<u>CRGV Project Update – one microscopic step....</u>

Could it be Aeromonas hydrophila?

Dr Fiona Macdonald BVMS MRCVS

Thanks to the generous support of many dog owners the investigation project which I run is progressing with some potentially interesting results.

The project consists of two main lines of investigation — a serology test which has been developed by Biobest Laboratories in Edinburgh and a lesion swab culture which Biobest organise as well to see if the causal agent is *Aeromonas hydrophila*.

Due to the sporadic nature of the syndrome, one of the challenges is in gathering sufficient cases for a detailed investigation. Sadly the majority of cases which are recognised as such very often die before there is chance of securing meaningful samples from them.

a. Lesion sampling

We have developed a strict protocol for taking lesion swabs on first presentation of a suspected case to a veterinary practice. It is very important that taking the swab is the first thing which is done when the dog is first examined, since even cleaning the wound with routine cleaning/disinfection agents are likely to kill the organism which has caused it if it is present in the lesion. Also treatment of the dog with antibiotics can kill the organism within a very short time after administration. Biobest have obtained specialist transport swabs specifically for *Aeromonas hydrophila*, and pass any enquiries they receive on to me. I send out a pack to the interested veterinarian, with swabs, information on the project, a detailed sampling protocol, appropriate consent forms, laboratory request forms and pre-paid labels to send any samples back to Biobest.

On receipt of the swabs, Biobest arrange specialist culture for the organism, with the back-up of the Veterinary Laboratories Agency (part of DEFRA) if there is any question over the exact identity of any organism recovered with DNA analysis if required.

b. Serology test

The serology test is still proving somewhat of a challenge to finally validate since there is no positive control from an animal which was known to have developed an antibody response to *Aeromonas hydrophila*. We use serum samples from other dogs which are not suspected of having contact with the organism as negative controls.

I am in the process of contacting colleagues in aquaculture to see if they have any positive fish samples since the organism does cause problems in fish and the species of the animal doesn't matter for serology as it is antibody bacterial agglutination reaction that we are measuring.

Results

Despite the limited number of cases which have come to our attention (I wrote two short communications for the Veterinary Record and the Veterinary Times last autumn to highlight

our project), we have had some interesting and encouraging results. We have had samples from around 20 dogs which out of an estimated 70 cases countrywide to date is very encouraging. However all of you can help by asking your own veterinary surgeon to contact me so I can send them a sampling pack to have in their surgery, just in case...

info@fish-treatment.co.uk

a. Lesion sampling

One dog from Oxfordshire had a positive isolation of *Aeromonas hydrophila* from a skin lesion, and we are in the process of repeat blood sampling of this dog to see what titres are present.

b. Serology

In order to try to overcome the challenge associated with not having known positive control material, Biobest are looking at repeat samples from each dog to see if there is any change in the antibody levels in the dog's serum.

General comments:-

Blood samples from each dog have been tested on receipt at the laboratory. It is now intended to run all stored samples together to rule out any variation as a result of carrying out the test at different times. We are also looking at serology from individual cases (for example the dog with the positive organism in its lesion) to see if there is a change in the antibody levels with the passage of time. We would expect a rise in these as the dog mounts a response to the organism, even in the presence of successful treatment. We are also looking retrospectively at dogs which survived the syndrome some time ago to see if they still have specific antibodies in their bloodstream, and also comparing them with in-contact dogs (from the same household or who for instance walked with them) to see what their levels are.

Since this is such a new syndrome we are trying to build up a picture to see if for example it is more widespread than first thought, but only affecting some dogs, while others develop immunity which will be constantly boosted by coming in contact with the organism.

At this point I would like to offer my sincere thanks for the support I have received from all the generous contributors (you know who you are!) with a special mention for Jessica Worthington who has raised a staggering amount of money running into thousands of pounds.

I would also like to acknowledge the professional support of Dr Susan Duthie BVMS PhD CertVR MRCVS from Biobest, and Stuart Matthews BA, MBA, MSB, CBiol. who have been great sounding boards for all of my theories and ideas!

Produced by Dr Fiona Macdonald BVMS MRCVS

for New Forest Dog Owners Group April 2016