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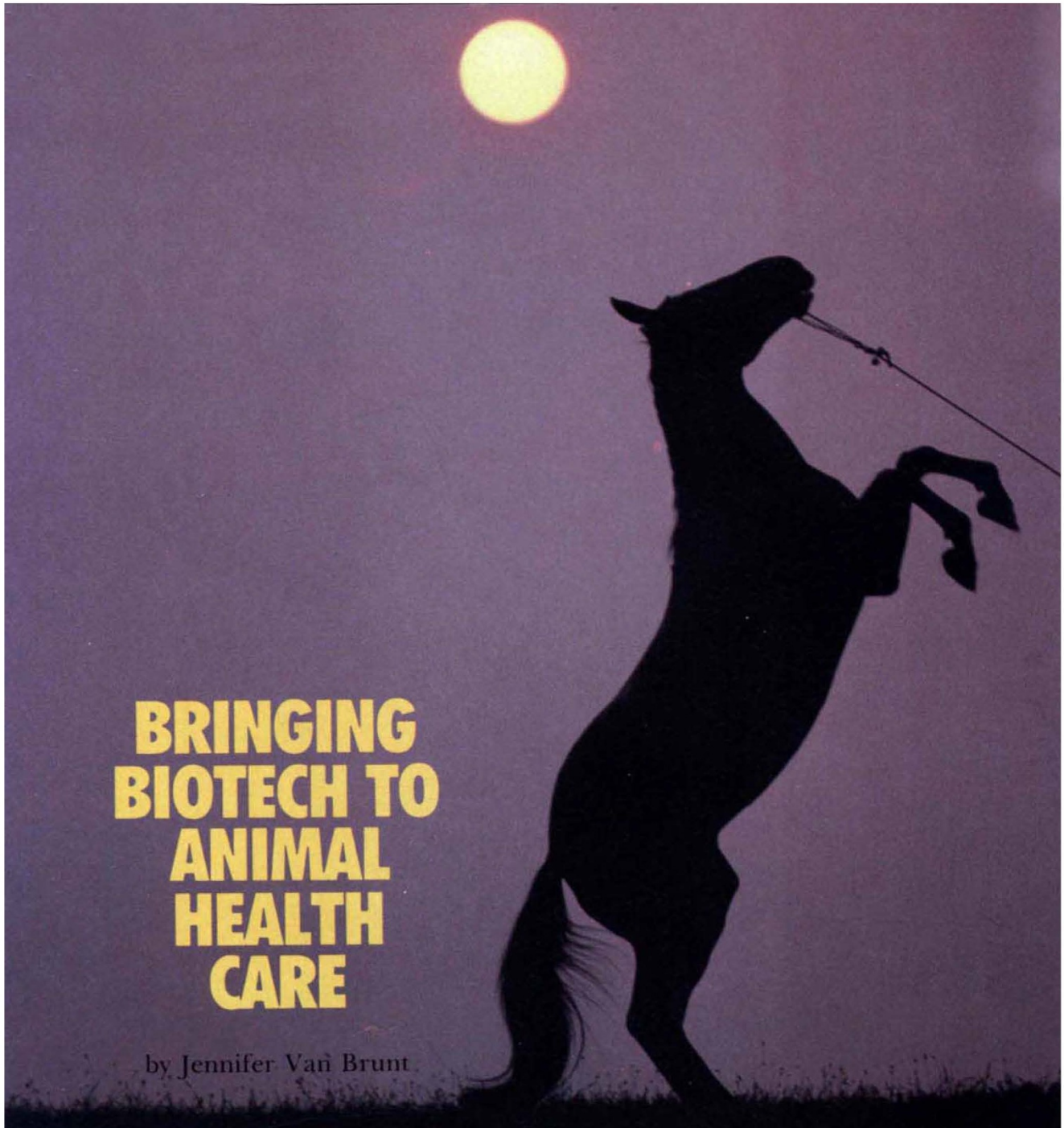

BRINGING BIOTECH TO ANIMAL HEALTH CARE

by Jennifer Van Brunt

RANDY DUCHAINE/THE STOCK MARKET OF N.Y.

The market for veterinary drugs and diagnostics is enormous. However, the profit on a product intended for large-animal use (about five percent) is much lower than for a complementary human pharmaceutical (15–20 percent). And biotech products must compete in this arena with conventional veterinary products, which are already inexpensive. In fact, studies show that a livestock farmer will only pay 10 percent of an animal's worth to keep it healthy. For race horses and companion animals (cats and dogs), the profits are better: Owners are usually willing to invest substantial sums of money to keep their pets and prize-winners healthy.

Because profits for veterinary products are lower than for human ones, and because some vaccines have been harder to develop than first anticipated, many of the biotech companies that first identified veterinary health care as a business objective have adjusted their sights. Other companies, however, have leapt to fill the void and now offer a wide variety of diagnostic tests, vaccines, and immune stimulators.

Pseudorabies Vaccine

Pseudorabies is a particularly insid-

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Norden Labs' brochure on feline leukemia, distributed to veterinarians' offices, answers questions about the disease, its symptoms, and its course of infection.

ious herpes virus that infects swine—it is often lethal to newborns and piglets. The virus infects nerve cells, where it becomes integrated into the genome and remains quiescent until it reverts, causing epidemics. Latency makes the virus difficult to detect. According to Roland Hendrickson, chairman of the Animal Health Institute (Alexandria, VA), "In my home state of Minnesota, animal health officials say pseudorabies is costing hog farmers there $1 million a month. Minnesota has four million hogs. Iowa has nearly 13 million. Nationally, it's estimated that about 10 percent of the country's 54 million swine are infected with pseudorabies virus. The annual cost to pork producers may be as high as $60 million."

The conventional pseudorabies vaccine consists of the killed virus; this gives only partial protection, however. A vaccine made from the live virus is more efficacious, but inoculated animals can still show disease symptoms.

"Omnivac," the first genetically engineered vaccine for pseudorabies, was introduced in 1986—accompanied by more fanfare than was expected. The vaccine became a cause célèbre for Jeremy Rifkin (Foundation on Economic Trends, Washington, D.C.), who advised the director of the National Institutes of Health (NIH) that the U.S. Department of Agriculture (USDA) had granted approval for the vaccine's use without complying with NIH's guidelines. The vaccine was temporarily pulled from the market while USDA performed an environmental impact assessment—which concluded that the use of the recombinant virus presented virtually no danger to humans or the environment.

Saul Kit, the inventor of the vaccine, had deleted the virus' gene for thymidine kinase, the enzyme that allows the virus to escape from nervous tissue. This virus is less virulent than the wild type, and there are no latency problems.

Omnivac is sold by The Biologics Corp. (Omaha, NE), a division of TechAmerica Group (Elwood, KS), which produces the vaccine under license from Kit's company NovaGene (Houston, TX).

The next genetically engineered, modified live pseudorabies vaccine likely to gain USDA approval will come with its own diagnostic kit, as well. Jim MacIsaac, vice president of marketing at Syntro Animal Health (Lenexa, KS), explains that the diagnostic will differentiate vaccinated animals from those that are infected by wild type viruses. The vaccine is currently in registration with USDA; MacIsaac expects product introduction by the end of this year. He claims that Syntro's vaccine is "similar to but different from" Omnivac and other vaccines under development.

Molecular Genetics (Minnetonka, MN) also expects regulatory approval in 1987 for a family of products to detect and prevent pseudorabies infection: "BreakStop" subunit vaccine, "LookOut" immuno-diagnostic test, and "LifeStart" monoclonal antibody for protecting newborns. These products, which have been in USDA registration for two years, were among the survivors of the company's recent revision of its infectious disease biologicals program.

Scientists at Applied bioTechnology (Cambridge, MA), a company formed by Robert Weinberg (and others) to commercialize this MIT scientist's cancer gene research, have used a different approach to vaccine development. Dennis Panicali, the vice president of R&D, explains that the researchers use a vaccinia virus vector, engineered to contain the sequences for several major glycoproteins of the pseudorabies virus. Panicali says the vaccine is highly efficacious in murine challenge studies, and large animal studies will begin soon. Panicali stresses that the scientists have devoted considerable time to developing the most attenuated—and host-range-limited—vaccinia vector possible. Vaccinia's broad host range, which is of considerable advantage in producing a vaccine, is of considerable *dis*advantage in satisfying regulatory agencies. Panacali adds that Applied bioTechnology's pseudorabies vaccinia vaccine, which should be in federal agency review late this year, will be vaccinia's "trial balloon."

FeLV Vaccines and Diagnostics

Feline leukemia virus (FeLV) was virtually unknown 23 years ago; today it is *the* feline killer, infecting more than 1.5 million cats in the United States alone.

The similarities between FeLV and HIV (the AIDS virus) are striking: FeLV is transmitted via saliva directly from cat to cat, and it causes an array of symptoms termed feline acquired immunodeficiency syndrome—including leukemia, lymphosarcoma, chronic anemia, pneumonia, and kidney failure. Moreover, conventional viral vaccines are ineffective against FeLV: in fact live or killed viruses usually end up promoting the disease.

Thus a subunit vaccine is the way to go. The first of these, Leukocell, was introduced by Norden Labs (Lincoln,

VACCINES AND THEIR DEVELOPERS

Avian:	Coccidiosis	Genex and A.H. Robins
	Coccidiosis	Codon and Salsbury Labs
	Newcastle Virus	Codon
Bovine:	Papilloma Virus	Molecular Genetics
	Viral Diarrhea	California Biotechnology
	Brucellosis	Ribi ImmunoChem
	Rinderpest	USDA and University of California, Davis
	Foot and Mouth	USDA and Genentech
	Rift Valley Fever	Molecular Genetics
Swine:	Parvovirus	Applied bioTechnology
	Dysentery	Codon
Equine:	Influenza	California Biotechnology
	Herpes	Applied bioTechnology
Companion:	Canine Parvovirus	Applied bioTechnology
	Feline Panleukopenia	Applied bioTechnology
	Antifertility	California Biotechnology

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Immune system modulators can be extremely effective in treating disease. The equine sarcoid tumor (*top*) was injected with the general immune stimulator Ribigen. Six months later (*bottom*), the tumor was gone.

NE), a subsidiary of SmithKline Beckman (Philadelphia, PA). Codon (Brisbane, CA) is currently developing a subunit vaccine, as well. Joel Kirschbaum, director of R&D, says the FeLV vaccine consists of the virus' major envelope protein.

Cambridge BioScience (Hopkinton, MA), which is also developing an FeLV vaccine, has already launched a diagnostic test. It is marketed by Norden under the trade name ClinEase. Diagnosis is important because vaccination is of no value in cats that have already been infected with the virus.

Synbiotics (San Diego, CA) markets two diagnostic tests for feline leukemia: "Virachek," an enzyme linked immunosorbent assay (ELISA) that detects the FeLV core protein (p27) in whole blood, and "Virastat/FeLV," which uses a proprietary technology to test saliva. The company claims that this second test can determine whether a cat is shedding the virus, and is therefore infective. Edward Maggio, Synbiotics' president, says this technology can be applied more broadly—for the noninvasive detection of retroviruses in saliva.

Immune System Modulators

Perhaps the most successful application of interferon will be in fighting animal diseases rather than human ones. As a general immune stimulator, interferon shows some promise in treating bovine shipping fever, a complex of respiratory infections brought on by co-mingling, crowding, and the stress experienced by cattle when they are transported *en masse*.

Amgen (Thousand Oaks, CA) has conducted extensive field trials on cattle using its consensus interferon (a synthesized molecule containing the predominant features of 13 natural interferons). Philip Whitcome, Amgen's director of strategic planning, says that results from the preliminary trials were encouraging, but inconclusive. He admits that the company has pulled back from these experiments, as more and more of its human pharmaceuticals reach clinical trials. This does not mean that Amgen has dropped the project; Whitcome explains that it is just not a priority at this time.

Agracetus (Middleton, WI), the joint venture between Cetus Corp. (Emeryville, CA) and W. R. Grace (New York, NY), has assessed interleukin-2's (IL-2) efficacy in treating shipping fever. Large-scale trials in feedlots were conducted in 1986. Winston Brill, Agracetus' vice president for R&D, says that there is definitely evidence that IL-2 is useful for treating shipping fever.

Immuno Modulators Labs (Houston, TX) has taken a broader approach to treating shipping fever: the company uses a lymphokine preparation, isolated from human leukocytes. The preparation—dubbed Agriferon A—contains interferon, of course, as well as other naturally occurring lymphokines. It is the interferon, though, according to Jerzy Georgiades, vice president of research, that is responsible for the preparation's effectiveness in preventing or modulating shipping fever. He emphasizes that it is imperative to administer the compound orally—not intramuscularly, intravenously, subcutaneously, or nasally. Not only does the interferon act as an anti-viral agent, Georgiades says, but it also stimulates the animal's hunger center, inducing sick animals to take in the nutrients that will aid them in recovering.

Biotech Research Labs (BTRL, Rockville, MD) has just introduced "Foalaid," a product to stimulate the immune system in newborn foals. Foalaid consists of equine immunoglobulins and a "promoter." Ray Mifflin, the developer of Foalaid and BTRL's director of equine veterinary services, says that foals are born immunoincompetent, and are at some risk for the first few weeks of life: This is especially true if the mare makes antibodies to the foal. The foal doesn't start to make its own antibodies until 4–6 weeks after birth. Foalaid, which is isolated from the blood of a hyperimmunized herd, according to Mifflin, promotes the transfer of antibodies from the mother's colostrum across the baby's gut wall. Mifflin concludes that administering Foalaid will assure the foal's immunity. The company will market a companion product, "Immuno-stik," to measure the foal's total antibody levels.

Ribi ImmunoChem (Hamilton, MT) has long recognized the value of overall immune system stimulation. The company's biological response modifiers consist of detoxified bacterial endotoxin and bacterial cell wall skeletons in an oil-in-water emulsion. The adjuvants, which are now being investigated for their effectiveness in human cancer therapies, have already shown their power in animal systems. "Ribigen," the company's first product (released in 1983), is used to treat sarcoid tumors in horses and eye tumors in cattle. The compound is injected directly into the tumor: Within weeks to months the tumor is gone.

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Filter immunoassay. A blood sample and reagents are filtered through a membrane that contains three calibration standards. The sample spot (top) is read against the calibration spots.

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

USDA scientists, as part of their effort to develop a multistrain vaccine for bluetongue virus (which is spread by a biting gnat), place a container filled with insects on a sheep's abdomen. The feeding insects will either transmit or become infected with the virus.

Nils Ribi, president and chief operating officer, says that company scientists have tried Ribigen in dogs, but they just didn't get good response rates. The product has been reformulated, and preliminary tests have been run, but, Ribi says, it is not an active pursuit at the moment.

Yet another approach to immune system stimulation has been taken by Neogen (East Lansing, MI). The company uses equine mixed leukokines (EML), isolated from equine blood cells, as a booster for current vaccines. Brinton Miller, vice president of research, explains that, when the company was first formed, one objective was to develop equine and bovine interferons from blood cells rather than via rDNA techniques. In 1984, when it became clear that all interferons were to be classed as drugs (with the development price tag running in the millions of dollars), the company turned its attentions to mixed leukokines instead.

Miller says the equine mixed leukokines (trademark Immunostim) are effective in boosting the efficacy of canine parvovirus vaccine. Parvovirus, a fatal intestinal disease, is widespread throught the United States. Dogs are routinely vaccinated, but, as Miller explains, it is tricky to find the *right* time to vaccinate puppies. Puppies are protected by their mothers' antibodies for their first 4–6 weeks. Although the titer then starts to decrease, there is still enough residual activity to prevent a modified live vaccine from being effective. And, Miller adds, if the dam had high antibody titers, the pup might not be vaccinatible until it is 21 weeks old. During this interim, the pup is at risk. When Immunostim is added to the vaccine, however, dogs respond 2–4 weeks earlier, with an increased titer that holds longer.

Neogen also markets two diagnostics for parvovirus: One measures the antibody titer of the dam and the pup; the other determines whether the pup is infected with the virus.

Miller adds that EML seems to stimulate the titer for certain bovine viruses, as well, but it does not work with all viruses. Live or highly infectious viruses—such as distemper—don't need any help, he concludes.

Monoclonals for Therapy and Diagnosis

The application of monoclonal antibodies for large-animal therapy has been limited. To date, the one product that stands out is "Genecol 99," Molecular Genetics' monoclonal against calf scours—a bacterial infection that causes diarrhea, dehydration, and death. Genecol 99, the first genetically engineered monoclonal for use in livestock approved by USDA, works by preventing *Escherichia coli* from adhering to the intestinal wall. ("Coli Tect 99" is the companion diagnostic test.) Molecular Genetics is currently developing a therapeutic monoclonal for *E. coli* mastitis in dairy cows, part of its agreement with the Kodak's Eastman Chemicals division (Kingsport, TN).

One of the major problems in treating animal disease has always been to identify the causitive agent soon enough—say, before half the herd is lost. The new generation of diagnostics addresses this need. Simple, reliable, rapid ELISAs are quickly replacing the old indirect immunofluorescent assays.

Diagnostic tests are either available or under development for brucellosis, equine infectious anemia, scours, trichinosis, blue tongue in cattle, canine distemper, feline infectious peritonitis, mastitis, canine heartworm, canine rheumatoid arthritis, and general serum immunoglobulin levels in the cow and horse.

Agritech Systems (Portland, ME) has several dozen veterinary diagnostic products. "FlockChek," for instance, is a computerized immunoassay that monitors the health of flocks of chickens. David Shaw, Agritech's president, says that a flock is routinely monitored every few months by statistical sampling; the assay includes about a dozen common analytes, for the major poultry pathogens.

On the smaller end of the scale, Agritech also markets assays that can be used for a single sample. These are membrane filter immunoassays; each filter contains a positive and negative control as well as the immobilized ligand. The tests for canine heartworm and feline leukemia give a simple "yes-or-no" answer. Shaw adds that Agritech's test for immune deficiency in foals is more quantitative; the filter contains several different reference spots which change color simultaneously with the sample.

Another large market for diagnostics is to assess ovulation and pregnancy in horses and cows. American Diagnostic Sales (Westport, CT, a coventure between University Genetics and International Embryos PLC) markets "Equicheck" (a progesterone test for mares that can determine if the female is in heat or pregnant), "Calfcheck" and "Heifercheck" (which measure progesterone levels to determine estrus or pregnancy), and "Calfcheck Confirm" (a late pregnancy test that checks for estrone sulfate, a hormone secreted by a live fetus). Synbiotics is also developing a bovine fertility test kit. And Monoclonal Antibodies (Mountain View, CA) has developed a pregnancy test for horses, "Marechek." This is the first of the company's diagnostics for the breeding management of horses and cattle.

There *are* drawbacks to ELISAs, however: they cannot distinguish between antibodies generated by a disease and those generated by a vaccine. And, veterinarians often feel uncomfortable running the assays themselves—even though they are being marketed as simple-to-use. Because the assays may display both false negatives and false positives, many vets fall back on the expertise of a diagnostic lab. The advent of second- and third-generation assays, however, should bring more tests directly to the animal's side. ◪

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