New developments in CMSM research

Talk given CKCS club
7th October 2010 - Leicester

(some images removed)
Copyright Clare Rusbridge

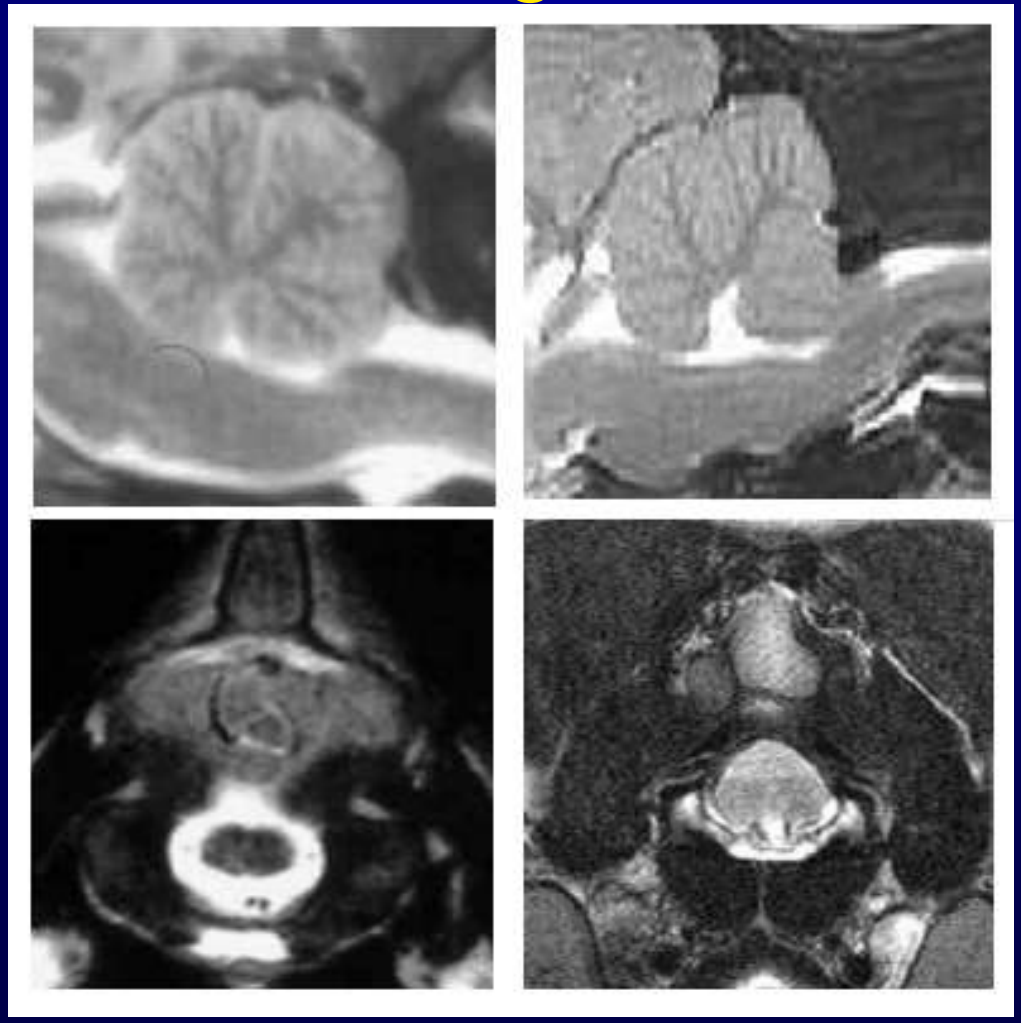
Lecture synopsis

- What causes Chiari-like malformation?
- Why do some dogs with CM get SM?
- Genetics of CM/SM
- What is the incidence of SM in the CKCS?
- The breeding guidelines – do they work?
- Treatment – anything new?
- Other questions to be answered

What is Chiari malformation

Overcrowding of the skull

Normal



CM

Chiari-like malformation

Overcrowding of the FM

large cerebellar herniation not required

8 months

Be cautious before declaring a dog safe to breed on the basis of appearance of the brain

8 years

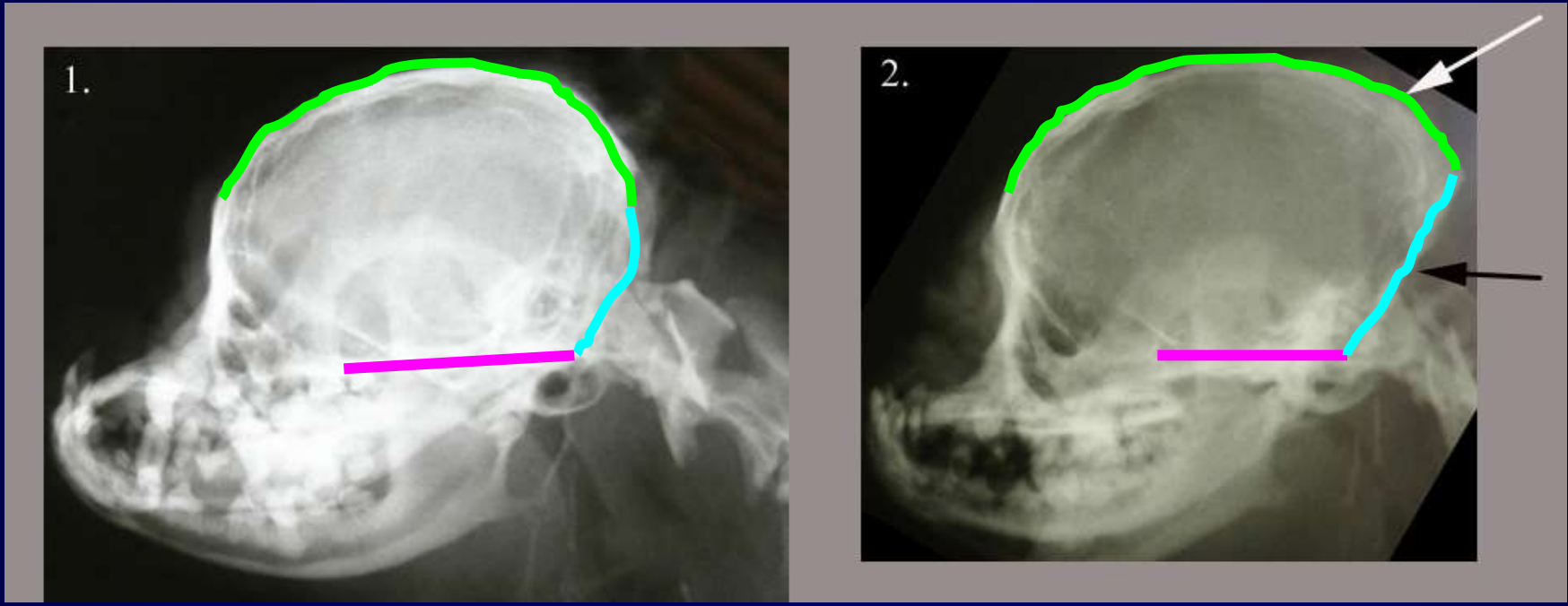
Which dog will get SM ?

Chiari-like malformation

most common cause SM in dog

Mismatch between brain and skull size

CM – characteristics
changes in proportions of the skull

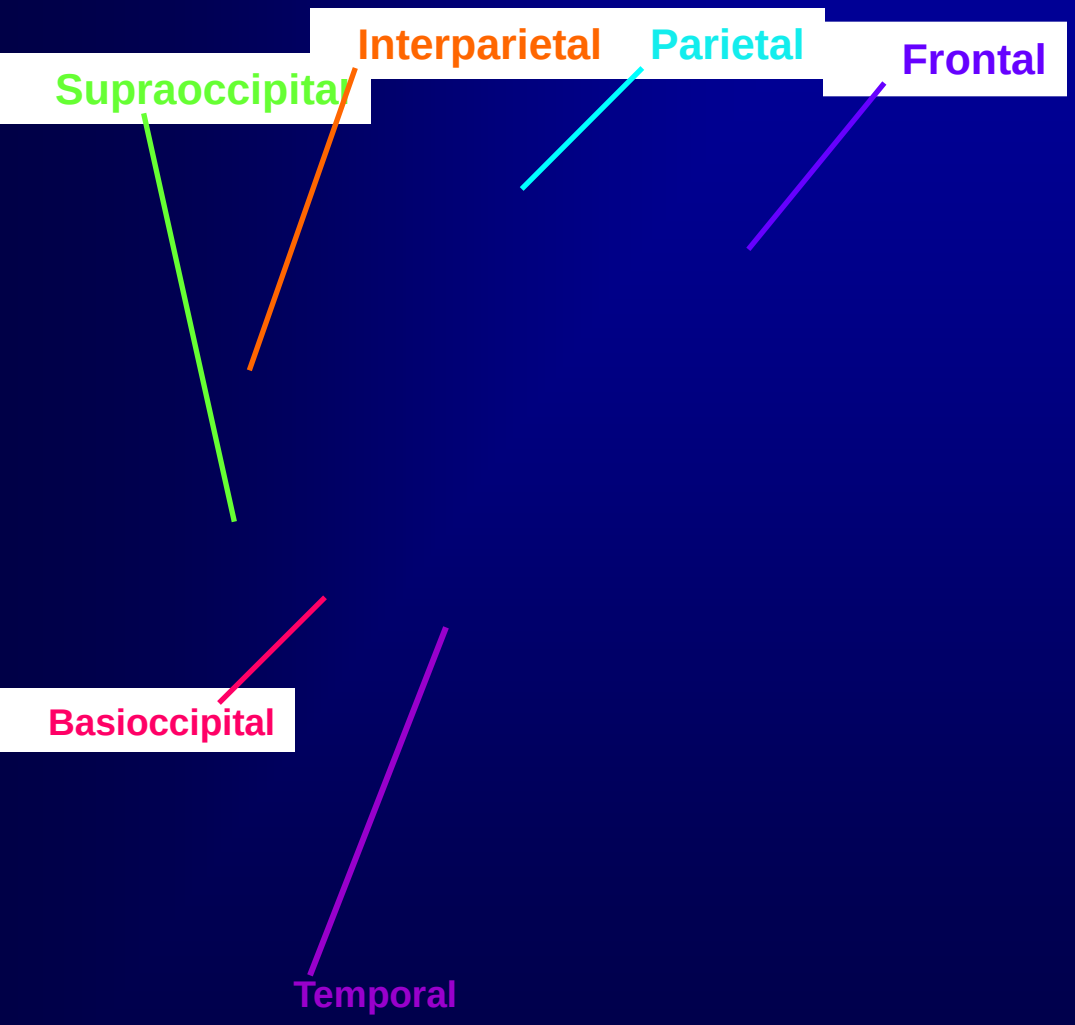


Griffon with no CM or SM

Griffon with CM and SM

basicranium (skull base) shortens
parietal bone lengthens
supraoccipital bone is shorter and straighter
smaller frontal sinuses

A theory for CM in the GB



Hypothesis for CM

Bone insufficiency

or

craniosynostosis?

Embryological origin skull bones

White – membrane bone

Stippled – cartilaginous neurocranium

Hatched – visceral arches

From The Life of Mammals

Young, Oxford University Press, 1957

CKCS with CM

Comparison of brain and skull volume

Cross H. R., Capppello R, Rusbridge C Comparison of cerebral cranium volumes between CKCS with chiari-like malformation, small breed dogs and Labradors *JSAP 2009 50 399-405*

CKCS with CM

- Similar skull volume to other toys
- More brain tissue within skull
  - Statistically similar volume to Labradors

Cross H. R., Capppello R, Rusbridge C Comparison of cerebral cranium volumes between CKCS with chiari-like malformation, small breed dogs and Labradors *JSAP 2009 50 399-405*

CKCS with SM

- Skull (caudal fossa) volume
  - No difference between CKCS with & without SM in a mixed age group
  - Significantly smaller for CKCS with early onset SM (<2y) compared to clear CKCS (> 5y)
- Volume of brain within skull
  - significantly greater for CKCS with SM
  - Especially in CKCS with early onset SM
- SM associated with brain / skull mismatch
- Early onset SM greater disparity

C.J. Driver C. Rusbridge H.R. Cross I. McGonnell H.A. Volk Volumetric Comparison of Brain Parenchyma within the Caudal Cranial Fossa of Cavalier King Charles Spaniels with and without Syringomyelia (unpublished)

CKCS with SM

- Larger brain within skull = larger syrinx (Pearson r=0.437)
- Larger syrinx = larger ventricles (Pearson r=0.592)
  - i.e. dogs with SM tended to have ventriculomegaly and big syringes were associated with big ventricles

C.J. Driver et al Volumetric Comparison of Brain Parenchyma within the Caudal Cranial Fossa of Cavalier King Charles Spaniels with and without Syringomyelia (publication pending)

Genetics

Inherited CMSM in humans

- Tartar population
- Work of Enver Bogdanov (Kazan Tartarstan)
- Typical appearance
  - Flat face (brachycephalic)
  - Short neck
- Variation in presentation and progression
  - Large syrinx progresses quicker
  - Mild cases may be asymptomatic
- Genome work in progress

Inherited CM/SM in dogs

- CMSM has moderately high hereditability in the Cavalier
  - SM = 0.37 (maximum is 0.64)
- It is a complex inheritance involving more than one gene.
  - Initial results suggest genes at two or more loci interact to give disease

Heritability of syringomyelia in Cavalier King Charles Spaniels.
Tom Lewis, Clare Rusbridge, Penny Knowler, Sarah Blott, John A. Woolliams.
Vet J. 2009; 183 (2010) 345–347.

Canine CM/SM genome project - 2 parts

Search for syringomyelia genes in Cavaliers

Search for chiari genes in Griffon Bruxellois

Canine SM genome project
Where are we now?

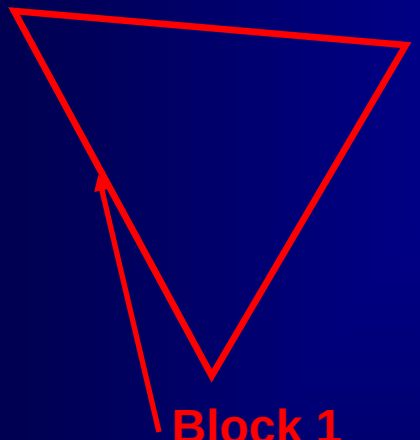
DNA samples taken after a MRI scan (saliva sponges)

Canine SM genome project

- **Recent progress**
  - **Identification of locus for SM associated with CM in the CKCS**
  - **Identification of a haplotype that infers protection against SM**
- Next steps
  - **Wider dense SNP coverage**
  - **Candidate gene sequencing**

Canine SM genome project

red triangle indicates significant region on the chromosome !



Block 1

***P* value = 3,2 x 10-15**

Linkage disequilibrium in fine-mapping region analyzed by Haploview v4.2 with R squared method

10-SNP window spans 1.3 Mb.

Genome wide linkage studies identify a novel locus for syringomyelia associated with Chiari-like malformation in the Cavalier King Charles Spaniel

Quoc-Huy Trinh, Penny Knowler, Alexandra Thibault, Marie-Pierre Dubé, Guy A. Rouleau ,Clare Rusbridge and Zoha Kibar

Canine CM genome project

- **Recent progress**
  - **Identification of 2 candidate loci (i.e. small areas of a chromosome) for CM**
- **Next steps**
  - **Fine mapping**
  - **Candidate gene sequencing**

Trinh VQH [1], Knowler P [2], Dubé MP [3], Blott S [4], Rusbridge C [2], Rouleau GA [5], Kibar Z [1]

1Centre de recherche CHU Sainte-Justine, Université de Montréal; 2 Stone Lion Veterinary Centre, Wimbledon; 3Institut de Cardiologie de Montréal, Université de Montréal; 4Department of Genetics, Animal Health Trust, UK; 5 Centre de recheche du CHUM, Université de Montréal.

Prevention of CM/SM

- EBV
  - Currently only CKCS
  - Ultimately GBV?
- Breeding guidelines
  - Eliminate early onset SM dogs (E)
  - Only breed young clear (C) to older clear (A)
  - Only breed late onset SM (D) to older clear (A)
  - Identify CM free dogs?
    - June 2009-10 229 MRI from breeding CKCS (CR) - no dogs without CM; 2 dogs with mild CM.

Incidence SM 55%
(slide content removed and replaced with abstract)

FP8. THE INCIDENCE OF SYRINGOMYELIA IN CAVALIER KING CHARLES SPANIELS

John Parker[1], *Penny Knowler*[2], *Nick Jeffery*[1], *T. J. McKinley*[1] *and Clare Rusbridge*[2]
[1]The Queen's Veterinary School Hospital, Department of Veterinary Medicine, University of Cambridge, Cambridge, Cambridgeshire, United Kingdom; [2]Stone Lion Veterinary Centre, Goddard Veterinary Group, London, United Kingdom

The epidemiology of cervical syringomyelia in a population of 804 Cavalier King Charles Spaniels (CKCS) was investigated using the results of a voluntary MRI screening programme that is ongoing in the United Kingdom (UK) and the Netherlands. The aim of the study was to establish the incidence of disease and to determine the risk factors for its development. The data was analysed using logistic regression to assess the influence of certain variables on the likelihood of detecting syringomyelia and to generate a predictive model for the outcome of screening.

The lifetime risk of developing syringomyelia in the study population was estimated to be 55%. Of the variables investigated, only the age at which a scan was performed significantly predicted the outcome of screening and the likelihood of detecting the disease increased with age-at-scan up to the age of four years. The predictive accuracy of the final model was 62.4% and factors not evaluated by this study are therefore also likely to contribute to the timing of disease manifestation.

It is concluded that syringomyelia is likely to be one of the most common disease conditions of the CKCS. The age at which an MRI scan is performed predicts the likelihood of disease detection and a susceptible individual may not express the diagnostic phenotype until the age of four. Performing screening before this time may give a false negative result for the lifetime risk of disease development.

Parker J. *et al* 2010 presented 23rd Annual Symposium ECVN and ESVN

Using breeding guidelines -
Incidence of “A” (>2.5y clear) dogs
(Slide contents removed and replaced with abstract)

FP12. INTERIM BREEDING GUIDELINES FOR SYRINGOMYELIA – 4 YEAR REPORT

SP Knowler[1], *AK McFadyen*[2], *C Rusbridge*[1]
[1]Stone Lion Veterinary Hospital, London, UK. [2]School of Engineering & Computing, Glasgow Caledonian University, Glasgow, UK

Chiari-like malformation and Syringomyelia (CMSM) is a complex trait with a moderately high heritability (Lewis *et al* 2010). Genome wide linkage studies in the cavalier King Charles spaniel (CKCS) have recently identified a novel locus for CMSM and a haplotype that infers protection against SM (Quoc *et al* 2010). In some toy breeds CMSM is prevalent and many breeders ascertain the MRI CMSM status of their dogs prior to breeding. In 2006, CKCS breeders sought guidance on breeding to reduce SM. A recommendation to exclude SM affected dogs from breeding may encourage genetic bottle-necking and fails to take account SM can occur as a late onset disease. Consequently guidelines were proposed that took account of age and also made provision for breeding older asymptomatic SM affected dogs (Grade D) that are free of other known inherited diseases (Cappello *et al* 2007). The aim of this study was to investigate the early outcome of these guidelines and to identify factors associated with increased risk of SM.

Using Microsoft Access ™ databases constructed for use in genome studies, a cohort of 465 dogs (307 females, 158 males) were identified which had either one (316 dogs) or both parents (149 dogs) with MRI confirmed CMSM status. Of these, 393 were CKCS and 72 were Griffon Bruxellois. All dogs were assigned an A - F CMSM grade according to the current breeding guidelines. Grade A implies a SM unaffected dog over 2.5 years old. In addition, to estimate the influence of late onset SM, an Grade A* was assigned to Grade A dogs over 5 years old. The CMSM grade of all offspring from all possible breeding combinations including using one parent of unknown status (Grade U) was ascertained.

Offspring without SM only occurred when there was at least one parent of Grade A status. There were.higher numbers of SM clear offspring if both parents had A status. In addition all A* offspring also had at least one A* parent and higher numbers of A* offspring resulted from crosses where both parents were A*. There was no influence of gender on SM affectedness. All offspring were SM affected if both parents were SM affected. SM affected offspring may also occur when SM unaffected dogs are used (15.4% from A x A parental crosses and 7.7% from A* x A* parental crosses). Using dogs of unknown status was risky for SM affectedness. Fifty percent of older offspring were SM affected in A x U parental crosses and there were higher numbers of SM affected dogs with other parental combinations that included one Grade U dog.

In conclusion, to increase the number of SM unaffected offspring, at least one parent should be ascertained to be free of SM by MRI at 2.5 years of age. Ideally both parents would be free of SM at 2.5 years of age and the true SM status of the grandparents at least 5 years old should be established. It is recommended that all breeding dogs from breeds susceptible to CMSM be MRI screened and results submitted to an officially recognised central database.

Knowler *et al* 2010 presented 23rd Annual Symposium ECVN and ESVN

Using breeding guidelines -
Incidence of “A*” (>5y clear) dogs

Knowler *et al* 2010

Conclusions

- To increase number SM free dogs
  - at least 1 parent should be ascertained to be free of SM by MRI at 2.5 years of age.
  - the true SM status of the grandparents at least 5 years old should be established.
  - Using dogs of unknown status is risky - all breeding dogs should be MRI screened.
  - all results should be sent to a recognised central database (EBV).

Conclusions continued

- If SM affected dog is used then ideally the chosen mate would either be selected on the basis of its EBV and/or would be a older SM clear dog (>5 years).
- The offspring of the proposed mating should also be scanned and ideally bred to older SM clear dogs.

Conclusions continued

- “D” status (or equivalent) will only be appropriate if the dog was first proved to be SM free before 2.5 years of age.
- Future breeding recommendations will also take account of dogs with central canal dilatation less than 2mm.

Proposed new guidelines

SM Grade	Age (years)	Breed to
0a	> 2.5	SM grade 0a,b, 1, 2, 3a*
0b	< 2.5	SM grade 0a, 1
1	> 5	SM grade 0a,b, 1, 2, 3a*
2	< 5	SM grade 0a, 1
3a	> 2.5	SM grade 0a, 1
3b	< 2.5	Do not breed
Any clinical signs	Any	Do not breed

Grade 0, normal (a = over 2.5 y; b = less than 2.5 y).
Grade 1, CCD < 2mm; dog > 5 y.
Grade 2, CCD < 2mm; dog < 5 y.
Grade 3, SM or pre-SM (a = over 2.5 y*; b = less than 2.5 y)
* Must have been clear of SM b4 2.5y

The need for estimated breeding values !

“but I know what is at the back of my dogs”

Conversation with breeder

- “I am worried about using ……**Dog X** who was …. scanned **A** but …..has fathered several progeny with SM”
- Only the vet and breeder of **Dog X** know that when younger **Dog X** was indeed an **A** … however since rescanned and is a **D**
- The MRI results of **Dog X** are confidential - i.e. vet cannot comment
- The breeder of **Dog X** keeps quiet
- EBV will help to protect breeders

- Funds MRI scans for CKCS >5 years and GB
  - Applicants to penny.knowler@ntlworld.com
  - participating vet centres
  - Aims to identify CKCS clear of SM and GB clear of CM

Treatment / prevention?

Before medication

After medication

Pain face

“Fear and aggression related behaviour traits have a positive correlation to clinical severity of CM/SM”

BEHAVIOUR ANALYSIS OF CKCS DIAGNOSED WITH SYRINGOMYELIA

L Rutherford at al ECVN Annual Symposium 2010

When they are gone……

Your Gift of Love

Freedom from Syringomyelia

The Syringomyelia Cavalier Collection Scheme

Be a part of saving the beautiful Cavalier King Charles Spaniel

The Cavalier King Charles Spaniel, as a breed, is prone to the painful and debilitating disease Syringomyelia or 'SM'. It is a disease which can hugely affect the quality of life for your dog'.

Currently, there is no cure.

The Syringomyelia Cavalier Collection Scheme is working to eliminate SM through genetic research.

If you have a Cavalier that has been diagnosed with SM then we would be extremely grateful if you would give consideration to agreeing to your dog taking part in this Scheme.

In the sad event of your dog passing away, the scheme will fund transport costs to the nearest suitably equipped veterinary centre. Here, they will take DNA samples which will also benefit five other research projects. On completion, your dog's ashes will be safely returned for you to decide on the final resting place.

We realise that this would be a sad and distressing time. Participation in this scheme needs careful thought while you are still enjoying the loving friendship that your dog brings you.

If you would like to help, please contact Margaret Carter by using the details below.

Please help us save this beautiful breed.

Tel: 01707 262 035

Email: mareve-ckcs@ntlworld.com

'My sunshine doesn't come from the skies,
It comes from the love in my dog's eyes.'
Unknown poet

Thank you for listening!

Any questions?

www.veterinary-neurologist.co.uk

Acknowledgments

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Syringomyelia DNA research
For the Love of Ollie fund
Rupert's Fund
Cavalier Friends
Ann Conroy Trust
American Kennel Club Health Foundation
CKCS club of USA Health Foundation
DNA archive for Companion Animals Manchester
Marshfield Clinic (NIH)
UK CKCS & Griffon Bruxellois clubs
The Cavalier Club of the Rand
French Cavalier Club
Cavalier Club of Canada
Frank and Lee Pieterse

Couldn't have done it without…….

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Genome scan

SM project

Vincent Quoc-Huy Trinh

CM project

Philippe Lemay

Overseen by

Penny Knowler
Zoha Kibar
Guy Roleau

Greatly assisted by

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Daniel Rochefort
Melanie Benard
Isabelle Thibault
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Yan Yang

Statistical analysis

Marie-Pierre Dube
Sarah Blott
Sylvie Provost

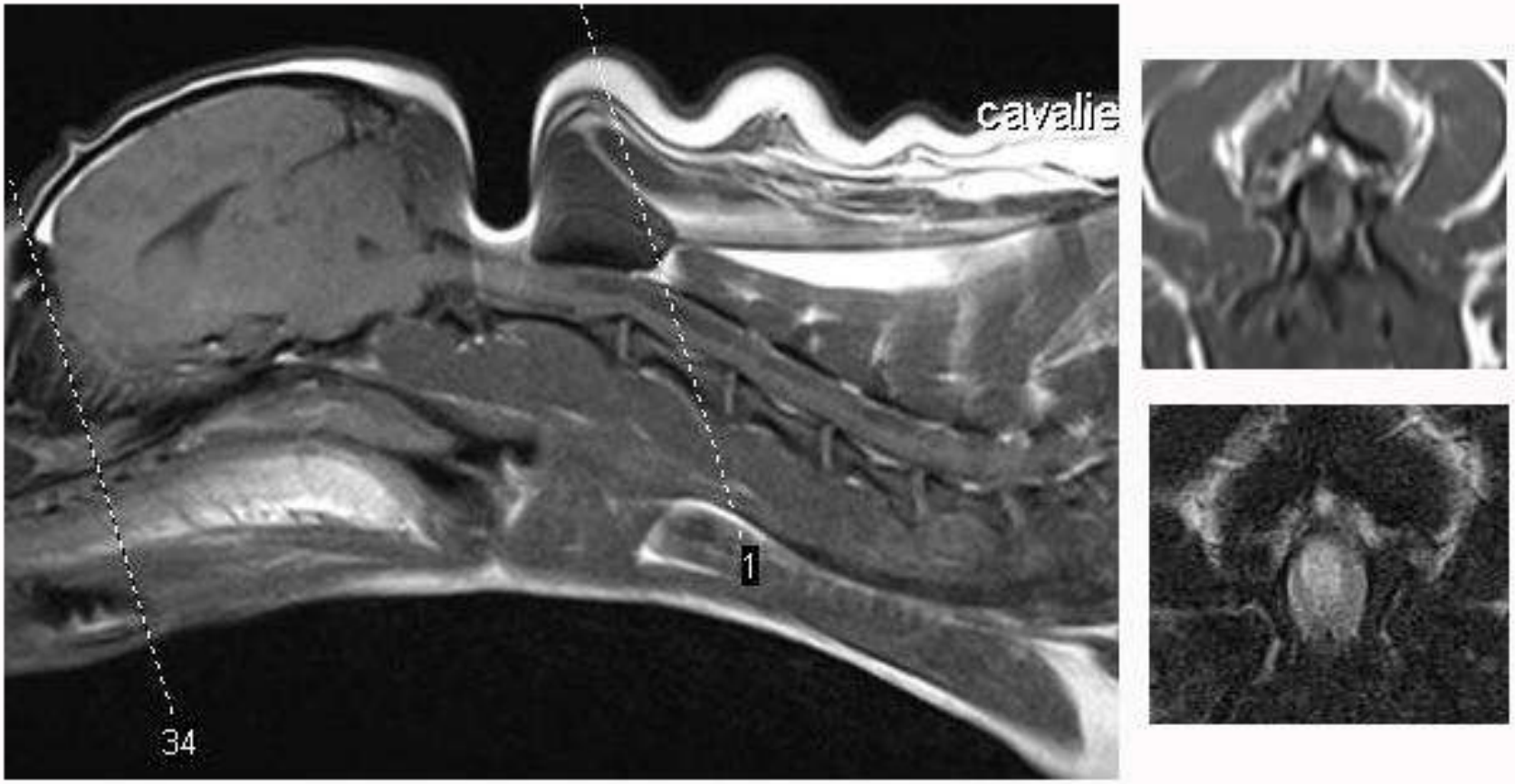
Slides which might have been required in question and answer session

“General anaesthesia, narcosis or deep sedation required”

General Anaesthesia

- Dexdomitor & butorphanol premedication
- Propofol induction
- Isoflurane & oxygen maintenance

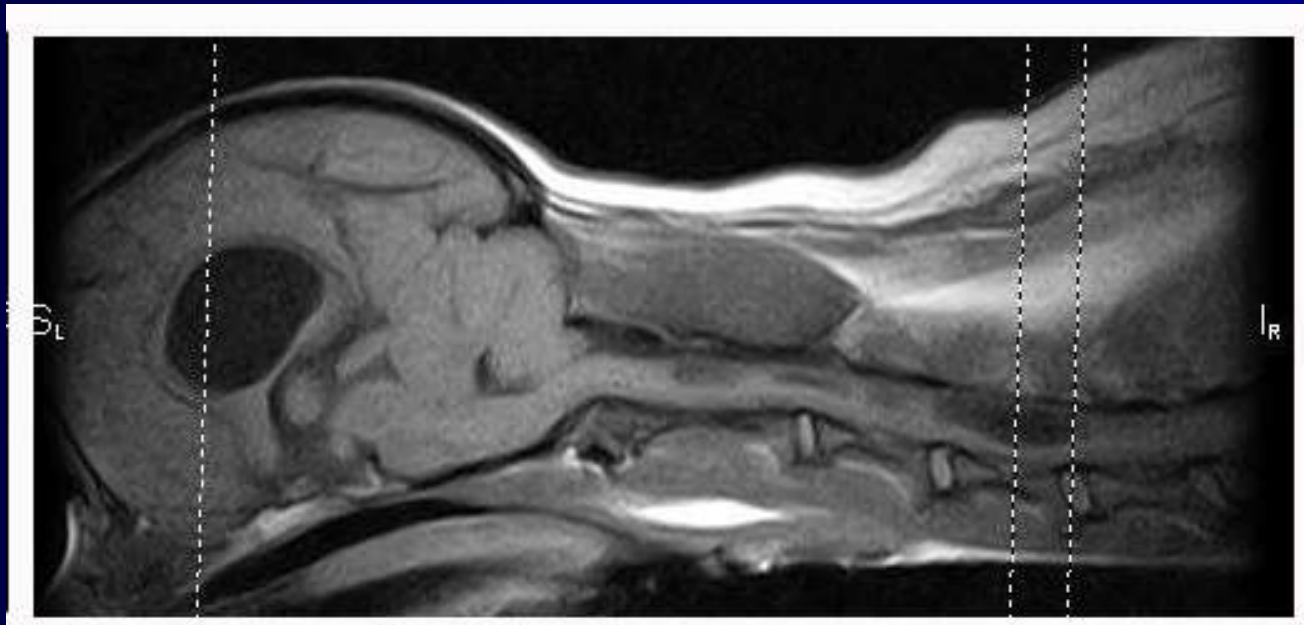
Wrong sequences

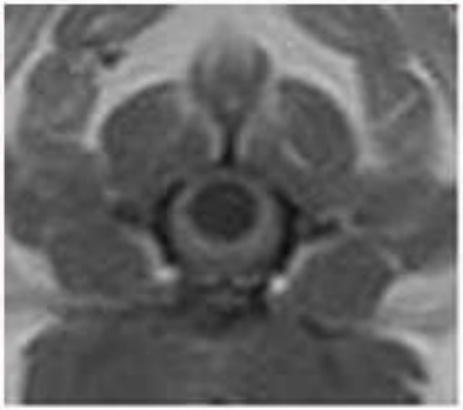


Head slightly flexed

Transverse images not perpendicular to spinal cord and not though widest part of syrinx.

Correct positioning and images

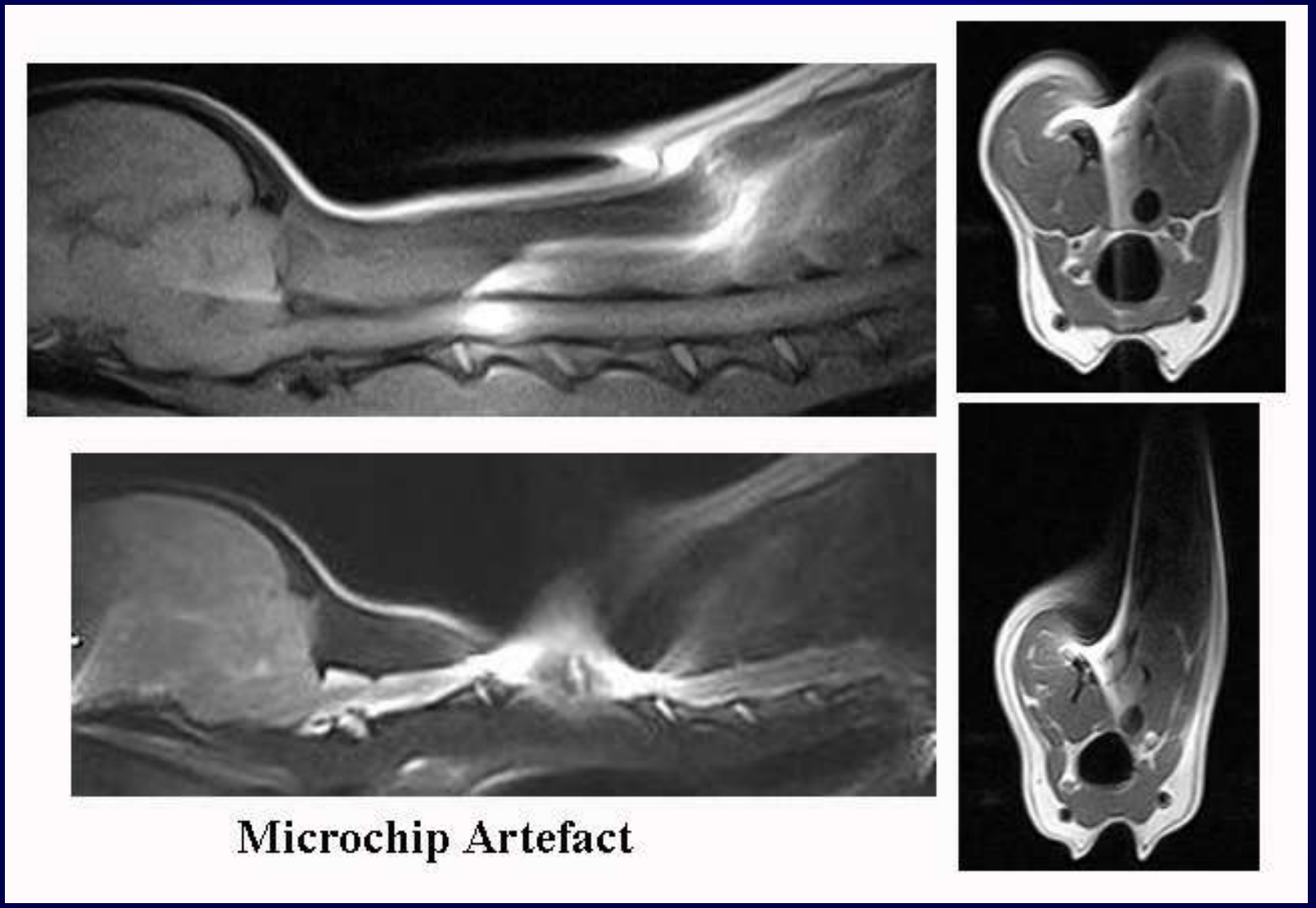




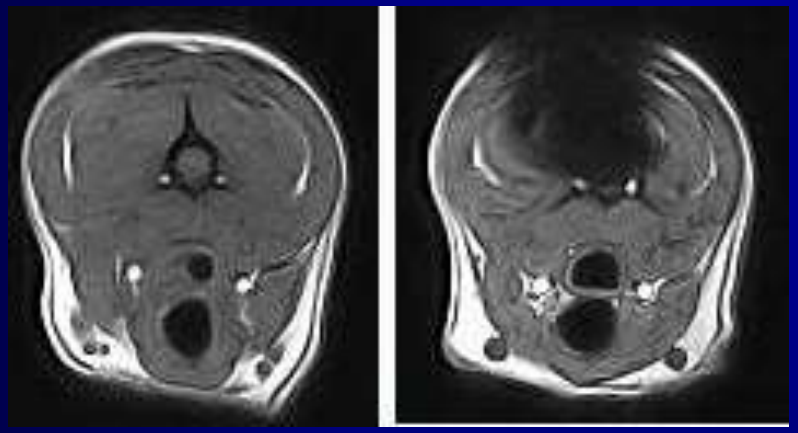
King Charles spaniel with head in extension

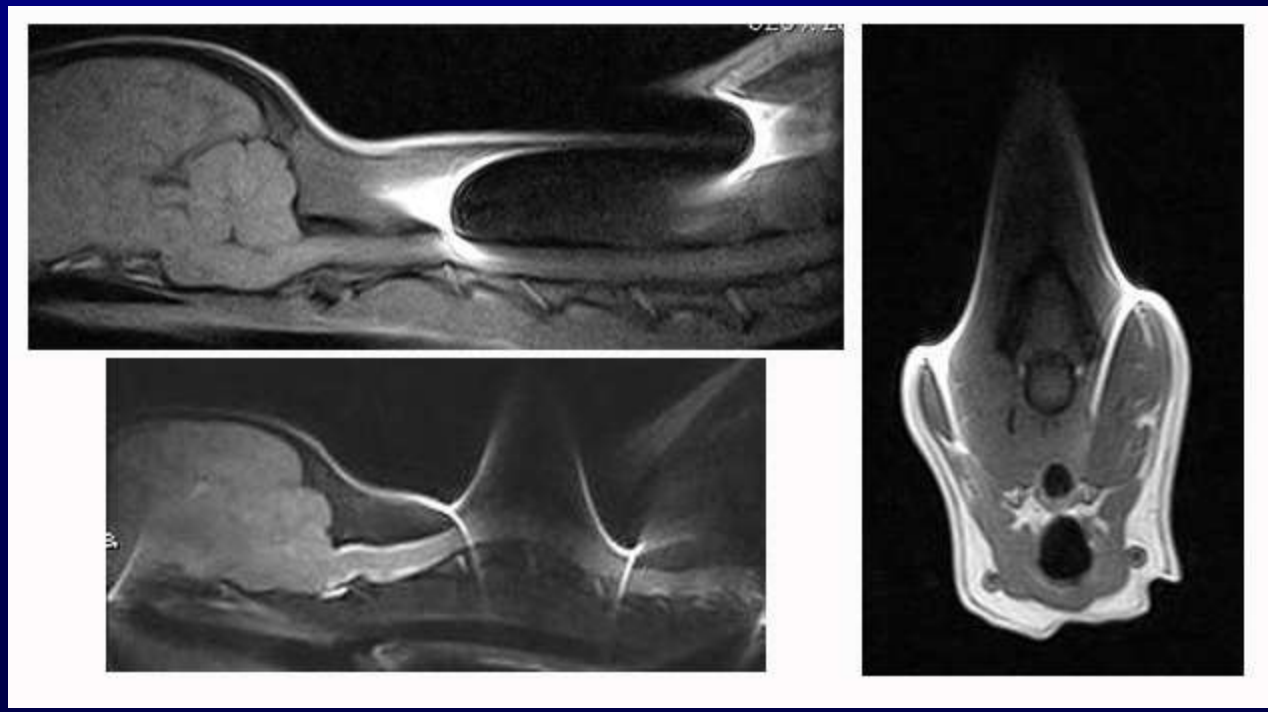
4mm slices perpendicular to the spinal cord with 1 slice though the widest part of the syrinx

Microchip artefact

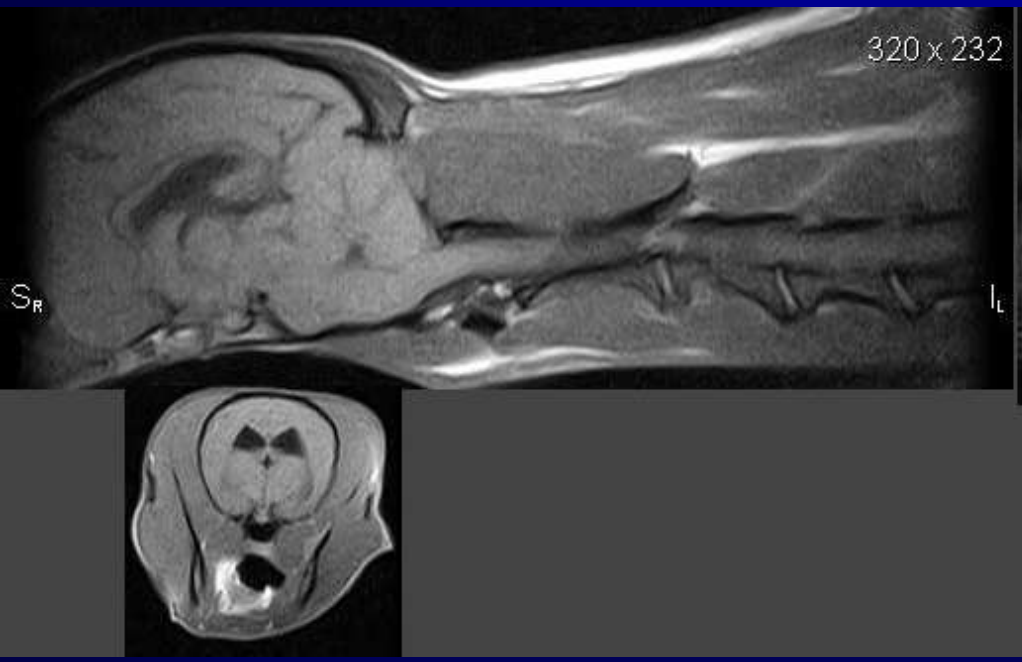


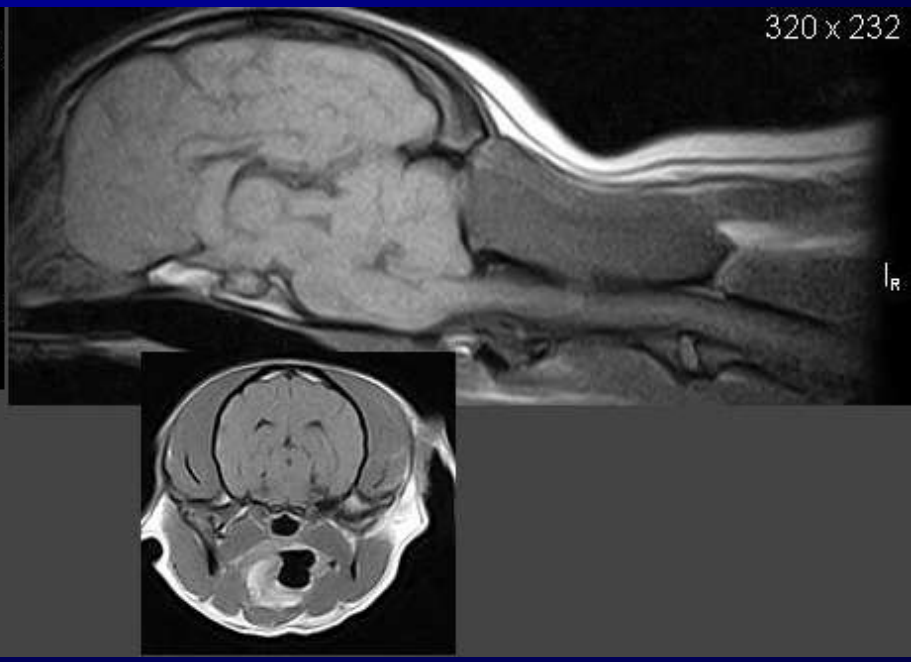
Microchip artefact





CM associated pain

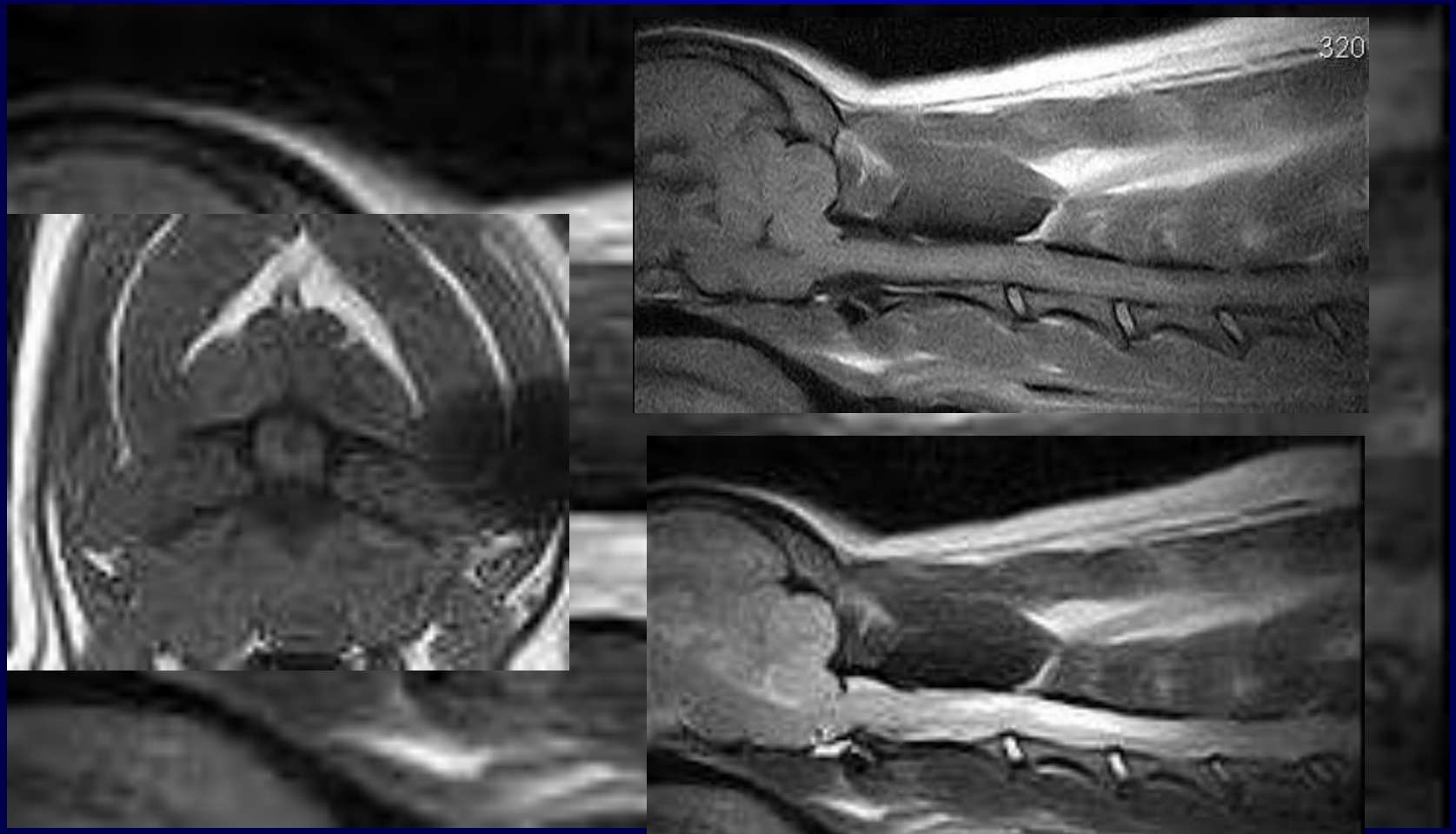




Signs of pain

No Clinical Signs

Why T2W? – confirmation suspicious lesion



Clinical signs of SM

- Not all dogs with SM have signs
- Depends on width and location of syrinx
  - Wide syrinx = pain +/- scratching
- Many dogs with SM have no / subtle signs
  - a “problem in waiting”
  - May produce offspring with SM
  - May produce offspring with pain from SM

MRI from 16 month CKCS in severe pain

Wide asymmetrical syrinx

White = fluid; light grey = spinal cord