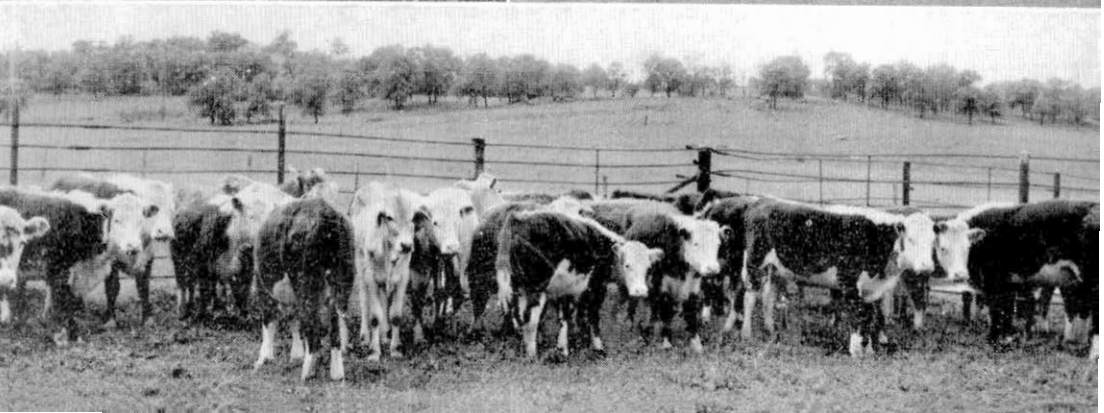
ANAPLASMOSIS in Oklahoma Cattle

Exp. Sta. Bul. No. B-323
September 1948

Oklahoma Agricultural
Experiment Station
and
Oklahoma Veterinary
Research Institute

LEFT: Best way now known to help prevent spread of **anaplasmosis** is careful sterilization of instruments between each animal in operations drawing **blood.**

BELOW: Some of the calves being used in research aimed at finding vaccines that will give cattle immunity.



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OKLAHOMA AGRICULTURAL EXPERIMENT STATION

Oklahoma A. and M. College, Stillwater

W. L. BLIZZARD, Director **LOUIS E. HAWKINS, Vice Director**

and

OKLAHOMA VETERINARY RESEARCH INSTITUTE

Herman Farley, Executive Director

ANAPLASMOSIS
in Oklahoma Cattle

By HERMAN FARLEY, LON E. FOOTE, C. C. PEARSON
and IRA OLIN KLIEWER*

Anaplasmosis, a blood disease of cattle, caused an annual loss to Oklahoma cattlemen for twenty-five years or longer. This disease kills approximately thirty percent of adult cattle that become infected. Death loss is less in young cattle, but is considerable even in this age group.

Oklahoma cattlemen estimate that losses from anaplasmosis in this State probably exceed $1,000,000 annually. Losses to cattlemen over the entire United States would possibly be in excess of $4,000,000 to $6,000,000 each year.

Because of the losses attributed to anaplasmosis, cattlemen in Osage County and other parts of Oklahoma were responsible for having the 1945 Oklahoma Legislature make a special appropriation of $100,000 to start research studies on this and other infectious diseases of livestock. The Oklahoma Veterinary Research Institute was organized to make these studies, and extensive working facilities were set up.

This bulletin describes progress to date, including a summary of earlier work done by the Oklahoma Agricultural Experiment Station in cooperation with the Bureau of Animal Industry of the United States Department of Agriculture. It also gives a general description of the disease, tells what is now known about ways of reducing losses, and indicates what is being done by the Veterinary Research Institute to find more effective ways of controlling anaplasmosis.**

Anaplasmosis has been found in a majority of the counties in Oklahoma, and is known to exist in at least 29 other states (See map, page 6). The Oklahoma counties where the disease has not been proved are in the west and northwest part of the State. However, anaplasmosis can be expected to develop in any part of the State at any time. Until definite means can be established for recognizing "carrier" cattle and a reliable vaccine can be produced, it will be necessary to continue research studies in an effort to find a satisfactory means of control.

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* Respectively, Executive Director, Associate Veterinarians, and Laboratory Technician, Oklahoma Veterinary Research Institute. The research reported in this bulletin was supported entirely by funds provided by the Oklahoma Agricultural Experiment Station.

** Additional details for the information of veterinarians and research workers are being prepared for publication as an Oklahoma Agricultural Experiment Station Technical Bulletin under the title "Anaplasmosis in Cattle."

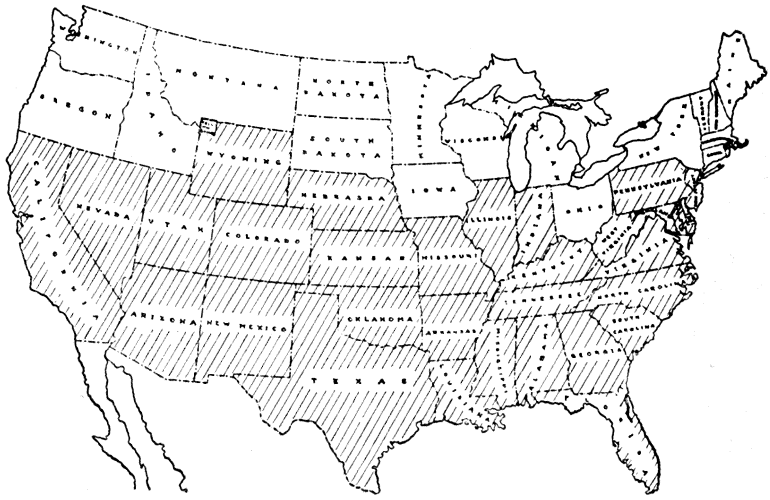
WHAT ANAPLASMOSIS IS

Anaplasmosis is a blood disease of cattle caused by the minute blood parasite *Anaplasma marginale.* It attacks and destroys red blood cells in cattle and produces a severe anemia. The *Anaplasma marginale* is too small to be seen without a microscope. It is transmitted from diseased to healthy cattle under natural conditions by bites of blood sucking insects. *Man is also responsible for causing outbreaks of the disease by careless use of surgical instruments and and hypodermic needles when dehorning, castrating, ear marking, and vaccinating cattle.*

Anaplasmosis exists in four forms: (1) mild; (2) acute; (3) peracute; and (4) chronic.

1. MILD INFECTION is confined to calves under 5 months of age. The diseased animal will probably not be noticed by even the most careful observer, because the calf seldom shows much evidence of sickness. In most instances it will be necessary to have a laboratory technician examine blood smears and find the disease producing organism (*Anaplasma marginale*).

2. ACUTE anaplasmosis infection is the type commonly observed on the farm and ranch. It is found principally among cattle nine months of age or older. The diseased animal loses appetite, and



Where Anaplasmosis Has Been Reported in the United States.
Cases of anaplasmosis have been reported from at least 29 of the 48 states, as shown by the cross-hatching on the above map. Oklahoma cattlemen have estimated that losses in this State alone run upward of one million dollars a year.

A Brief History of Anaplasmosis

The first evidence of anaplasmosis in the United States was in 1893. Research workers who were studying Texas fever (*Piroplasmosis*) noticed bodies in red blood cells that were not typical for this disease, but they were not recognized at the time as the cause of a separate disease of cattle. In 1910 it was proved that anaplasmosis was a definite and distinct disease of cattle in South Africa. However, it was not diagnosed in its pure form in this country until 1926, when a veterinary practitioner, Dr. D. P. Darlington of Chanute, Kan., diagnosed anaplasmosis in herds in southeast Kansas. Soon after Darlington's discovery the disease was diagnosed in California, Louisiana, Arkansas, Texas, Florida, Mississippi, Oklahoma, Missouri, and a few other states. The disease is now known to exist in at least 29 states, and it will probably continue to spread to additional states until definite means of control are developed and put into use.

The first cases of anaplasmosis in Oklahoma were observed by Dr. George W. Stiles in 1927. Dr. Stiles at that time was employed by the Bureau of Animal Industry of the U. S. Department of Agriculture and was working cooperatively with the Oklahoma Agricultural Experiment Station.

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there is an increase in temperature, pulse rate, and respiration. The animal has a weak, wobbly gait and tends to lag behind the herd or lie down often. Anemia is always present; and in some cases (icterus) there is a yellow discoloration around the muzzle, eyes, tailhead, and inner surface of the thighs. An examination of prepared blood smears by a trained observer will usually show great numbers of *Anaplasma marginale* in the red blood cells of the sick animal.

3. PERACUTE infection is not as common as acute or mild types of the disease. It attacks principally milk cows that are high producers. However, it has been found in beef cattle. Usually the animal is dead when found or it dies soon afterwards. There is a severe anemia or bleaching of membranes around nose, eyes, teats, and tailhead. Occasionally a yellow discoloration is also observed in these cases. The death rate runs from 50 to 100 percent in this type of infection.

A Typical Case of Acute Anaplasmosis.

This animal at the time the picture was taken had a temperature of 104.5 degrees Fahrenheit, pulse of 108, respiration of 55, hemoglobin 45% (Talq.), and red blood count of 2,500,000. Outward symptoms of anaplasmosis include anemia, depression, loss of appetite, cessation in rumination, and muscular weakness.

4. CHRONIC infection is long lasting, the animal lingering for weeks or months before dying, or making a recovery. A yellow discoloration is always noticed, and the animal is thin. It is thought that chronic infection is more common among range animals, that are compelled to follow the herd and make their own way, than it is among dairy or feed lot animals.

PREVENTIVE METHODS

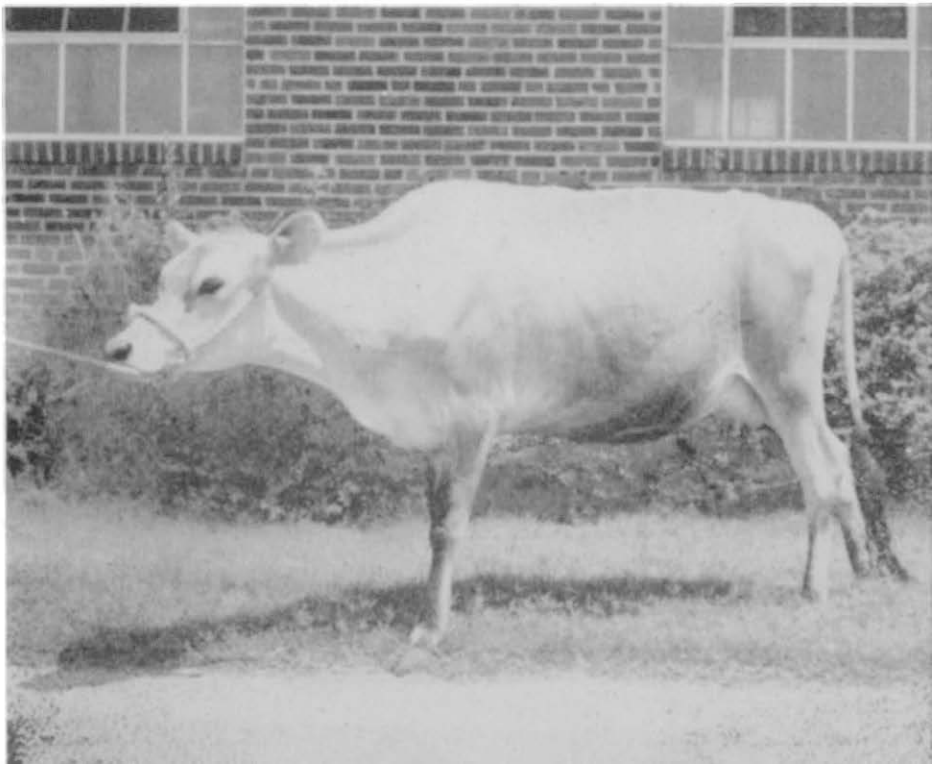
Cattle which recover from any of the four types of anaplasmosis mentioned above retain "carrier" infection in their blood for an indefinite period, perhaps for the remainder of their lives. Such animals are healthy in appearance and are readily accepted for food by the packers, but if left in a herd are a continuous source of danger to normal or susceptible cattle. *Anything that transfers blood from one of these carrier animals to a healthy or susceptible animal can transmit the disease.* It is known that certain biting insects transmit anaplasmosis, and instruments used in such operations as dehorning, castrating and vaccinating also help spread the disease when not properly sterilized.

"Carriers" Are Hard to Identify

It is not unusual for a herd to include from one to several innocent looking "carrier" calves. The only means at present known for detecting carrier infection in cattle is too expensive for general use. It involves taking a blood sample from the suspected animal and injecting it into a cow, steer, heifer or bull that has not had the disease. Thus the sacrifice of an expensive animal is necessary to complete the test.

A diagnostic test to detect carrier infection would be a forward step in the control of anaplasmosis, and an active search for such a test is being made by the Oklahoma Veterinary Research Institute. Until such a test is available, other means must be used to prevent spread of the disease.

A Three-year-old Jersey With Chronic Anaplasmosis.
Three months after this animal had acute anaplasmosis, the following clinical symptoms were evidence of chronic anaplasmosis: Anemia icterus, hemoglobin 6.5 grams per 100 c.c. of blood, red blood count three million per cubic millimeter, and from 6 to 10 percent of the red blood cells showing anaplasma bodies. The cow had poor appetite and showed muscular weakness.



Sterilization of Instruments Important

Mechanical transmission by man is probably responsible for a greater number of outbreaks of anaplasmosis than is generally suspected. In fact, it may be the most common means of spreading the disease. Dehorning shears, knives, saws, hypodermic needles, ear notchers or tattoo instruments are the usual means for transmitting anaplasmosis from the carrier or otherwise infected animal to susceptible cattle. Many outbreaks can be attributed to the individual who vaccinates his herd for blackleg, "shipping fever," or Bangs disease without observing necessary precautionary measures.

The instruments used in operations on cattle should be disinfected between each animal in order to eliminate the possibility of carrying diseased blood from one animal to another. It is recommended that syringes, needles, castrating instruments and other materials capable of drawing blood be disinfected between each operation. Instruments can be disinfected by boiling, or by washing in 5 percent creolin or other reliable disinfectants and then using a brush or cloth to remove blood cells.

Control of Insects Would Help

It has already been proved that certain species of horse flies (Tabanidae), ticks, stable flies, and at least two species of mosquito will transmit anaplasmosis from carrier or diseased animals to healthy ones. Entomologists and veterinarians all over the country are seeking sprays or dips that will prevent these insects and ticks from feeding upon cattle and thereby spreading anaplasmosis infection. But up to now no effective poison has been found.

It is likely that other insects are also busy transmitting this disease, but that has not been definitely proved as yet.

Investigators at the Oklahoma Agricultural Experiment Station started working on insect vectors and other possible means of transmission soon after anaplasmosis was diagnosed in the State in 1927. Early transmission studies were under the supervision of the late Prof. C. E. Sanborn, who was then head of the Department of Entomology. He and his co-workers were the first to prove that horse flies (Tabanidae) would transmit anaplasmosis. Some species of ticks had already been proved transmitters. Professor Sanborn also proved additional ticks as transmitters of anaplasmosis, and suggested the possibilities of other biting insects as transmitters of the disease. Later, Dr. D. E. Howell of the Oklahoma Station entomology staff, proved a number of additional species of flies (Tabanidae), ticks and mosquitoes as transmitters of anaplasmosis. Dr.

Howell is at present in charge of further studies at this Station on the vector control problem as it pertains to the control of anaplasmosis.

In all, nineteen species of ticks, nine species of horse flies (Tabanidae), the stable fly (*Stomoxys calcitrans*), and two species of mosquitoes have been proved transmitters of anaplasmosis by in-

This Carrier Animal Looks Healthy, But . . . .

This yearling calf was made a carrier of anaplasmosis when two months old. There was very little change in the blood picture during the course of the disease. Such a light case is common with younger animals. As a result, they often escape notice and remain in the herd as carriers. This animal is now used as a source of anaplasma infected blood needed in research.

Headquarters of Veterinary Research Ranch.

For study of anaplasmosis and other livestock diseases under range conditions, a 900-acre ranch is operated in connection with the Institute's field laboratory at Pawhuska. The ranch headquarters is equipped with barns, feed bins, corrals, and other usual equipment for handling cattle.

vestigators working in various parts of the world. Transmission studies are well in advance of other phases of study such as diagnosis, treatment, and prevention.

TREATMENT

In addition to seeking methods of preventing outbreaks of anaplasmosis, the Oklahoma Veterinary Research Institute is testing drugs in an effort to find one or more which will help cure animals after they become ill. At least two of the 15 tested since 1946 show some promise, although further testing is needed.

All cattle which recovered continued to retain "carrier" infection, regardless of the type of drug used in treating the active stage of the disease.

*S. N. 7618-5,** an antimalarial, has given the most satisfactory results and is apparently the best drug used to date. It has been used on 28 field cases and 12 laboratory cases. Approximately 85 percent of the diseased animals treated made a recovery. Most cases were treated late in the course of the disease without affecting the recovery rate. Treatment has been by the intravenous method. Most cases were not treated until the total red blood cells had been reduced to half or less.

PALUDRINE HYDROCHLORIDE, an animalarial, was administered to 17 cows affected with the acute type of the disease. Four died. Two of the four died while fighting restraint. This drug proved highly satisfactory when given in the feed or when administered intravenously. Four cows treated intravenously with Paludrine made a rapid recovery. The drug apparently acts rapidly when administered in this manner. Recoveries were approximately 85 percent in all cases treated.

* 7-chloro-4-(4'-diethylamino-1'-methylbutylamino)-quinoline diphosphate.

Disinfecting Instruments Helps Prevent Anaplasmosis.

A ranch worker here shows how instruments used in dehorning, castrating, and vaccinating should be cleaned to avoid carrying anaplasmosis from one animal to another in the blood. Between each animal, instruments are first washed in a 5 percent solution of creolin and then washed in distilled water. This should be standard practice wherever and whenever instruments which draw blood are used. Carelessness in disinfecting instruments is believed to be one of the most important ways of spreading anaplasmosis from a carrier animal to an uninfected one.

Aricyl, an arsenical, was administered (subcutaneously and intravenously) to 16 cows that were affected with acute anaplasmosis. Three died. If treatment was started early in the course of the disease, chances for recovery were good.

Chlorophyll in ethyl alcohol has been used intravenously in treating two cases of acute anaplasmosis infection under laboratory conditions. A one-year old splenectomized steer recovered without running a typical course of infection. Treatment of a nine-year-old cow was started late in the course of the disease and she died. It is too early to predict the value of this type of treatment, but the fact that a splenectomized animal made a rapid recovery following treatment was encouraging.

Copper sulfate administered intravenously apparently produced an unusual condition in one cow thus treated. She continued to show a high percentage of *Anaplasma marginale* (6 to 10 percent) seventy-two days after developing the disease. Copper sulfate in the amount administered apparently inhibited the production of red blood cells. It is likely that copper sulfate will not be used alone in the treatment of anaplasmosis.

Chemozine was given, in accordance with directions, to eight cows that were in the early stages of the disease. Three died and five recovered. When two injections were made at 48-hour intervals the animals failed to survive. Chemozine administered to a normal cow in accordance with recommended dosage caused a reduction of approximately 2,000,000 red blood cells per cubic millimeter. This drug has been discarded because it not only failed to alter the course of the disease but proved to be harmful.

Other treatments used on from one to three acute cases of anaplasmosis without apparent benefit included: Neosalvarsan, Giemsa's stain, mercurochrome, mapharsen, potassium iodide (in feed and water), sodium iodide, clorarsen plus mercurochrome, whole blood, and potozidin. All were administered intravenously except potassium iodide.*

OTHER ANIMALS AS POSSIBLE "CARRIERS"

All of the anaplasmosis research reported to date shows that it is a disease peculiar to cattle. All breeds are susceptible. Anaplasmosis, as it exists in the United States, has not been known to produce active infection in any animal other than the bovine.

* In testing drugs, each sick animal was examined at 24- to 48-hour intervals during treatment, and the following data recorded: Temperature, pulse rate, hemoglobin, total red blood cell count, and the percentage of red blood cells that showed typical anaplasma bodies.

Deer Are Being Tested as Possible Anaplasmosis Carriers.
This one-year-old deer, native to Oklahoma, was intentionally made a carrier of anaplasmosis by inoculating it with blood from a cow that had the disease. The carrier condition had existed for six months at the time this picture was taken. The test must be repeated with other deer for positive evidence, and so far there is no proof that carrier infection can be induced in deer by insect bites. But until further information is available, it appears deer should be considered a possible source of infection in areas where they range with cattle in the presence of biting insects.

It has been suggested that deer, wild or in capivity, may be accidental carriers of anaplasmosis. Sheep and goats have also been suggested as possible carriers.

In recent work at the Veterinary Research Field Laboratory at Pawhuska, an Oklahoma deer purposely inoculated with blood from an infected bovine remained a carrier for at least six months. However, only one deer was used in this study, and additional studies with several others will be needed before deciding to what extent deer can be caused to serve as carriers of the disease. So far, carrier infection induced in deer by bites of flies and ticks has not been demonstrated; but it appears deer should be considered as a possible source of infection in areas where they range and feed with cattle in the presence of horse flies, ticks, and species of mosquitoes that are proved transmitters of anaplasmosis in cattle.

Almost every type of animal common to the United States has been examined by various researchers for the presence of carrier infection; but in all instances except the purposely inoculated deer mentioned above the results have been negative. Until it has been proved under controlled conditions that anaplasmosis can exist under natural conditions in the bodies of animals other than cattle, it will be necessary to look elsewhere for the cause of outbreaks of this disease that cannot be traced to means of transmission already known.

RESEARCH UNDER WAY.

Current anaplasmosis studies of the Oklahoma Veterinary Research Institute are aimed at:

1. Development of a safe and practical vaccine that will give animals lasting immunity to the disease.

2. Finding a practical and inexpensive method of identifying "carrier" animals.

3. Learning more about the behavior of *Anaplasma marginale* in the blood stream of cattle, as a basis for applied research in prevention and control.

4. Finding a research animal less expensive than the bovine, to reduce the cost of research and thereby make more rapid progress.

These studies are in addition to the work aimed at curing animals already ill with the disease.

The Veterinary Research Field Laboratory.

Field work requiring isolation of animals and readily available scientific equipment is housed in this unit at Pawhuska. Another isolation barn and more extensive laboratory facilities are located at Stillwater. The Pawhuska unit shown above has four screened stalls and has water, lights, concrete floors, feed room, and a well equipped laboratory. It is located on a well fenced 35-acre tract of good grazing land, and has corrals, feed racks, stanchions, loading chute, and other facilities for handling cattle.

Vaccine Studies

TISSUE VACCINES.—Four different types of tissue vaccine have been produced, using spleen, blood, bone marrow, and spleen and lymph glands mixed, taken from cattle recently dead from anaplasmosis. Each of these types was tested* on 10 head of calves, including two controls. The animals vaccinated with vaccines from blood tissue origin did not develop as severe a type of anaplasmosis as those in the other three groups, and the clinical blood studies were also favorable to this type of vaccine. Therefore blood vaccine is being currently studied in a test using three groups of 10 calves each.

This type of research is necessarily slow. There are differences in tissue vaccines as well as variations in the resistance of individual animals. There is also the possibility of variation in strains of *Anaplasma marginale*. It will be necessary to continue these vaccination studies, using both calves and older cattle.

ATTENUATION PROCEDURES.—An attempt to produce an immunizing agent is also being made by trying to weaken the *Anaplasma*

* Testing procedures will be described in detail in a forthcoming publication.

marginale organism which causes the disease. Substances so far tested, without success, on one or more animals include: Para-amino benzoic acid; merthiolate plus an antimalarial (09916); riboflavin (vitamin B_2); sheep serum; a mixture of adrenalin, horse serum, and thiamin hydrochloride (vitamin B_1); and mapharsen plus 09916.

Diagnostic Tests

Three general types of tests, and variations of each, have been tried so far in the effort to find a way of positively identifying carrier animals. To date, no promising leads have been uncovered. Methods tried so far include: Experimental antigens prepared from spleen, lymph glands, and whole blood; intrademal extracts from spleen, thymus, and bone marrow; and complement fixation tests.

Development of Research Methods

Methods now in use for producing vaccines and diagnostic agents for other diseases have not worked with anaplasmosis. Therefore it is necessary to do fundamental research on the nature of anaplasmosis, and to develop the necessary laboratory methods. This work is being pushed by the Oklahoma Veterinary Research Institute as rapidly as conditions permit.

ARTIFICIAL CULTIVATION OF *Anaplasma marginale*.—The behavior of *Anaplama marginale* in the blood stream of cattle is another unknown factor. Therefore different culture media are being used in an attempt to learn something about the development stages of the organism. A developmental stage of *Anaplasma* that could be demonstrated with consistency would furnish another avenue of attack on the disease. This method of study has revealed no satisfactory information to date, but additional types of media are being tried.

SEARCH FOR LABORATORY ANIMALS.—Anaplasmosis research could advance much more rapidly and less expensively if a laboratory animal smaller than the bovine could be found. In seeking such an animal, the spleen was removed from rabbits and guinea pigs by a relatively simple surgical operation. It was hoped this would cause these animals to develop anaplasmosis when inoculated with anaplasma infected blood, or at least retain the infection, but the results were negative. Splenectomized calves have not been of value except for diagnostic purpose.

SUMMARY

1.Fifteen drugs have been used experimentally in treating active cases of anaplasmosis. Paludrine hydrochloride and quino-

Some of the Calves Being Used in Vaccine Experiments.

More than seventy calves are currently being used in experiments aimed at finding a vaccine for prevention of anaplasmosis. The calves shown here were vaccinated with experimental tissue vaccine (blood origin). Three other tissue vaccines are also being tested at the present time.

line diphosphate have given excellent results in that 85 percent of all cases treated have recovered.

2. An Oklahoma deer was made a "carrier" of anaplasmosis when inoculated with anaplasma infected blood of cattle origin. These animals have not been known to become carriers under natural conditions.

3. Four types of tissue vaccines have been used experimentally. Experimental vaccines of blood origin showed promise when used in one lot of ten calves. Additional vaccines of blood origin have been put into use on thirty head of calves.

4. Attempts to attenuate *Anaplasma marginale* by use of drugs, vitamins, and serums have not given satisfactory results to date.

5. Attempts to obtain additional information about *Anaplasma marginale* by trying to cultivate the causative agent in artificial culture media have shown no encouraging results to date.

6. Diagnostic tests used experimentally include agglutination, intradermal, complement fixation, and splenectomy. With the exception of splenectomized calves that are used to detect "carrier" infection, these studies have not been satisfactory.

The Oklahoma Veterinary Research Institute

HERMAN FARLEY, *Executive Director*

The Oklahoma Veterinary Research Institute was created by the Board of Regents of the Oklahoma A. & M. College July 1, 1945, in comformity with an Act of the 13th Legislature of the State of Oklahoma providing for research work at the College in animal disease control.

The purpose of the Oklahoma Veterinary Research Institute is to study the various livestock diseases of Oklahoma. It enables the College to conduct research directed at some of the most important livestock diseases that are proving deterimental to our livestock industry.

Livestock diseases are investigated in the order of their importance. Research studies are directed at cause, economic importance, and the best means of control.

The Institute keeps contact with other State and Federal disease control agencies that are studying livestock diseases common to Oklahoma livestock in order to further strengthen the disease control program in this state.

Present board members and staff of the Institute are:

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