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Haemophilic dogs at the University of North Carolina's blood-research laboratory are helping researchers to learn about the disease and develop treatments.

ANIMAL MODELS

Dogged pursuit

In the study of haemophilia, man really does have a best friend.

BY EMILY SOHN

Austin, a fluffy white-and-black Old English sheepdog, was still a puppy when his owners called the University of North Carolina's Francis Owen Blood Research Laboratory in Chapel Hill four years ago. After deciding that the children were finally old enough to get a dog, the family had quickly bonded with the rambunctious pup. But within six months of bringing Austin home, they had spent US$10,000 on veterinary bills to deal with extreme bleeding from small scrapes. Austin was also suffering from spontaneous bleeding into his joints and uncontrollable nosebleeds caused simply by overexcitement. The family loved him, but could not take care of him.

Timothy Nichols, director of the North Carolina lab, gets enquiries about haemophilic dogs from around the world four or five times a year. Sometimes he offers advice and information. Other times, he goes and gets the dog. After blood tests confirmed that Austin had haemophilia, two of Nichols' lab members flew to the family's home in New Orleans, Louisiana, where they rented a car, packed it with a cool box full of medication and drove Austin back to Chapel Hill. There, the dog joined a colony that for nearly seven decades has been quietly transforming understanding of haemophilia.

Unlike the rats favoured as animal models for many other diseases, dogs develop haemophilia naturally, have enough blood to contribute to research studies and live long enough to reveal long-term outcomes of treatments. "We have a 60-year track record now showing that if it works well in dogs, it's likely going to work well in humans," says Nichols.

LIKE HUMAN, LIKE DOG

The earliest recognized cases of haemophilia in dogs were documented in 1935 in three related Scottish terriers. About a decade later, a lawyer in New York contacted the North Carolina blood-research lab to discuss two Irish setters that were bleeding frequently, both inside and out. Already eager to acquire an animal model of haemophilia, the lab's then-director, Kenneth Brinkhous, adopted the aristocratic, long-haired dogs and began searching for breeding partners for them. Since then, colonies of haemophilic dogs have sprung up at Queen's University in Kingston, Canada; the University of Alabama at Birmingham; and Nara University in Japan. There are also a few dogs at Cornell University in Ithaca, New York. Today, these colonies breed both haemophilic and healthy dogs to maintain populations with specific variants of the disease.

It did not take long for dogs to become pivotal to scientists' understanding of the disorder in humans: the disease works in the same way in both species. Early breeding efforts in the 1940s, for example, made it clear that in dogs, the genes responsible for haemophilia lie on the X chromosome — which later proved to be true for people, too. Except in rare cases, only males get the disease; females are carriers. "The genetic and laboratory studies from breeding these dogs and testing their blood helped establish the classic parallel example of humans

and animals having the same genetic defects," says Jean Dodds, a veterinary surgeon in Santa Monica, California, who has been working with haemophilic dogs since 1959.

More recently, gene-sequencing studies have revealed that identical genes with parallel mutations account for many cases of the disease in both dogs and humans. Both species can have either haemophilia A or haemophilia B, versions of the condition caused by defects in the genes that produce the clotting proteins factor VIII and factor IX, respectively. Symptoms are remarkably similar across species: both people and dogs with the disease are unable to form clots, so cuts can bleed uncontrollably. Bleeding in the bowel can lead to diarrhoea. And lumps of blood can form in joints and muscles.

Dogs are also good models for practical reasons. Most of them are bigger than small children. They react to medicines much like humans do, allowing researchers to look to dogs first as they calculate doses. And the animals cooperate well. "The dogs here are around people all the time," says Nichols. "If you need to draw blood, they put their paws out."

DOGS FIRST

Dogs in haemophilia colonies often win researchers' hearts. Veterinary surgeon Clint Lothrop of the University of Alabama at Birmingham has adopted several from his colony, and he treats them at home when they bleed. The Queen's University dogs run, climb and play with balls and other toys every afternoon, says Queen's haematologist David Lillicrap. The North Carolina dogs have access to an outdoor play area. With severe haemophilia, animals can bleed simply from wearing collars, so handlers are careful to prevent fights or rough play.

Between play sessions, dogs give blood for research. Those donations have allowed scientists to make key discoveries about why the disease develops.

By the 1950s, researchers knew that normal blood could correct clotting defects, but they were not sure which components of blood mattered most. With the help of dog blood, Brinkhous and others deduced[1] that clotting factors were in the plasma rather than mixed in with platelets or blood cells. Giving healthy plasma to haemophilic dogs made them better. Once scientists had identified factors VIII and IX, and could distinguish between healthy and haemophilic dogs, Brinkhous and his colleagues were able to develop a test for measuring levels of the factors in plasma on the basis of how long it took for clots to form in test tubes.

In the 1940s, life expectancy for humans with haemophilia had been about 20 years, often plagued by painful bleeding into muscles and joints, says Nichols. Plasma-replacement therapy transformed the quality — and duration — of life, as did the ability to concentrate the factor in plasma, developed by the mid-1960s.

In the 1970s and early 1980s haemophilia treatment went through a dark period: contaminated plasma infected many recipients with hepatitis or HIV. Dogs helped people out of this tragic stage.

Scientists thought that they had found the light at the end of the tunnel in 1984, when the cloning of the gene for factor VIII allowed them to make artificial factor in the lab[2]. But after years of dealing with blood-borne infections and a cultural fear of such genetically modified products, it was hard to get people to try the synthetic factor. Then studies[3] in dogs showed that the treatment worked without complications, and a 43-year-old North Carolina state legislator agreed to be the first person to sign up. "He knew of Brinkhous's work and he knew of the dogs at Chapel Hill and it helped him to know that it had really helped the dogs and was safe," says Nichols.

To everyone's relief, the treatment worked. In fact, infusion of the factor was so uneventful that the recipient, known as GM, pretended to be a hamster during the procedure (the product had been produced in hamster cells). After the treatment was licensed, GM spoke at a celebration at the Genetics Institute in Cambridge, Massachusetts. "After slowly and painfully climbing to a balcony half way up the stairs, he delivered a powerful story about what it was like to grow up with hemophilia without adequate treatment, how as a child he had lost a beloved older brother from a bleed, and how important the development of safe recombinant factors was to him and all people with hemophilia," wrote Gilbert White, director of the Blood Research Institute at the BloodCenter of Wisconsin in Milwaukee, in a paper[4] describing 35 years of advances in haemophilia research. "His comments had the entire company in tears."

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POINTING THE WAY

Research in canines often foreshadows what is coming for humans. Over the years, more than 25 products that had been tested in dogs have been licensed for clinical use in people. One of the first studies to show the feasibility of gene therapy[5], published in 1993, involved three factor-IX-deficient dogs and an extremely invasive procedure, in which researchers removed two-thirds of each dog's liver. Over the course of three days, they injected the regenerating organs with a potentially dangerous HIV-like virus loaded with the healthy gene. The procedure boosted levels of factor IX from zero to 1% of normal — enough to fuel optimism that a more efficient procedure might one day be possible.

By 1999, dog studies[6] began to show that one injection with a much safer vector called an adeno-associated virus could deliver a healthy factor IX gene, boosting levels of the clotting factor to 2% — enough to reduce spontaneous bleeds. "We were able to move past that rapidly and have had levels of 10% for a long time," says Katherine High, a haematologist at the Perelman School of Medicine at the University of Pennsylvania in Philadelphia. Dogs can now get simple, 10- to 15-minute infusions of factor-bearing viral vectors. Similar work with factor VIII is close behind, says Nichols.

Some of the first dogs to receive factor IX gene therapy with just a single injection have lived full and happy lives. Brad and Semi were two basenjis — African hunting dogs — who lived in the Alabama colony. After receiving the treatment, one died at 13, the other at 14, neither from haemophilia-related causes. Several clinical trials are now assessing gene therapy with factor IX in humans (see page S160).

Other studies are testing the possibility of administering factors VIII and IX orally instead of with an injection — a technique that has been shown to work in mice and is now being tested in dogs. And ongoing work by Lothrop and his colleagues suggests that replacement factors might become available as longer-lasting, less-invasive subcutaneous shots instead of intravenous injections.

Dogs are also helping scientists to develop strategies for combating the inhibitor antibodies that many patients develop in response to factor-replacement therapy. One approach[7] gives dogs a gene to express another clotting factor, factor VIIa, completely bypassing the need for factors VIII and IX. The technique can reduce the number of bad bleeds each year from five or ten to one or even none.

In other lines of work, dogs have undergone bone-marrow transplants to express factor VIII in their platelets, shielding them from inhibitors. And Nichols' team has acquired a strain of dogs deficient in clotting factor VII, allowing it to test therapies for rare bleeding disorders that may not occur in enough humans to allow large clinical trials.

It is unlikely that any of these next-generation approaches would have been possible without canine models. "The role of haemophilic dogs in the preclinical development of novel therapies for haemophilia during the past three decades has been enormous," says Lillicrap. The disease once seemed insurmountable, but in the years ahead, he says, dogs will continue to provide insights that will make life better for humans. ■

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