Musladin-Lueke syndrome of the Beagle: first report in Italy

Musladin-Lueke syndrome (MLS), also called Chinese Beagle syndrome, is a genetic disorder that affects Beagle dogs. It is due to a mutation in the *ADAMTSL2* gene. This report describes the first two cases of MLS in Italy. Two Beagle littermates were presented for evaluation of abnormal gait. Physical examination identified the typical phenotype: a flat skull, higher ear set, ear folds and slanted eyes. Upon orthopaedic examination an evident stiff gait with reduced range of motion was observed. The dogs showed a characteristic "ballerina-like" stance, with only the digital pads of the 3rd and 4th digits resting on the ground. X-rays and computed tomography showed increased radiopacity of the soft tissues of the limbs and mineralisation of the hock joint capsule. The diagnosis of MLS was confirmed by a genetic test performed on whole blood.

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Key words - Musladin-Lueke syndrome (MLS), Beagle dog, genetic disease.

INTRODUCTION

Musladin-Lueke syndrome (MLS) is a genetic disease that affects dogs of the Beagle breed. The disorder, described for the first time in the 1970s as "Chinese Beagle syndrome" because of the characteristic slanted eyes of affected animals, takes its name from Tony and Judy Musladin and Ada Lueke who showed, in 1998, how the disease was the result of a recessively inherited genetic defect[1].

The disease originates from a mutation in the *ADAMTSL2* gene, responsible for the regulation of fibrillin-1 protein, which has an important role in the formation of elastic fibres and is involved in the regulation of growth factors[1,2].

The same alteration of fibrillin-1 in humans has clinically different consequences and may result in Marfan's syndrome, geleophysic dysplasia or stiff skin syndrome. Marfan's syndrome primarily affects connective tissues of the lung and cardiovascular system[3]. Geleophysic dysplasia is a skeletal dysplasia characterised by short stature, a distinctive "happy" facial expression, and short limbs and fingers. Stiff skin syndrome is manifested by cutaneous fibrosis and reduced joint movements[5]. The strong link between fibrillin-1 mutation and cutaneous fibrosis is also found in the murine species, in which a condition referred to as "tight skin" was described in 1998[6].

In Beagles, MLS has been reported to occur with an incidence of 2-3% in England and Australia in the late 1990s[2]. Other cases were reported in the USA and Japan in 2010[2].

The phenotype of the MLS is characterised by short stature, thick, taut skin, elongated palpebral fissures, high-

MLS is a disease caused by a genetic defect that results in abnormal formation of elastic fibres.

Received: 24/06/2015 - Accepted: 16/12/2016

This work was presented as an oral communication at the 83rd International SCIVAC Congress, Palacongressi Rimini Riviera, 29 May-1 June 2014.

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set, thick ears, increased musculature, reduced range of joint mobility and a hopping "tiptoe" gait. These signs are found, in various associations, in Marfan's syndrome, geleophysic dysplasia and stiff skin syndrome in humans. Affected Beagles usually have a very sociable temperament, a peculiarity that has also been reported in children with geoleophysic dysplasia. The manifestations of the disease start to appear at 2-4 months of age in puppies and stabilise at about the age of 1 year. The condition is not fatal, but affected animals frequently develop degenerative joint disease[2].

In this article we present two cases of Beagles diagnosed with MLS.

CASE REPORTS

Two 9-month old Beagles, one male (case 1) and one female (case 2), from the same amateur-bred litter in the province of Grosseto (Tuscany, Italy), were referred to the "Mario Modenato" Veterinary Teaching Hospital of the University of Pisa for an orthopaedic evaluation.

The dogs' parents were working Beagle dogs born in Italy, without a pedigree, and had never had any orthopaedic disorders.

The puppies were adopted at 2 months of age and vaccinated normally at 60 and 90 days. The owners reported that the dogs had always had a peculiar gait, which had become more accentuated as time passed.

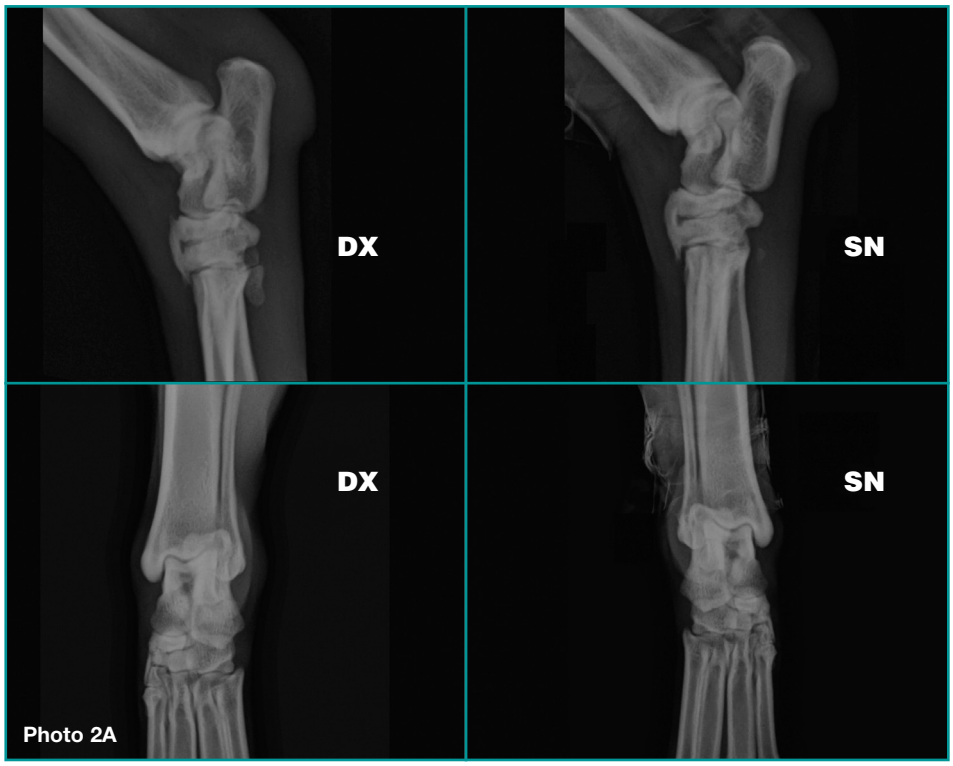
The general physical examination highlighted stunted growth compared to the breed standard, but in proportion, with the animals weighing 11 kg (case 1) and 8 kg (case 2). Both dogs had particular facies, with elongated, narrow palpebral fissures, this feature being more pronounced in the male, and thickened ears (Photo 1A and B). On standing, the limbs rested exclusively on the pads of the 3rd and 4th digits, without any support from the 2nd and 5th digital pads or the plantar footpad. This stance is described as being "on tiptoe" or, as reported in the literature, "ballerina-like" and is considered a symptom pathognomonic of MLS[2].

Affected dogs show a characteristic phenotype: they are small, have elongated palpebral fissures and a stiff, hopping gait, and walk on their tiptoes.

The subjects had a hopping gait characterised by extreme joint stiffness, especially of the carpo-metacarpophalangeal and tarsal-metatarsal-phalangeal joints (Video 1). This rigidity, which was more pronounced in the male, caused a reduction of joint movement and abduction of the limbs to compensate for the lack of elasticity and strength. The orthopaedic examination confirmed the reduced range of motion, especially at the carpal-metacarpal and tarsal-metatarsal joints, again more noticeable in the male, in the absence of pain, crepitus or capsular ectasia. The stiffness persisted also when the animal was anaesthetised.

Video 1 - The video shows the characteristic hopping gait with abduction of the limbs and tiptoe stance, the joint stiffness during the orthopaedic examination and the facial phenotype.
http://cms.scivac.it/it/v/13289/1

Photo 1
Case 1 **(A)** and case 2 **(B)**: 9-month old Beagle dogs. Note the small size, slanted eyes and the stance, with all four limbs resting on the 3rd and 4th digits.



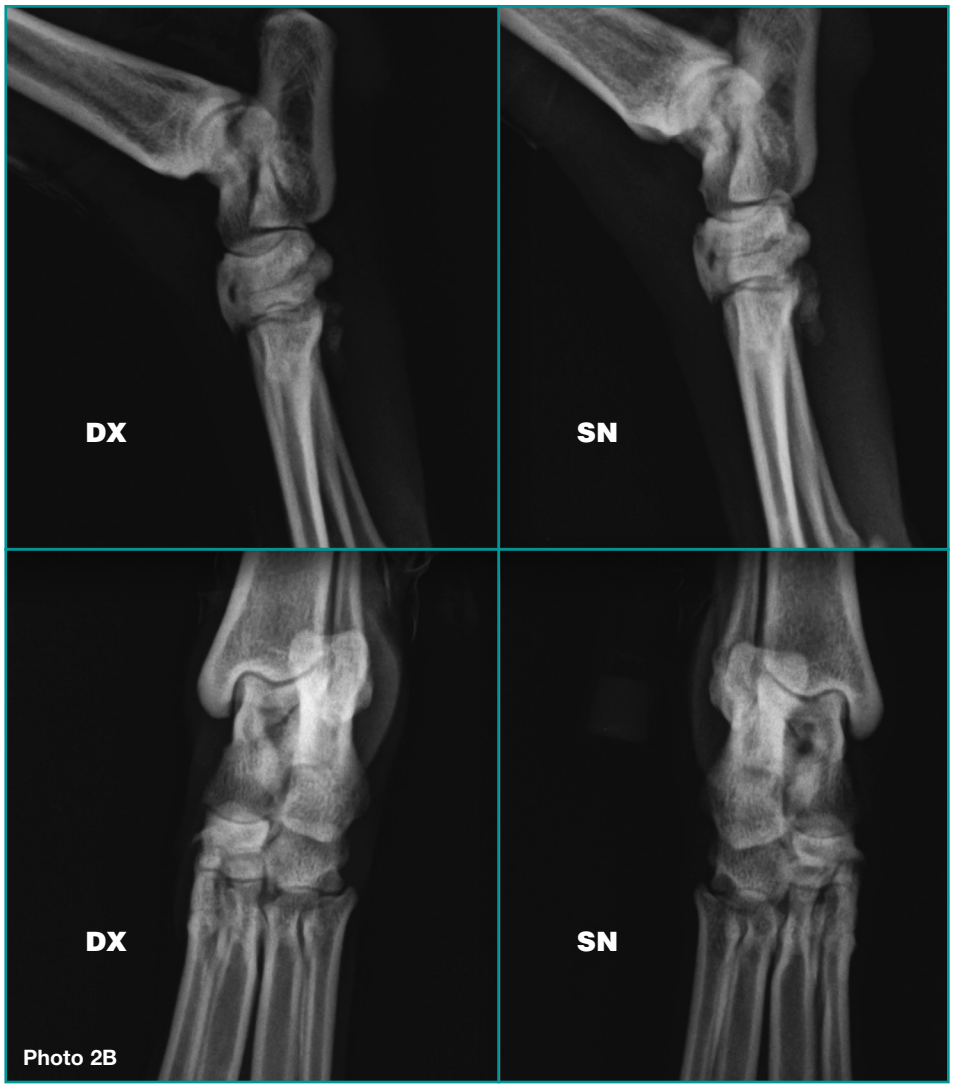


Photo 2A and B
Medial-lateral and posterior-anterior X-rays of the hocks of case 1 **(A)** and case 2 **(B)** showing the presence of a band of mineralisation on the dorsal surface of the second and third rows of the tarsal bones. Case 2 also has a narrowed joint space between the central bone of the tarsus and tarsal bones II and III.

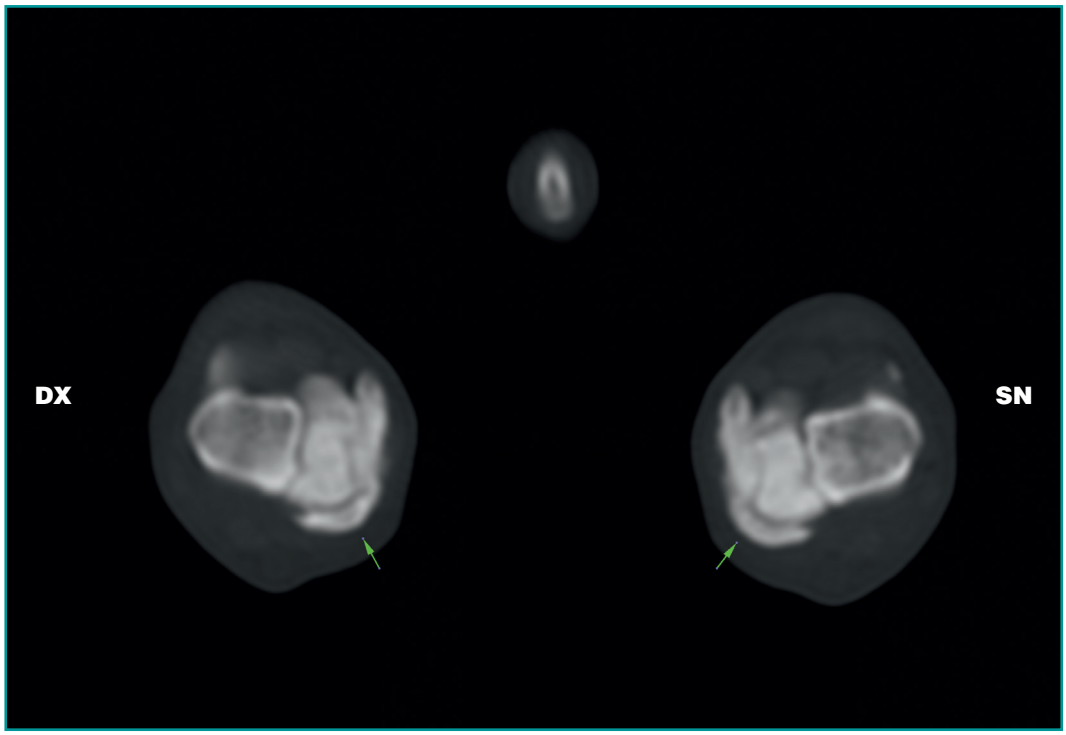


Photo 3
Computed tomography scan of the hock region, at the level of the second row of the tarsal bones, right and left.
The arrows indicate a curved line of mineral density contiguous with the dorsal surface of tarsal bones II and III, which is mineralisation of the joint capsule.

There were no significant abnormalities of the blood-biochemistry profile of either dog.
Radiographic examination of the front and hind legs in the two perpendicular axes and a ventrodorsal view of the pelvis showed a widespread increase in the opacity of the soft tissues of the limbs. Bilaterally in the hocks of both subjects there was a clearly defined, regular band of mineral radiopacity, apparently fused with the dorsal cortex of the second and third rows of the tarsal bones; case 2 also showed reduction of the joint space between the central bone of the tarsus and tarsal bones II and III (Photo 2A and B). No alterations of the forelimbs were seen.
The joint heads and articular congruity of the pelvis and knees were normal.
To investigate the lesions found in the hocks further, computed tomography of the hind limbs of both subjects was performed, with scans of 1 mm from the pelvis to the metatarsals.
The images revealed bilateral mineralisation of the joint capsule of the hock (Photo 3).
The particular clinical presentation, in the absence of significant blood-chemistry and radiographic changes, raised the suspicion of MLS.
Whole blood samples were, therefore, sent to the Genefast Laboratory (Bazzano, Emilia Romagna, Italy) for specific testing for mutation of the *ADAMTSL2* gene; the test revealed that both subjects were homozygous for an *ADAMTSL2* mutation, caused by the substitution of an arginine by a cysteine at codon 221 (R221C).
The dogs were re-evaluated after 3 and 6 months and no clinical changes were found.

The genetic test is the only diagnostic test that is informative for the diagnosis and selection of breeding stock.

DISCUSSION

MLS is a rare disorder that affects Beagle dogs. Only a few cases have been described in the literature[2], and so far there had been no reported cases in Italy.
The clinical literature available indicates that the first signs of the disease become evident as soon as the affected puppies begin to walk, and that as the months pass the animals do not grow as much as their peers[2,8].
In the two cases described here, the owners, probably due to inexperience, had underestimated the abnormal gait and attributed the smallness of their animals to a different breed standard[1,7,8].
The worsening of the signs, with the obvious hopping, stiff gait and the even more peculiar "tiptoe" stance, was the reason that an orthopaedic evaluation was requested. On the basis of the clinical features, the differential diagnosis included some other diseases reported in the Beagle breed, namely carpal hyperflexion syndrome, chondrodystrophy and cerebellar ataxia[8-10].
Carpal hyperflexion syndrome is a disorder of undetermined aetiology involving tendons and muscles. It is

characterised by asynchronous development of skeletal tissue and the muscle-tendon apparatus and affects only anterior limbs[8,9]. Chondrodystrophy is an autosomal recessive genetic disorder that causes dysharmonic dwarfism[8]. Cerebellar ataxia, or cerebellar abiotrophy, is a genetic disorder that is manifested by incoordination and exaggerated movements; it causes degeneration of the neurones of the cerebellum which have an inhibitory function and control movements. The disease is progressive and has a dismal prognosis; in some breeds, including the Beagle, the symptoms may occur already in the first month of life[8,10].

The clinical presentation of the two subjects examined, in particular the combination of the peculiar gait with slanting eyes, thickened ears and small size, allowed us to exclude these diseases, and raised the clinical suspicion of MLS, subsequently confirmed by genetic testing.

CONCLUSIONS

MLS is a rare disease, for which instrumental diagnostic methods are not helpful. It is, therefore, important to recognise the phenotype at the time of an examination and then immediately perform a genetic test, which is the gold standard for making the diagnosis.

The causative gene is recessive and, therefore, for an animal to express the phenotype of the disease, both parents must be carriers.

Genetic testing is important for both the diagnosis and prevention when selecting breeding stock; identifying healthy carriers and excluding them from breeding will prevent the affected gene from spreading within the Beagle breed.

KEY POINTS

- This report is the first description of cases of MLS in Italy.
- MLS has only been described in purebred Beagle dogs.
- The particular facies and the characteristic gait allow exclusion of other disorders reported to occur in the Beagle breed.
- A diagnosis of MLS can be suspected from the clinical examination and confirmed by a genetic test.

REFERENCES

1. Musladin JM, Musladin AM, Lueke A. The New Beagle: a dog for all seasons. Macmillian General, 1998.
2. Bader HL, Ruhe AL, Wang LW *et al.* An *ADAMTSL2* founder mutation causes Musladin-Lueke syndrome, a heritable disorder of beagle dogs, featuring stiff skin and joint contractures. PLoS One 5 (9): pii:e12817, 2010.
3. Steindl K. Marfan syndrome and related connective tissue disorders. Praxis 102(24):1483-8, 2013.
4. Le Goff C, Morice-Picard F, Dagoneau N *et al.* ADAMTLS2 mutations in geleophysic dysplasia demonstrate a role for ADAMTLS-like proteins in TGF-beta bioavailability regulation. Nature Genetics 40(9):119-23, 2008.
5. Loeys Bl, Gerber EE, Riegert-Johnson D *et al.* Mutations in fibrillin-1 cause congenital scleroderma: stiff skin syndrome. Science Translational Medicine 2(23):23ra20, 2010.
6. Kielty CM, Raghunath M, Siracusa LD *et al.* The Tight skin mouse: demonstration of mutant fibrillin-1 production and assembly into abnormal microfibrils. The Journal of Cell Biology 140:1159-1166, 1998.
7. Jebavy L, Cancikova A, Svobodova I *et al.* Somatometry of beagle dogs. 16th Conference about Laboratory Animals, Kutna Hora, Czech Republic, 2013, pp 2.
8. Bell JS, Cavanagh KE, Tilley L *et al.* Veterinary Medical Guide to Dog and Cat Breeds. Teton Newmedia, 2012, pp 50-51.
9. Martellaro CM, Petazzoni M, Vezzoni A. ATLANTE BOA, Breed-oriented Orthopaedic Approach - Approccio Ortopedico Orientato alla Razza. INNOVET, 2005, pp 86-87.
10. de Lahunta A, Glass E. Cerebellum. In: de Lahunta A, Glass E. Veterinary Neuroanatomy and Clinical Neurology. Philadelphia: WB Saunders Co, 2009, pp 363-369.