Inaugural lecture: Lyndy McGaw **8 September 2021**

Professor Mosia, Professor Naidoo, colleagues, family and friends, good evening and thank you for the opportunity to present this address. As was mentioned in the introduction, I am going to be discussing ethnoveterinary medicine as a link between the past and future mainly in terms of animal healthcare, but with an underlying One Health theme.

Our research group is located at the University of Pretoria in the northeast of the country. It houses the only veterinary faculty in the country and is one of the leading research-intensive universities. Based at the Faculty of Veterinary Science, which is some distance north of the Pretoria city centre, one of the major research areas of the Phytomedicine Programme is Ethnoveterinary Medicine. We are fortunate to be in a position to collaborate with many veterinary and other colleagues on projects of mutual interest. The Veterinary Faculty boasts state of the art facilities for student training, as well as world-class research laboratories, and we have established links with many leading international institutions. The Phytomedicine Programme has been based at the Faculty of Veterinary Science since 2002. It was founded in 1995 by Professor Kobus Eloff and was initially hosted at the Department of Pharmacology at the Prinshof Campus. Prof Eloff is currently affiliated with the Programme in an Extraordinary Lectureship position and, together with my permanent appointment, we have a permanent laboratory technologist, Mrs Sanah Nkadimeng. There is an active research programme in our group, with approximately 100 MSc and PhD students graduating in the past 20 or so years. Currently we have 15 MSc and PhD students, and two postdoctoral fellows.

To give an outline of the presentation, I will first cover the use of plants as medicinal agents. Then, a definition of Ethnoveterinary Medicine and its relation to One Health will be provided. The historical use of plants in human and animal health is an aspect of the “Past” referred to in the title. The current crisis of antimicrobial and anti-parasitic drug resistance will be mentioned in terms of the potential contribution that can be supplied by investigating further the medications largely based on plants that were historically the medicine chests of our ancestors. I will finally go on to discuss some of the past and present projects involving several postgraduate students to highlight some of our focus areas with a view to contributing solutions for the future.

I would like to introduce some concepts here that are important for my area of research in ethnoveterinary medicine. Plants have been used as medicines for millennia. The first commercial pure natural product introduced for therapeutic use was morphine marketed by Merck in 1826. The first semi-synthetic pure drug, aspirin, was based on a natural product, salicin isolated from *Salix alba*, introduced by Bayer in 1899. There are many other plant-derived medicinal compounds used currently, both in human and in animal medicine. Some of the third year veterinary students in the VME310 module on Ethnoveterinary Medicine are surprised to note how many current veterinary drugs were originally derived from plants. Plants were a major component of veterinary pharmacopoeias until the 1960s.

Natural products or secondary metabolites are produced by bacteria and fungi as well as plants. Up to 50% of approved drugs during the last 30 years originated directly or indirectly from natural products, and higher plants contribute about 25% to this total. In terms of cancer, from the 1940s to date, of 175 small molecules used, 85 are natural products or direct derivatives. Only 35 000 – 70 000 of plant species globally of a total of about 250 000 have been investigated for their potential medicinal use.

In 2015, the Nobel Prize for Physiology and Medicine was jointly awarded for two discoveries. The first half of the prize was awarded for the discovery of avermectin from a microorganism growing in a handful of Japanese soil. Avermectin was then chemically modified to increase its activity and safety resulting in development of the antiparasitic drug ivermectin. The second half of the prize was given to Youyou Tu for discovering artemisinin from a Chinese medicinal plant, *Artemisia annua*, which was traditionally used to prepare a tea to treat fever.

Coupled with the incredible plant diversity is the diverse cultural heritage, as well as the rich traditions present in South Africa, including the continuing use of plants for medicinal and other purposes. In South Africa, about 3 000 plants are used in traditional medicine with 350 species most commonly used and traded. Traditional Health Practitioners (or THPs) and rural livestock keepers have essential roles in primary health care for humans and animals respectively owing to the acute shortage of Western-trained doctors/veterinarians, health clinics and rural veterinary clinics. Then the needs of animals are superimposed on those of humans and the environment, drawing together the concept of One Health.

Giving impetus to continued research on plants is the emergence of many commercially available products based on useful South African plants. This is also true for the area of animal health, more specifically for companion animal health.

To return to the title of the presentation, we need to have a good understanding of what is involved when we talk about ethnoveterinary medicine. Essentially ethnoveterinary medicine (or EVM) is a complex system of beliefs, skills, knowledge and practices relating to animal husbandry and general animal care. The concept was more fully defined by Constance McCorkle, the renowned veterinary anthropologist. EVM includes the use of diagnostic procedures, animal husbandry practices, surgical methods and use of ethnoveterinary plants to prevent and control disease. It most likely dates back to the domestication of animals.

Together with a proliferation of scientific articles in recent years, some books have been published in the field. The one on the left-hand side was edited by South African colleagues in 2010, and one published last year was edited by myself and a Senior Postdoctoral Fellow, Dr Muna Ali Abdalla. At the Annual Meeting of the Society for Medicinal Plant and Natural Product Research, a day-long pre-conference symposium is held by the Networking Group on Medicinal Plants and Natural Products in Animal Healthcare and Veterinary Medicine. This symposium is increasingly well-supported.

EVM is commonly used by rural livestock keepers in areas where access to orthodox remedies is limited. Diseases have a significant economic impact on livestock, not only in terms of loss of animals but also loss of transport, farming aids and decreased output of products such as milk and meat. Cattle, goats and sheep may suffer from non-specific indications like diarrhoea, coughs and wounds. For these conditions, EVM could be promoted more widely as a possible alternative or complement to Western medicine once more research has been done.

It should be kept in mind that EVM involves a complex system of management, not only the use of plants in animal health. Plants are used to treat livestock as well as poultry and sometimes companion animals. The owners of the livestock generally treat animals with their own medicinal plant knowledge rather than consulting traditional healers. Before the introduction of synthetic pharmaceuticals, plants were predominantly used to prevent and treat illness in animals and humans.

Many of the pathogens associated with epidemics of human disease have evolved into multidrug-resistant (MDR) forms subsequent to antibiotic use. Additionally, resistance is also developing in parasites of economic importance, such as the barber pole worm, or *Haemonchus contortus*, affecting small ruminants, particularly sheep.

The most prevalent Gram-negative pathogens, such as *Escherichia coli*, *Salmonella enterica*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, cause a variety of diseases in humans and animals. A strong correlation between antibiotic use in the treatment of these diseases and antibiotic resistance development has been observed over the past half-century. The use of antibiotics at sub-therapeutic concentrations in animal production has also contributed significantly to the development of antimicrobial resistance, leading to bans of such practices in many countries in the European Union and other areas.

The term "superbugs" refers to microbes or microorganisms causing enhanced morbidity and mortality following multiple mutations which allow high levels of resistance to the antibiotic classes specifically recommended for their treatment. Therapeutic options for infections caused by these microbes are reduced. In some cases, super-resistant strains have also acquired increased virulence and enhanced transmissibility.

There are many definitions of One Health. The Food and Agriculture Organization (FAO) describes it as "A collaborative, international, cross sectoral, multidisciplinary mechanism to address threats and reduce risks of detrimental infectious diseases at the animal-human-ecosystem interface". Never before has the importance of a "One Health" approach to fighting disease been more appropriate than in these times. The One Health concept relates to integrated, interdisciplinary collaboration and communication among health professions to improve health care for humans, animals and the environment. This is particularly relevant where we are experiencing increased incidences of emerging and re-emerging diseases, 75% of which are zoonotic and may have originated from wildlife. The antimicrobial resistance crisis we find ourselves in has provided impetus for the search for complementary strategies to reduce the burden of infectious disease and a One Health approach to this goal is critical.

Although EVM comprises a number of different aspects, in our research group we have a specific focus on plants used in preventing and treating diseases in animals. There is a great lack of knowledge on what plants are being used and this is complicated by issues of Intellectual Property rights, although South Africa has stringent regulations to protect this.

In literature surveys that we have conducted, ten years apart, it was highlighted that a large amount of work needs to be done to document and protect indigenous knowledge regarding the use of plants in animal health. Documentation of plant use can be done in several ways but there are recently published methods available in top journals providing guidance for such endeavours. There are also many ways in which to present the results and make sense of them in broader contexts.

The graphic on the left refers to South African provinces in which some surveys have been done up until 2008, and the one on the right depicts provinces covered up until 2019. It is clear that much work needs to be done in this regard. There are many challenges involved with conducting EVM surveys. For example, seasonality is often an issue as different plants may be used at different times of year pending availability. Trust relationships need to be established and not infringed, with clear indications of intentions and benefit sharing should this become relevant. The need for involvement of a veterinarian or animal health technician is vital to ensure correct interpretation of traditional diagnoses and relating them to clinical symptoms.

This slide represents a study that one of our MSc students undertook to survey plants used for animal treatment in the Mnisi community of Bushbuckridge. The University of Pretoria runs a community veterinary clinic in the area, and has a research facility nearby. On the left, one of the diptanks where the interviews were conducted is shown. Then Thato is interviewing a livestock keeper with one of the Animal Health Technicians from the clinic. This was followed by evaluation of traditionally prepared plant-based remedies in the lab for antimicrobial efficacy, compared to extracts prepared using typical organic solvents. Interestingly the methods of traditional preparation appeared to result in extracts with enhanced activity compared to the other solvent extracts.

EVM studies provide valuable knowledge supporting the development of innovative products for prevention or treatment of common livestock diseases. Research into developing standardized, low-cost preparations would take care of the issues of seasonable availability, variation, as well as known efficacy and safety. Only four of nine provinces have been surveyed, and even then, the areas have not been fully covered. There is much to be done to complete the EVM inventory of South Africa. The use of certain plant species for similar ailments by different ethnic groups can provide interesting leads for prioritising research.

Continuing on, I would like to discuss some examples of the activities we have been involved with over the past few years. These largely concern *in vitro* investigations of biological activity and toxicity of plant species with potential for further development.

Diarrhoea is a condition affecting humans and animals, particularly neonates, with negative economic and other consequences. *Escherichia coli* serotypes are particularly problematic and other species belonging to genera such as *Salmonella* are also of concern. Many plants are used traditionally against diarrhoea, and several students have tested plant extracts against model bacterial strains as well as clinical isolates from diarrhoea cases. Combination studies of plant extracts, fractions and pure compounds together with current antibiotics have shown enhanced antibacterial activity in some cases. Apart from direct antibacterial activity, chemicals found in plants may also affect bacteria in other ways.

What we have learned from the antimicrobial and anti-parasitic resistance crisis is that resistance to newly introduced compounds develops extremely quickly. We need to embrace a more holistic approach to tackle the problem from different angles simultaneously.

Traditional remedies often comprise decoctions or infusions and we’ve found that although water extracts often don’t have direct antibacterial activity, they may have different mechanisms to combat bacterial infections. For example, bacteria are able to secrete quorum sensing molecules to communicate with one another. Most infectious diseases are caused by bacteria which multiply within quorum sensing (QS) facilitated biofilms. A biofilm usually begins to form when a free-swimming or planktonic bacterium attaches to a surface. Quorum sensing chemicals are released to attract other bacteria to form a biofilm and the consortium of bacteria secrete extracellular polymeric substances or EPS. This EPS assists in sheltering the bacteria from harmful factors in the environment, such as desiccation, antibiotics, and the host body's immune system. Biofilm or quorum sensing inhibitors are therefore sought after and several plant extracts have shown promise in this area. In addition, bacteria need to adhere to host cells in sufficient numbers to be able to produce a clinical infection. Anti-adhesion factors from plants could also be useful antibacterial agents. Resistance mechanisms of bacteria include efflux pumps, which function to pump out antibiotics from the bacterial cell, reducing their efficacy. Certain chemicals, including some from plants, are able to inhibit these efflux pumps, reversing the resistance. Using either Scanning or Transmission Electron Microscopy, we are able to detect ultrastructural changes or damage induced by plant extracts or compounds on target bacteria.

One such study was that of a previous PhD student, currently a postdoctoral fellow in our group. This work resulted in several papers on the efficacy of plant species from the Myrtaceae family on bacteria. Biofilm inhibitory effects were noticeable and electron microscopy studies highlighted the direct effects of some of the plant extracts on the ultrastructure of the *E. coli* isolates investigated.

Gastrointestinal infectious diseases caused by foodborne pathogens results in enormous health and economic concerns worldwide. Salmonellosis caused by the genus *Salmonella* is one of the most common foodborne diseases in both animals and humans. The emergence and spread of antimicrobial-resistant *Salmonella* species has become a worldwide health challenge.

In this PhD study, extracts of several plant species used for stomach ailments in humans and animals were tested against a wide range of foodborne pathogens. When evaluated for cytotoxicity some extracts had good selectivity index values so were more toxic to the bacteria than to mammalian cells. *Loxostylis alata* was one of the most promising species and several active compounds were isolated and identified for the first time from the leaves.

Also as part of this study, the susceptibility profile of *Salmonella* isolates of animal origin indicated that approximately 84% of isolates tested were susceptible to a range of antibiotics, while approximately 7% were resistant to between five and seven currently used antibacterial drugs. Pulse field gel electrophoresis (PFGE) analysis of *Salmonella* Enteritidis and *Salmonella* Typhimurium isolates indicated there was no genetic relatedness between resistant strains.

Methanol and hot water extracts of *Loxostylis alata* and one of the isolated compounds had very interesting efficacy against biofilm formation as well as against preformed biofilms of a number of different species of foodborne pathogenic bacteria. The pure compound had very good anti-inflammatory effects against lipoxygenase and inducible nitric oxide (NO) synthase, as well as very good antioxidant activity in various assays.

The emergence and spread of antimicrobial-resistant *Salmonella* species has become a worldwide health challenge. Animals are carriers of a number of *Salmonella* serovars. The development of multi-drug antibiotic resistance among *Salmonella* serovars isolated from animals in South Africa is an indication of the need to enforce strict monitoring and follow up of resistance among bacteria isolated from animals. This will help prevent the emergence and distribution of drug resistant salmonellosis in humans.

An MSc study highlighted the potential of South African plants as quorum sensing and biofilm inhibitors. Our research also focuses on Gram-positive pathogenic bacteria, including *Staphylococcus aureus* and non-aureus staphylococci implicated in mastitis in humans and dairy cattle.

Some plant extracts tested had good activity against isolates from mastitis cases in cows. Several had good results relating to inhibiting biofilms. The extracts were not toxic to bovine dermis cells at the highest concentration tested. Selected extracts also had interesting anti-inflammatory and antioxidant effects, contributing to their value in future studies.

Current studies are focusing on quorum sensing inhibition. The best extracts are undergoing further tests relating to chemical composition using various methodologies. Formulations are being prepared for *in vitro* testing, prior to which a pilot study will be undertaken to determine their efficacy in reducing the bacterial load in dairy cattle. This is funded in part by the Translational Medicine Research Theme of the Faculty.

Another project relates to anti-parasitic activity of plant extracts, particularly anthelmintic efficacy. Biological activity assays against parasitic nematodes are difficult to conduct as the organisms can’t be cultured in the laboratory and require an animal host. The correlation between efficacy against the free-living model nematode *Caenorhabditis elegans* and the parasitic nematode *Haemonchus contortus* was investigated. Some extracts had good efficacy and also strong anti-inflammatory and antioxidant activity.

A further PhD project revealed plant species with promising activity against nematodes including plant parasitic *Meloidogyne* species, which cause major economic losses in crops worldwide. Seasonal and geographical variation in plants for selection of high-performing strains for cultivation is also an area of research. Chemical markers or active compounds also need to be identified to standardize active preparations.

Research in this project focuses on protecting chicken feed against contamination with *Aspergillus* and *Fusarium* species (aflatoxins and fumonisins). Additionally, the plant-based feed additives aim to protect poultry against infection, f or example colibacillosis, aspergillosis, salmonellosis and campylobacteriosis. Mostly *in vitro* work has been done to date. Some interesting articles have been published in recent times regarding resurgences of *Campylobacter* infections.

A number of studies have been conducted by students regarding the use of plants to enhance poultry production. A survey was conducted in Zimbabwe to determine which plants were most commonly used for poultry disorders. These and other plant species were selected and tested against various poultry pathogens on the basis of traditional use in literature.

The most frequently reported zoonotic disease in humans in the European Union in recent years is campylobacteriosis, and the bacterial species most often implicated is *Campylobacter jejuni*. Broiler chicken meat contaminated with *C. jejuni* is considered to be a major source of human campylobacteriosis. Interestingly, broiler chickens are asymptomatic *Campylobacter* carriers. The most important primary contamination site of *Campylobacter* is at the farm level, because *Campylobacter* exists widely in the outside environment.

In vitro activity of plant extracts was demonstrated against a large number of poultry pathogenic bacteria and fungi together with other beneficial properties. A promising plant species was selected for a feed additive pilot study currently being conducted in broiler chickens experimentally infected with *Campylobacter jejuni*. This study is also partly funded by the Translational Medicine Research Theme.

Additional research in our group focuses on anti-inflammatory and antioxidant efficacy effects as has been previously mentioned. This relates to the beneficial effects of the plant extracts on the host as well as the detrimental effects on the pathogen.

The impact of invasive plants is severe and therefore deserves serious attention. It is not impossible that control through utilisation without encouraging propagation may be one of the options to consider. With this in mind, invasive plants may be good sources of medicinal compounds which serve as alternatives to highly exploited plants with similar medicinal properties. Many weeds have been incorporated into the traditional South African *materia medica* and are used in the treatment of several disorders. These plants may be developed into low cost, low technology plant-based remedies on a more commercial scale.

Similarly, endophytes, or microbes living inside plants, are an extremely under-researched area and it has been found in some studies that bacteria or fungi isolated from well-known medicinal plants can produce highly active medicinal compounds, such as the anti-cancer drug taxol.

Ethnoveterinary medicine may provide exciting leads for antimicrobial, anthelmintic and other interesting bioactivities. Cytotoxicity is also an important aspect to consider in tandem with bioactivity studies to determine specific anti-pathogenic activity and not general toxicity. Anti-inflammatory, antioxidant and immune modulatory activity are important host-specific activities to consider. Plants may be useful sources of antibiotic feed additive alternatives.

Ethnoveterinary medicine provides us with leads to produce active, safe, potentised plant extracts or fractions produced by selective extraction, or purified compounds for further research and development into useful products. A focus on One Health is critical in the next era of animal and human medicine.

There is a growing realisaton that conventional, formal sector healthcare and husbandry interventions from the developed world cannot sustainably meet the basic stock-raising and food security needs of most rural people in the developing world, where every rural community keeps animals. EVM can potentially contribute to enhancing the overall health of animals amongst other aspects as part of an integrated approach to One Health.

In closing I would like to express appreciation for those who have mentored me throughout my career. The path to becoming an academic is a long one and I have been assisted greatly by several mentors. I can’t mention all of them but will select a few who have been with me for many years, continuing with advice and mentorship. Prof Hannes van Staden was my PhD supervisor and over the 20 years since then we have remained in contact and collaborated on many different projects. His personal interest, kindness and care for his students is legendary.

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Thank you very much for your attention.