CAMEROON,
CENTRAL AFRICA'S MEAT POT

BY BABETTE ABANDA

Cameroon is the meat pot of Central Africa. Vast fertile grazing land, relatively mild climate on the Adamawa plateau, and – most importantly – no Tsetse flies make Cameroon the meat supplier of the region. The Gudali cattle breed, a hybrid between the Indian Zebu and European Friesian, is the most important economically in the area due to their climatic adaption and the high productivity for milk and meat. Those cattle have the potential to reach their maximum weight of 600 kg under ideal conditions, with constant and appropriate food supply and free of harmful microbes and parasites. However, depending on pathogen pressure cattle may lose appetite and weight, have bloody urine or feces. They can develop strong skin alterations, have their hoofs or mouthparts destroyed, and may even die. Because the death of an animal is an immense economical shortfall, herds are normally systematically treated after the slightest signs of weakness.

The success of the treatment used, is by its broad spectrum of activities targeting more than one pathogen at the time, and focusing on the most endemic in the geographic area. This strategy allows even herdsmen to treat animals in remote areas where veterinarian services cannot be reached. The problem with this approach is that the residuals of the drug are accumulating in the organs and by that leading to resistances against further treatments.

Inhibiting factors of livestock production

There are numerous reasons

explaining the stable, but compared to the industrialized world, considerably low rate of production of cattle livestock in Cameroon, varying from parasite pressure to food sustainability. There are many important factors, such as changing climate conditions, lack of adequate agricultural technology, or different pathogen strains that ultimately make a regional approach for solving the problems necessary. Simply put, what works well in one place may not work in another.

When looking at the notable constraints surrounding productivity improvement, infectious diseases can be categorized or divided into two groups: 1. highly contagious and 2. vector-borne. For my research I am focusing on the second category of vector-borne diseases, that are transmitted by an intermediary organism, generally a blood feeding insect, tick or snail.

Information on tick-borne pathogens in Cameroon has been scarce and lacks a recent update. Available data is mostly based on identification under the microscope. Molecular tools, like polymerase chain reaction (PCR) or antigen detection methods have hardly been used. The screening of tick-borne, and other vector-transmitted pathogens in cattle is a current hot topic because of its implication on the One Health concept. The One health concept, which describes how the permanent human exposure to cattle pathogens may permit the invasion and establishment of the animal pathogen to the human host. Such zoonotic infections have happened many times in human history. They are nowadays recognized as one of the highest dangers to Public Health.

Vectors are a highly efficient carrier of infectious agents from one host to another. A vector can be of biological origin, which means the parasite needs it to complete its life cycle, or merely mechanic, meaning it


Female Cattle Chip


Male Cattle Chip

transmits the germs like an infectious needle would do.
For my current work a team from the Agricultural Research Institute for Development at the IRAD Wakwa Centre gathered around 1300 cattle blood samples and 2000 ticks. These blood samples were taken with vacutainer tubes containing anti-coagulants, and the ticks were put in 70% alcohol for conservation. After bringing the samples to the laboratory, the ticks were morphologically classified by specially trained students and staff at the Programme Onchocercoses field station of the Tübingen University in Ngaoundéré (more info on www.riverblindness.eu). At the IRAD Wakwa Centre, the blood samples were screened microscopically for blood parasites. Additionally, the DNA was extracted for later molecular investigation at the University of Tübingen.

At the moment only basic analyses can be done in Cameroon. Nonetheless, the samples should be sequenced for final results, a service currently not available in Ngaoundéré, which meant our specimens had to be sent to Germany whenever possible. A better and more efficient way would be to get results on site, and shortly after sampling, to be able to give a feedback to cattle holders and owners. The potentially harmful pathogens need be identified before appropriate treatment can be given, when this is not possible the infected animal is to be isolated. Under the present circumstances, results come late, possibly years after the sampling has been done, perhaps never reaching the cattle owner, ultimately allowing for the pathogen to develop and spread over time.

My research objective

As one way to address this issues my PhD project focuses on the design of a new chip-based microarray, which can quickly identify some of the most common and dangerous tick-borne pathogens in the region. All this can be done with only needing a few pieces of equipment for easy implementation in a small diagnostic laboratory. The technology has been developed by the German biotech company Chipron GmbH, based in Berlin. It is a low-density chip - array (5 cm2 plate surface) and has been used for the identification of biological substances based on their genetic code. The chip holds eight samples at a time. Generally, the method is based on the Reverse Line Blot hybridization technique, in which the PCR-amplified target DNA of the sample binds to its complementary strand which is covalently attached to the chip surface. Once the specific DNA has been bound, an enzymatic reaction catalyzes a color change which can be detected by a scanner, or even the naked eye. By exact positioning of a number of those specific DNA strands ('probes') on the chip, the simultaneous detection within the same sample is possible in less than an hour.
Apart from a functional PCR facility, the whole method requires only the chip, some reagents, a washing box, a scanning reader and a software program to be installed on any laptop. As a special feature, the chip can also find similar, but not further characterized species, of the same genus by using 'catch all' probes, which can then be sent away for sequencing. That can even allow for the identification of new or emerging pathogens in previously unreported areas.

How to develop such a microarray

For the development of a chip with the right properties, the correct DNA probes, that only bind to the respective pathogen, have to be identified and tested. This sounds easy, but in fact, it is not. First, I had to screen the whole database of published sequences of the pathogens of interest, and look also for those which are similar to it. The species-specific probes I used were located at the 16S and 18S ribosomal subunit RNA, respectively. For genus identification the probes were extracted from the invariable portion of the same locus. Primer pairs and cycling conditions for the PCR were either retrieved from the literature, or self-designed and optimized. The detected pathogens were grouped as a strategy to catch all pathogens from the same genus present in a sample. The bacterial genera Anaplasma, Ehrlichia and Rickettsia, and the eukaryotic piroplasmids Babesia and Theileria. All are intracellular parasites of red or white blood cells and are known to cause serious disease in cattle. Some even pose a threat of infection for exposed humans.

Cameroons diversity of cattle breeds

In Cameroon there are two inherently different groups of cattle: the humpbacked zebu breeds originating from the Indian subcontinent, and the African taurine breeds which are generally of small size. Common taurine breeds are the Kapsiki and Namchi, which occur only regionally in North Cameroon. The zebus comprise roughly 99% of the total cattle population, because they grow much faster and larger. Apart from the Gudali breed, the white Fulani and red Fulani, large animals with impressive horns, are the most abundant. Until the beginning of the 1980's the indigenous taurine and nomadic zebu breeds were not allowed to enter the Adamawa highland plateau, because of their endemic diseases and, for taurine cattle, their annoying grazing behavior. These cattle feed the complete plants with the roots attached making the regrowth process longer.

One of the interesting results revealed by the screening of pathogens on different cattle breeds is that they differ considerably from their susceptibility. This is one of the astonishing observations in the DFG-cattle herd of the University of Tübingen, which is now kept for 8 years on a paddock close to the river Vina du Sud near Ngaoundéré. Individuals from the same herd, raised under the same conditions and exposed to the same parasites, show extremely different parasite loads. For filarial Onchocerca worms, which apparently do not cause pathology in cattle, some animals carry more than 2000 adult worms, and others less than 5. Some individuals even show the complete absence of a certain pathogen, whereas other animals need treatment from the same infection. Furthermore, some animals are more attractive for ticks to bite while other carry none at all.

This difference in susceptibility is an issue with different hypotheses for explanation. Some are based on the morphological aspect of the animal, like abundance or absence of hair, immune response acquired during individual life or not, or the genetic background. To evaluate the genetic implication of the difference in susceptibility, a complementary project was launched which looks for genetic diversity and associated manifestations of the phenotype in susceptibility. A genome-wide association with the level of parasite burden is currently underway in collaboration with the Institute of Animal Genetics at the University of Hohenheim (Dr. Siegfried Preuss).