Case Report

Phenotypic diagnosis of dwarfism in six Friesian horses

W. BACK*, J. J. VAN DER LUGT[†], P. G. J. NIKKELS[‡], A. J. M. VAN DEN BELT[§], J. H. VAN DER KOLK and T. A. E. STOUT

Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 114, NL-3584 CM Utrecht; [†]Department of Pathobiology, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, NL-3584 CL Utrecht; [‡]Department of Pathology, Faculty of Medicine, University Medical Center Utrecht, PO Box 85500, NL-3508 GA Utrecht; and [§]Division of Diagnostic Imaging, Department of Companion Animal Sciences, Yalelaan 108, NL-3584 CM Utrecht, The Netherlands.

Summary

An extreme form of abnormal development, dwarfism, is common in man and some animals, but has not been officially reported in horses. Within the Friesian horse breed, congenital dwarfism has been recognised for many years, but no detailed report exists on its phenotype. The most salient feature of the dwarf syndrome is the physeal growth retardation in both limbs and ribs. Affected animals have approximately 25% shorter fore- and hindlimbs and approximately 50% reduced bodyweight. Post natal growth is still possible in these animals, albeit at a slower rate: the head and back grow faster than the limbs and ribs leading to the characteristic disproportional growth disturbance. Thus, mature dwarfs exhibit a normal, but a relatively larger head conformation, a broader chest with narrowing at the costochondral junction, a disproportionally long back, abnormally short limbs, hyperextension of the fetlocks and narrow long-toed hooves. Furthermore, a dysplastic metaphysis of the distal metacarpus and metatarsus is radiographically evident. Microscopic analysis of the growth plates at the costochondral junction shows an irregular transition from cartilage to bone, and thickening and disturbed formation of chondrocyte columns, which is similar to findings in osteochondrodysplasia.

Introduction

The ultimate phenotypic appearance of an individual horse is the result of the interaction between environmental factors and the underlying genetic constitution. Normal increase in height and size (i.e. growth and development) results primarily from the growth plates of the long bones and ribs, combined with adaptations in limb and thorax conformation. Growth rate has been measured noninvasively in various breeds using skin markers with video- and still photography (Green 1969, 1976; Goyal *et al*. 1981; Thompson 1995; Back *et al*. 1995; Hunt *et al*. 1999; Anderson and McIlwraith 2004) and using ultrasound (Aleman *et al*. 2002). Invasive techniques, relying on radiography combined with metal orthopaedic bone growth markers or cortical screws have also been described (Heinze and Lewis 1968; Campbell and Lee 1981; Fretz *et al*. 1984; Smith *et al*. 1991).

Abnormal development results in aberrant formation of bones and tendons (Speed 1960; Hermans 1969; Watson *et al*. 2003; Kroekenstoel *et al*. 2006). An extreme form of abnormal development, dwarfism, is common in man (Gruber *et al*. 1990) and in some animals (Martinez *et al*. 2007; Thompson 2007), but has not yet been reported in horses (Nicolas 2000; van Weeren and Barneveld 2001). In any case, the molecular mechanisms underlying growth, growth plate development and growth disturbances resulting in dwarfism are still poorly understood (Kronenberg 2003). Within the Friesian horse breed, congenital dwarfism has been recognised for many years, but no detailed report exists on its phenotype (Osinga 2000). Apart from being of interest for the Friesian breed and studbook, the Friesian dwarf phenotype may serve as a model for growth disturbances in general (Sande and Bingel 1983).

The purpose of this report is to describe the typical clinical, radiographic, and pathological features of dwarfism in 6 Friesian horses as a basis for further molecular genetic studies.

Case details

Five Friesian dwarf foals (2 colts and 3 fillies) were presented to the Equine Clinic of the Department of Equine Sciences of Utrecht University and followed until age 3–36 months with signs of poor growth (Table 1). After a clinical and radiological examination to confirm the diagnosis they were finally subjected to euthanasia for pathological examination (*Cases 1–5*). In addition, a 6-year-old mature dwarf Friesian gelding (*Case 6*) was admitted to recover from a colic episode and left the Clinic after 2 weeks of hospitalisation.

Clinical examination

All 6 horses underwent a general clinical, orthopaedic and reproductive examination at admission. Dwarf foals had a

TABLE 1: The case details and presenting history of the 6 Friesian dwarfs and their final outcome and age when discharged from the Clinic

Case	Age (months)	Sex	Presenting signs	Outcome
1	3	Filly	Poor growth	Euthanasia
2	6	Colt	Poor growth	Euthanasia
3	12	Filly	Poor growth	Euthanasia
4	24	Colt	Poor growth	Euthanasia
5	36	Filly	Poor growth	Euthanasia
6	72	Gelding	Colic	Sent home

TABLE 2: Typical example of the body measurements of 4 individual Friesian horses recorded at the same date: a normal Friesian horse at foal and 'old' age and a dwarf both at foal and 'young' mature age (*Cases 4* and *5*)

| | Foal age | | Adult age | |
	Normal (n = 1)	Dwarf (n = 1)	Normal (n = 1)	Dwarf (n = 1)
Age (months)	9	9	173	19
Weight (kg)	230	130	611	231
Height at the withers (cm)	142	101	166	120
Height at the croup (cm)	143	97	164	116
Length of the back (cm)	105	82	129	97
Circumference of the chest (cm)	162	128	199	152
Circumference third metacarpal bone (cm)	18	15	26	19
Vertical distance carpus - ground (cm)	48	33	46	37
Vertical distance tarsus - ground (cm)	54	36	54	42

characteristic conformation (Fig 1), being markedly smaller than normal foals (Fig 2) and exhibiting hyperextension of varying severity of the fetlock joints in both fore- and hindlimbs (Fig 1), contributing to a reduced vertical carpus/tarsus to ground distance (Table 2). Bodyweight of the young mature filly (*Case 5*) was 231 kg and height at the withers 120 cm (Table 2), while the first phalanx of both a dwarf foal (*Case 3*) and a young mature dwarf (*Case 5*) were of similar length, but approximately 25% shorter than of a normal Friesian foal (Fig 2; Table 2).

The owners ascribed these anomalies to premature birth and only recognised the retarded foal as a dwarf a few weeks later. The typical flexor tendon laxity, often seen in newborn foals, failed to recover as in normal foals, but tended to increase further, so that these animals typically developed an abnormal gait in which the elbows and hocks underwent extreme outward rotation.

While being a constant sign, there was considerable variation in the degree of hyperextension between individual dwarfs and between the fore and hindlimbs of individuals. The laxity was not restricted to the flexor tendons, also hypermobility of fetlock, carpal and tarsal joints could be observed and knuckling over the fetlock, particularly when stepping over a small obstacle. Nevertheless, the dwarf animals were able to walk, trot and canter and, at pasture, could jump and gallop despite their hyperextending joints. The dwarfs had a normal looking head, their hair coat was not different to that of normal animals and they had normal appetite. The thorax was typically abnormal with 'a dip' in the ribcage at the level of Th10-16. The abdomen had a weak and rounded appearance, and musculature over the body was poorly developed, making the spinous processes protrude along the back. The sexually mature dwarfs had an anatomically normal reproductive tract (*Cases 4, 5*). Serial transrectal examination of the dwarf mare (*Case 5*) revealed a normal ovulatory oestrous cycle, while the post pubertal colt (*Case 4*) produced clinically normal semen.

Radiographic examination

Dwarf Friesians exhibit radiographically normal growth plates and bone density. The first phalanx of the young mature dwarf (*Case 5*) was of similar length to that of a dwarf foal (*Case 3*), but had become broader (Fig 2). Dwarfs also showed dysplasia of the distal metaphysis of the metacarpus/metatarsus (Fig 3).

Pathology

Methods: Extensive macro- and microscopic pathological examination of the 5 dwarf animals was performed with special attention to limbs and thorax. For histology, 5 mm sections of the ribs at the costochondral junction were fixed in 10% neutral buffered formalin for 24–48 h and, subsequently, decalcified using

an aqueous solution of 18% formic acid and 3.5% sodium formate for 1–2 weeks at room temperature under continuous slow agitation. Following routine paraffin embedding, 4 μm sections were cut and stained with haematoxylin and eosin (H&E) and Ladewig's trichrome stain.

Macroscopic pathology: This revealed abnormally short long bones and a distorted ribcage with enlarged and widened costochondral junctions and an inward protrusion of the junction in the caudal part of the ribcage (Fig 4). The dorsal bony part of the rib deviated firstly to caudal and more ventrally to cranial, while the distal cartilaginous part deviated from inward to outward. In combination, this resulted in a somewhat S-shaped appearance of the ventral portion of the ribcage.

Histology: The costochondral junctions of the ribs in 2 dwarfs (*Cases 1* and *4*) was compared with material from 2 age-matched clinically normal Friesian horses. Histology revealed an irregular metaphyseal growth zone, increased width of the hypertrophic zone and irregular, distended chondrocyte columns (Fig 5). In the 24-months-old dwarf (*Case 4*), chondrocytes in the distal portion of the growth plates were diffusely disorganised when compared to the age-matched control (Fig 5). There was slight retention of cartilage in the primary spongiosa, although vascular ingrowth of cartilage and the secondary spongiosa appeared unaffected. When compared to the controls, larger numbers of osteoblasts and osteoclasts were lining the bony trabeculae.

Discussion

Dwarfism in Friesian horses is a congenital defect with an estimated incidence of 0.25% (1:400), as postulated by Osinga (2000) on the basis of a proposed heterozygosity in the population of 10%. The Friesian breed shows low levels of variation in protein and microsatellite markers, possibly due to a reduction in the number of breeding stallions after World War II (Luís *et al.* 2007).

The most salient feature of the dwarf syndrome is the physeal growth retardation in both limbs and ribs. Affected animals have markedly shorter fore- and hindlimbs due to the resulting reduction in bone length. Osinga (2000) reported 455 kg as an average bodyweight and 160 cm as an average height at the

withers in a population of 20 normal Friesian stallions, which was similar to that found in 33 Friesian stallions (mean 160.3 cm) of a South African population of 232 Friesian horses (Pretorius *et al.* 2004). In this study, the bodyweight of the young mature dwarf filly (*Case 5*) was 231 kg (approximately 50% reduction) and the height at the withers 120 cm (approximately 25% reduction). Individual differences, however, do exist among individual horses, both normal and dwarf (Table 2). Post natal growth is still possible in these animals, albeit at a slower rate: the head and back grow faster than the limbs and ribs leading to the characteristic disproportional growth disturbance. Therefore, mature dwarfs exhibit a normal, but relatively larger head conformation, a

broader chest with narrowing at the costochondral junction, a disproportionally long back, abnormally short limbs, hyperextension of the fetlocks and narrow long-toed hooves.

In normal horses, growth is fastest during the first 3 months *post partum* and over 90% of the height at the withers at age 2 years is achieved within the first 12 months (Thompson 1995; Hof 2001). Normal longitudinal, proportional bone growth from the carpus and tarsus ceases at age 140 days (Thompson 1995); more specifically, growth in the distal metacarpus, metatarsus and proximal first phalanx is limited to the first 10 weeks (Fretz *et al.* 1984). Bones where the growth plate closes last (proximal humerus, femur and tibia and distal radius) show the fastest growth (Campbell and Lee 1981; Fretz *et al.* 1984). Further increase in height is achieved when the proximal joints become more extended and the foal therefore adopts a more upright conformation (Back *et al.* 1995; Anderson and McIlwraith 2004).

The growth plates are reported to close radiographically at different ages (Brown and MacCallum 1975; Vulcano *et al.* 1997). It should be realised that radiographic analysis can only demonstrate the existence of cartilage within a growth plate, but does not give any information on the growth capacity of cartilage cells (MacCallum *et al.* 1978). Growth of bones always coincides with partial destruction of previously formed tissues and concurrent new bone formation. Therefore, it is possible that, although the basic model is retained during growth, considerable changes in bone conformation may still occur. This continuous
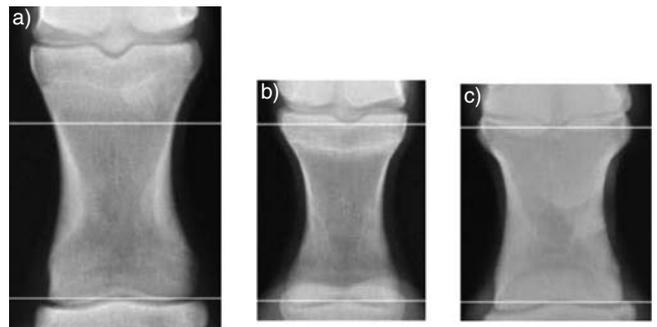

Fig 2: Anterior-posterior (A/P) radiographs of the first phalanx of the left front limb of a) a normal Friesian foal, b) a dwarf at foal age (Case 3) and c) a dwarf at young mature age (Case 5). Note the approximately 25% reduced bone length in the dwarfs at foal and mature age relative to a normal Friesian foal.

Fig 1: Typical examples of dwarf Friesian horses at foal age (Case 2), as young mature (Case 4), and at mature age (Case 6). Note the hyperextended fetlock and low-heeled conformation of the dwarfs together with the considerably shorter limbs than normal and the disproportionally long back.
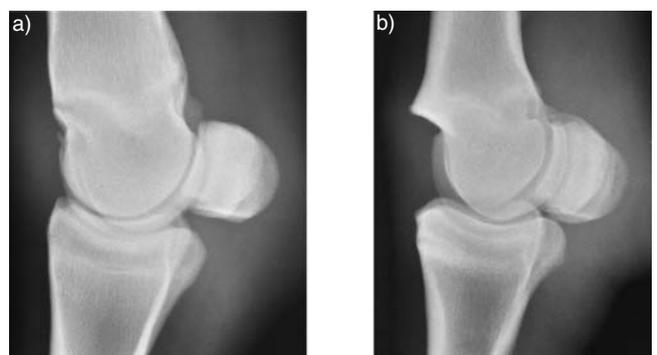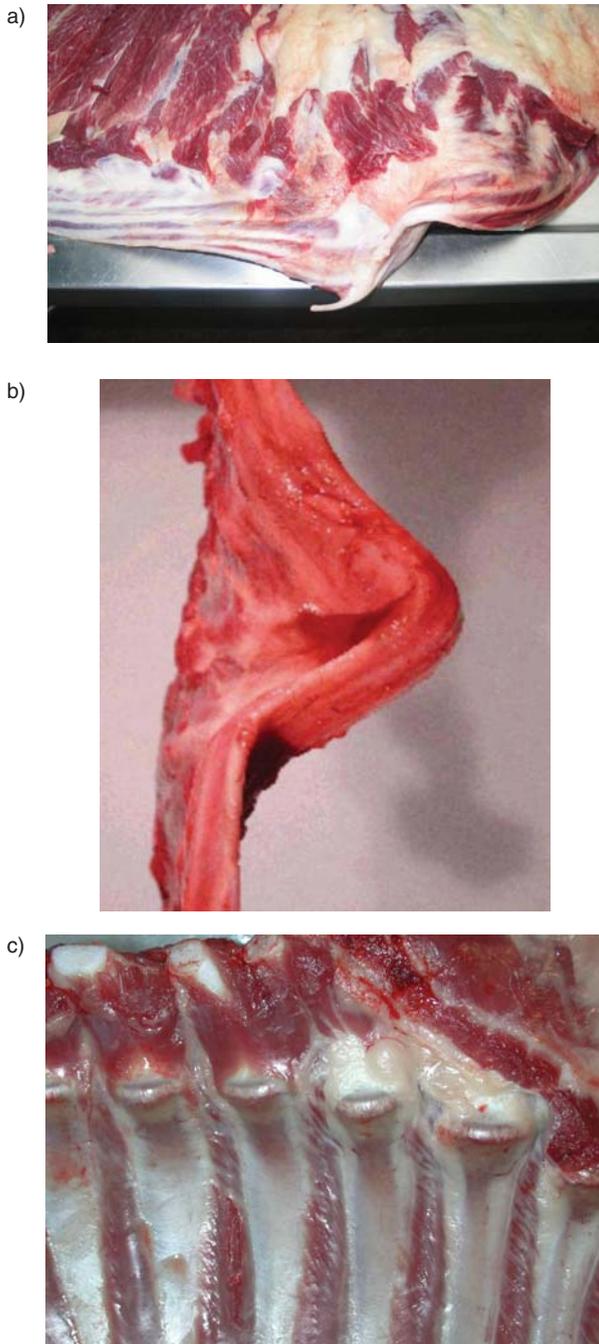

Fig 3: Latero-medial (L/M) radiographs of the fetlock of the left front limb of a) a normal and b) a dwarf Friesian colt (Case 4). Note the osteochondral chip at the dorsal, sagittal ridge of the metacarpus of the normal foal and the dysplastic distal metacarpal metaphysis in the dwarf.

remodelling process continues after completion of mineralisation, enabling the bone to adapt to the forces exerted on it (Wolff 1892; Campbell 1977). In the dwarfs, this occurs at the distal metacarpus and metatarsus, in which the dysplasia is enhanced probably by the persisting fetlock hyperextension (Figs 1 and 3), and also at the S-shaped ribcage (Fig 4).
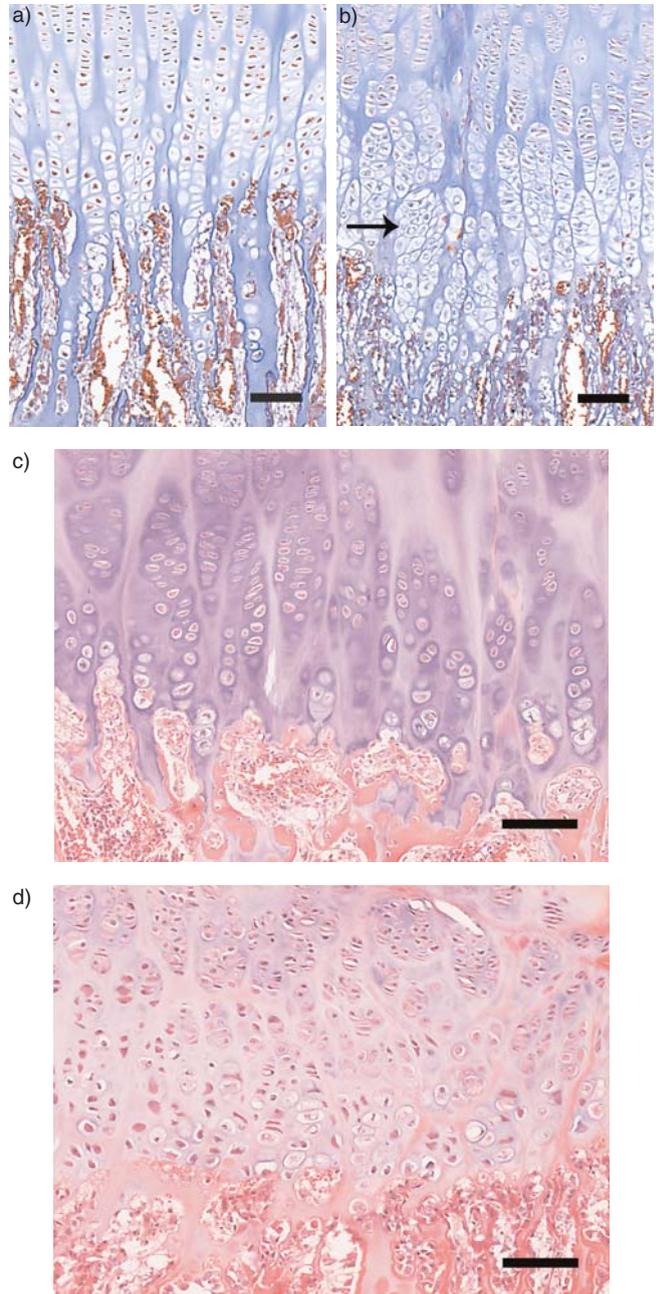
Microscopic analysis of the growth plates at the costochondral junction that showed gross abnormalities revealed an irregular transition from cartilage to bone, and thickening and disturbed formation of chondrocyte columns, similar to findings in osteochondrodysplasia (Martinez *et al.* 2007; Thompson 2007). When the normal bone remodelling process is disturbed in horses, abnormal defects in the growth plate may result (Mason and Bourke 1973; Ellis 1976; Vaughan 1976; Brown and MacCallum 1976; Gee *et al.* 2005).

Growth plate development is under control of many autocrine and paracrine factors (Kronenberg 2003). Simple, central pituitary

Fig 4: Typical example of a distorted, S-shaped costochondral junction of the left ribcage of a dwarf Friesian foal (Case 3) showing an inward protrusion: a) lateral view, b) caudal view, c) view from the inside of the thorax illustrating the thickened costochondral junctions.

Fig 5: Microscopic view of normal and affected growth plates at the costochondral junctions of the ribs: a) costochondral junction of a 3-month-old clinically normal Friesian horse (Ladewig's trichrome stain, bar = 100 µm); b) age-matched dwarf (Case 1): chondrocyte columns are disorganised and thickened (arrow) (Ladewig's trichrome stain, bar = 100 µm); c) growth plate of a 24-month-old clinically normal Friesian horse (H&E, bar = 100 µm); d) disorganisation of chondrocytes in the distal portion of the growth plate in an age-matched dwarf (Case 4, H&E, bar = 100 µm).

underproduction of GH would lead typically to proportional dwarfism, as seen in the German Shepherd dog (Andresen and Willeberg 1976; Hanson *et al*. 2006). In the Friesian dwarfs, however, a disproportional growth disturbance is seen, which would imply a local defect or disturbance in one of the regulatory systems for growth plate development.

Single mutations can cause a dwarf phenotype in man (Bellus *et al*. 1995; Merritt *et al*. 2006) or result in smaller sized dogs (Sutter *et al*. 2007). Assuming that the dwarf syndrome in the Friesian horse is inherited in an autosomal, recessive, monogenic fashion, as proposed by Osinga (2000), a whole genome scan approach in affected compared to animals with no evidence of dwarfism in their pedigree, may help to identify the specific genetic defect involved. A concomitant, in depth biochemical analysis of the abnormal growth plates in these dwarfs may then further elucidate the molecular mechanism behind this form of osteochondrodysplasia. It will be the responsibility of the studbook to decide what to do once the genetic defect has become known. Eradication from the population as an unwanted trait lies at hand, but the purposeful development of a 'new toy or pony version' (*Case 6*) is also an option. In that latter case, it will be the responsibility of the wider equine community, with the many aberrations in dog-breeding in mind, to start discussing whether or not such a policy would be acceptable from an animal welfare and a broader ethical viewpoint.

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