas these findings were absent in all 10 unaffected dogs. Moderate to advanced elbow joint osteoarthritis with periartricular new bone formation at the medial aspect of the humeral condyle, anconeal process, and radial head was seen in 12 affected dogs. Moderate to severe sclerosis of the incisura trochlearis of the ulna was detected in 14 affected dogs and in 1 unaffected dog, associated with a blunt cranial margin of the medial coronoid process and a poorly defined contour, respectively. Six affected dogs younger than 9 months of age had a retained cartilage core in the distal ulnar metaphysis, but this was not present in mature affected dogs or unaffected dogs. In 14 affected dogs and none of the unaffected dogs, a shortened ulna was noticeable. A sharply demarcated spur-like formation of new bone arising from the mid-caudal portion of the ulna and extending in a caudo-proximal direction was seen in 5 affected dogs, but in none of the unaffected dogs.

The angulation of the radius at mid-diaphysis was greater, and the radial head had a marked lateral deviation in affected dogs, compared with unaffected dogs (Figure 3). Mean ± SD degree of radius

AJVR, Vol 71, No. 8, August 2010 885

Unauthenticated | Downloaded 11/25/23 07:59 AM UTC
angulation was 14.9 ± 6.1°, with a range from 9.3° to 30.3° in affected dogs (n = 16) and from 0° to 6.6° (mean ± SD, 4 ± 1.8°) in unaffected dogs (10), and the difference between the 2 groups was significant. Mean ± SD a/b ratio was 1/0.99 ± 0.29 and 1/1.54 ± 0.48 in affected and unaffected dogs, respectively (P = 0.009). Mean ± SD percentage of the proximal part of the radius, compared with the overall length of the radius, a/(a+b), was 51.7% ± 9.7% (range, 42.2% to 85.9%) in affected dogs, whereas in clinically nor-

Figure 1—Photographs of 3 Bouviers des Flandres with hereditary radial head subluxation. Notice the abnormal forelimbs, including valgus deviation of the feet and disproportionately short forelimbs.

Figure 2—Radiographic appearance of hereditary radial head subluxation in Bouviers des Flandres. A—Craniocaudal and lateral radiographic views of the forelimb of an affected dog at 4 months of age with a flared ulna, retained cartilage in the ulnar metaphysis, and marked lateral deviation of the radial head. B—Craniocaudal and lateral radiographic views of the forelimbs of 2 affected adult dogs. Notice abnormal elbow joint configuration, incongruity, and various degrees of subluxation of the radial head (white arrowheads). The cranial margin of the coronoid process is abnormal, and there is moderate to severe sclerosis of the trochlear notch. Notice a spur-like lesion on the mediolateral view at the caudal aspect of the ulna (open arrow) in 1 dog.
mal dogs, a mean of 39.8% ± 6.5% (range, 31.4% to 46.3% \( P = 0.002 \)) was determined.

Cranial bending of the olecranon was marked in 8 affected dogs, as indicated by the line between the top of olecranon process to the distal end of the ulnar styloid process (Figure 3). In these dogs, at least 50% of the line was within the boundary of the projection of the ulna, whereas 100% of the line was outside the boundary in all clinically normal dogs. The pelvis and hip joints were normal in all affected dogs.
except for 1 dog with mild osteoarthritis of both hip joints.

**Cytogenetic and molecular genetic studies**—A pedigree diagram was constructed for 31 affected dogs (13 males and 18 females; Figure 4). Twenty-one affected dogs were born in 3 litters in which no clinically normal dogs were born. One dog sired 2 of these litters and was the grandsire of the third litter. This dog also sired another litter with an affected dog. Another dog sired 2 litters, 1 in which all dogs were affected, and the same was true for 1 of the dams. Segregation analysis was not possible because not all of the litters with probands were completely ascertained.

We hypothesized that loci with alleles contributing to the phenotype were fixed or nearly fixed in the breeding line evaluated. To explore the genetic divergence of the breeding line from the general population, the allele frequencies of a set of 1,536 SNPs, evenly located along the canine chromosomes, were compared in the related and unrelated dogs. Of the set, 1,319 SNPs passed the quality control test and were not monomorphic in all dogs. Two hundred five SNPs were homozygous in the affected dogs, and 163 of these were homozygous in the relatives of those dogs as well. Only 3 SNPs were homozygous in the group of unrelated control dogs. The mean level of heterozygosity was 29% in affected dogs and 35% in unrelated control dogs. The SNP allele frequencies in affected dogs, related controls, and unrelated controls are available on request.

Whether the deformity could be the result of a gross chromosomal rearrangement or instability was examined. Analyses of 10 Giemsa-stained metaphase preparations of cells from an unaffected bitch and from one of its affected pups revealed that both dogs had 76 telocentric autosomes and 2 submetacentric X chromosomes (78, XX). Besides the X chromosomes, no other biarmed chromosomes were observed.

**Discussion**

Reports on hereditary subluxation of the radial head in Bouviers des Flandres were not found in the literature. In the dogs reported in the present study, the disease was characterized by moderate to severe angulation of the radius, shortening of the ulna, laterocaudal displacement and subluxation of the radial head, and elbow joint osteoarthritis of varying severity.

Because of the angulation of the radius, neither the location of the pivot point nor the angulation of the axis measured on standard radiographs should be considered as precise measurements, but they were suitable for analysis. In the unaffected dogs, a pivot point with an angulation between the proximal and distal axes of < 6° was seen, located at 39.8% ± 6.5% of the total length of the radius. Because the proximal growth plate of the radius contributes 28% to 38% of the growth in length of the radius,\(^{11,13}\) it is likely that this pivot point was originally localized in the midpoint of the primary ossification center of the radius in these unaffected newborn dogs. Because this pivot point was located more distally in the affected dogs with a reduced total length of the radius, we concluded that the distal radial growth plate contributed less to the growth in length of the radius in affected dogs. The growth in length of the ulna, which is almost entirely dependent on the growth of the distal ulnar growth plate, was also diminished, as reflected by the observation that the ulnar styloid process did not reach the accessory carpal bone. Five affected dogs had evidence of a retained cartilage core of < 2 cm; this did not necessarily hamper growth\(^{15}\) because 11 dogs with radial head subluxation without a cartilage core also had diminished growth. A cartilage core of 1 cm or less is not considered to be clinically relevant.\(^{15}\) Thus, this abnormality is not a radius curvus syndrome caused by ulnar growth plate trauma,\(^{16}\) osteochondrosis,\(^{17}\) or excessive intake of calcium\(^{18}\) or vitamin D.\(^{19}\) Therefore, we concluded that this developmental abnormality originated in the mid-diaphysis of the radius, with decreased growth in the length of the distal portion of the radius. The angulation was located at the insertion site of the interosseous ligament, which firmly connects the radius and ulna.

The growth in length of the proximal part of the radius did not seem to be reduced in affected dogs. However, because of the angulation at the pivot point, the radial head passes the humerus and the humeral condyle rotates around the anconeal process. The radiographic signs of osteoarthritis can be explained by the abnormal architecture of the elbow joint or the occurrence of fragmentation of the coronoid process,\(^{19}\) although a common cause of chondrodysplasia and osteoarthritis cannot be ruled out.\(^{20}\)

Several authors have suggested that soft tissue is involved in this disorder.\(^{1,3}\) Underdevelopment of the collateral ligament could be considered a consequence of abnormal growth in the length of the radius and ulna. Subluxation of the radial head of the elbow joint and bending of the olecranon have not been reported in other cases of asynchronous growth of the radius and ulna, including radius curvus syndrome or short radius syndrome. Thus, we speculated that, apart from the angulation of the radius, abnormal forces from the supinatus muscle may also play a role in luxation of the radial head and that forces from the triceps brachii muscle may play a role in the cranial bending of the olecranon. These forces probably act in conjunction with the laxity of the lateral collateral ligament and annular ligament, which allows the radial head to move away from the ulna,\(^{1,3}\) whereas the midradius is strongly held by the interosseous ligament.

The spur-like lesion close to the point of insertion of the deep digital flexor muscle in the midcaudal portion of the ulna, seen in 5 affected dogs, has not been reported in other cases of hereditary elbow joint subluxation or in clinically normal Bouviers des Flandres. The cause of this lesion is not known.

The radial head subluxation arose at the distal growth plate of the radius and affected the function and configuration of the elbow joint. In the present study, all affected dogs also had malfunctioning elbow joints. This type of subluxation of the elbow joint is inciden-
Akitas. Our findings were similar to those described in congenital elbow luxation type I, where the radial head moves away from the humeral condyle. However, the clinical findings were also similar to those of chondrodyplasia described in isolated cases in Saint Bernards, Newfoundlands, Lapphunds, and German Shepherd Dogs. In these breeds, no hereditary aspects have been detected. The valgus deformity is the consequence of asynchronous growth of the radius and ulna, resulting in abnormal development of the carpal and elbow joints. This finding has also been described in dogs with traumatic radius curvus syndrome in which often only 1 limb is affected, therefore directly resulting in loss of normal joint configuration. Therefore, we concluded that valgus deformation in these Bouviers des Flandres was not per se a consequence of hereditary luxation of the radial head.

All affected dogs were closely related over 5 generations (Figure 4) and had at least 1 founder in common. The disease was seen both in male and female dogs (ratio, 1:1.4). Because the phenotype of radial head subluxation seems to have accumulated in this group of dogs with a high level of inbreeding and the parents did not have the phenotype, recessive inheritance would be the most likely mode of inheritance. However, in recessive disorders, it is uncommon that all siblings are affected, as was seen in 3 litters. Linkage between the inheritance of the SNPs and the subluxation of the radial head was analyzed in the pedigree under assumption of recessive inheritance with reduced penetrance. The result of analysis of chromosome 8, with the highest logarithm of odds score of 1.8, is available on request. With a supposed dominant inheritance pattern, the lack of the phenotype in the parents would indicate incomplete penetrance of the genotype at risk. The observation that all 21 puppies of 3 litters were affected makes reduced penetrance in combination with dominant inheritance unlikely.

The observations of the present study could suggest that environmental factors play a role in combination with a genetic effect. Although traumatic or nutritional factors cannot be ruled out at this stage, the fact that the affected dogs came from different breeders makes it unlikely that they all made similar errors in husbandry or preparation of the diet. Severe growth plate abnormalities such as valgus deviation or short radius syndrome can also occur as a result of trauma and oversupplementation, however, these diet-induced abnormalities never coincide with radius angulation and elbow joint subluxation, but instead occur with radius curvus syndrome.

A novel phenotype can arise if a new mutation occurs abruptly at some point in the breeding line, if there is chromosome rearrangement or replication, or if there is accumulation of the mutation in the breeding line. Chromosome rearrangement might cause a particular chromosome region to be duplicated, lost, or fused to a chromosome region that disrupts its regulation, thereby contributing to the phenotype. Although conventional chromosome analysis revealed that the 2 dogs investigated had a total number of chromosomes that reflects the normal female karyotype (78, XX), more detailed chromosome analysis is needed to exclude the presence of structural abnormalities. Because of the relatively low number of metaphases and the poor quality of the metaphase spreads, GTG banding was not carried out in the present study. More detailed chromosome analysis, for instance by use of comparative genome hybridization, is needed to investigate the involvement of structural abnormalities in the phenotype.

A complex inheritance pattern with involvement of several genes and accumulation of deleterious alleles by inbreeding seems to be possible. Alternatively, genomic instability in the form of gradual expansion of a DNA repeat over generations in this breeding line could also explain the data. This repeat could disrupt the expression of an associated gene, and disease severity could be associated with the length of the repeat. Zlotogora postulated a similar model of repeat expansion with an autosomal dominant effect and reduced penetrance for split-hand-foot malformation with long bone deficiency in humans, even though the disorder was observed in a number of consanguineous families. Naveed et al determined that this disorder was inherited in dominant digenic mode with reduced penetrance in a large consanguineous family.

Single nucleotide polymorphism genotyping of the Bouviers des Flandres with radial head subluxation revealed a high level of homozygosity in the affected pedigree. Finemapping is required to limit the number of positional candidate regions and to evaluate the involvement of the suggestive linkage region on chromosome 8. High-throughput DNA sequencing of these regions may be required to confirm the presence of an expanding repeat and to understand the developmental process that underlies radial head luxation in Bouvier des Flandres.

References


