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Editor's page:

Antimicrobial resistance, threat in veterinary medicine and what are we going to do next?

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Antimicrobial resistance is one of the key threats in human medicine. It is also a burning issue in veterinary medicine including livestock and companion animal practice. Sometimes, veterinarians are blamed for the main course of the emergence of antimicrobial resistance in the food chain. Although, it is not true, livestock and companion animals also contribute a role in dissemination of antimicrobial resistance in the environment.

The specific classes of antimicrobials have been identified as threatening issues such as resistance to quinolone in poultry, resistance to beta lactams in livestock and companion animal, resistance to macrolides in poultry and aquaculture, resistance to tetracycline in poultry and aquaculture. A high frequency of resistance has been reported for beta lactam, macrolides, and tetracycline. However, limited classes of antimicrobials are being used in veterinary medicine except in companion animal practice in Sri Lanka. Gaps in legislation and malpractices have been identified as one of the reasons for over usage of different classes of antimicrobials in veterinary medicine. Therefore, the Department of Animal Production and Health (DAPH) has already taken necessary action to amend the animal disease act to impose necessary laws and regulations in the field.

As mentioned previously, antimicrobial resistance is not limited to a single sector. All relevant stakeholders in the country work together against antimicrobial resistance as the Ministry of health, DAPH, NARA and the Ministry of Environment.

The following strategies are followed as described in National Strategic action plan 2017-2022 by the Ministry of Health, Sri Lanka.

1. Improve awareness and understanding of antimicrobial resistance through effective communication.
2. Strengthen the knowledge and evidence base through surveillance and research.
3. Reduce the incidence of infection through effective sanitation, hygiene, and infection prevention measures.
4. Optimize the use of antimicrobial medicines in human and animal health.
5. Prepare the economic case for sustainable investment and increase investment in new medicines, diagnostic tools, vaccines, and other interventions.

(Source: National Strategic action plan 2017-2022, Ministry of Health, Sri Lanka)

In addition to antimicrobial resistance, antimicrobial consumption and antimicrobial usage are being monitored in the veterinary sector by DAPH. The relevant information can be taken from DAPH and World organization of Animal Health websites too.

AMR awareness week 2022 at Ministry of Health
(Representatives from Ministry of Health, DAPH, Ministry of Environment, Sri Lanka College of Microbiologist, NARA)

Table: 1 Importation of antimicrobials to Sri Lanka from 2016 to 2020: Source: VDCA:DAPH

Antimicrobial Class	2016 (Kg)	2017 (Kg)	2018 (Kg)	2019 (Kg)	2020(Kg)	Total (Kg)
Aminoglycosides	5983	1295	829.0626	926	1046.704	10079.7666
Amphenicols	0	0	0	0	0	0
Arsenicals	0	0	0	0.2	0	0.2
Cephalosporins (all generations)	6.3	24.6	0.9	1	1	33.8
1-2 gen. cephalosporins	1.3	6.6	0.9	1	1	10.8
3-4 gen cephalosporins	5	18	0	0	0	23
Fluoroquinolones	2422	2865	3501.67	4339	3723.364	16851.034
Glycopeptides	0	0	0	0	0	0
Glycophosphoipids	0	0	0	0	0	0
Lincosamides	0	0	0	0	0	0
Macrolides	9596	7026	14385.38	42610	5346.645	78964.02937
Nitrofurans	0	0	0	0	0	0
Orthosomycins	0	360	0	0	0	360
Other quinolones	0	0	80	122.5	95	297.5
Penicillins	3520	5116.6	9153.344	16976	18883.82	53649.7676
Pleuromutilins	2140	174	1227.55	1899	695.7	6136.25
Polypeptides	54600	4	280.0739	0	0	54884.0739
Quinoxalines	0	0	0	0	0	0
Streptogramins	1450	0	0	0	0	1450
Sulfonamides (including trimethoprim)	3900	32042	3813.786	8064	10224.67	58044.4594
Tetracyclines	8325	32493	6615.769	7936	5264.75	60634.5194
Others	0	22.5	167.25	172	1947.216	2308.966
Aggregated class data	0	0	0	0	0	0
Total (kg)	91942.3	81422.7	40054.79	83045.7	47228.88	343728.166

Locally produced FMD vaccine: What do we know?

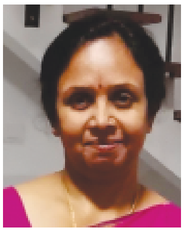
FMD is an economically important and highly contagious disease of livestock worldwide. Vaccines are among the most cost-effective health interventions against infectious diseases, saving millions of livestock globally. The currently used vaccine for FMD globally is an inactivated vaccine that will produce immunity for up to 4-6 months duration depending on the adjuvant. The FMD vaccine can reduce clinical signs, protect against clinical infection, and reduce the transmission of the live virus to susceptible animals. There are 7 serotypes of the virus, referred to as type O, A, C, Asia 1, SAT1-3. Infection with one serotype does not provide cross-protection for a different serotype. Only one serotype ("O") is present in Sri Lanka which is comparatively easier to prevent FMD by vaccination.

VRI has a long history of vaccine production. The first vaccine, against fowl pox, was produced in 1934 by veterinary diagnostic laboratory which was later renamed as Veterinary Research Institute in 1967. The production of FMD vaccine was started in 1962 and it was suspended due to technical issues. However, FMD vaccine production was resumed at Animal Virus Laboratory and first batch was issued after several years in 2012. The vaccine used local isolate as seed and grown in BHK21 cells. After chemical inactivation of the virus, vaccine was formulated by using Aluminum hydroxide as an adjuvant.

Since the recommended dose and route of the FMD vaccine were 4 ml and subcutaneous administration, this vaccine was not very popular in the field due to difficulties in administering vaccine in the herds especially in extensively - managed herds.

Any manufacturing process needs continuous research and development, and vaccine production is also not exceptional. Montanide ISA 201, a double oil adjuvant, was introduced in 2015 and the oil adjuvanted FMD vaccine was successful in developing protective immunity in cattle. Further, the dose of vaccine has been reduced to 3ml and the recommended route of administration is intramuscularly. These developments facilitate the easy administration of vaccine in the field situation. Further, the currently produced FMD vaccine by VRI can be used in goats and pigs similar to imported FMD vaccine and the shelf life of the vaccine has been increased up to one year. It should be noted that animals are protected when the vaccination is correctly done with the recommended dose and route. Further, FMD vaccine is an inactivated vaccine, cold chain maintenance is utmost essential to develop protective immunity.

VRI has the mandate to produce FMD vaccine enough to cater to the whole country in near future and thereby move forward towards freedom from FMD.



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High dietary potassium can reduce ethanol stability in milk

Milk has complex structure and is made of two phases, colloidal and aqueous. The casein micelle in milk is formed by casein sub-units (α, β, and κ) interconnected by micellar calcium phosphate (Walstra, 1999) and present in the colloidal phase of milk. These casein micelles are shown to have a lattice structure in which both casein-calcium phosphate aggregates and casein polymer chains act together to maintain its integrity and steric repulsion among these micelles stops aggregation. The micelles are not in a static state and it is found that the micelles are in an equilibrium which can be affected by different factors such as pH, temperature, ionized calcium level in aqueous phase and salts can affect ethanol stability (Guo et al., 1998).

Stability of milk proteins is an important issue in the dairy industry, especially for the manufacture of milk products that require intense heat treatments, such as ultra-high temperature (UHT) and powdered milk. During heating process, the milk protein casein in milk should withstand the heat effect and sustain its integrity. If casein protein is unstable at high temperatures, the protein can be precipitated compromising the product shelf life which can result a major financial loss. Therefore, milk collectors use the ethanol stability test as an indirect measure of milk heat stability.

Ethanol stability test is originally regarded as a measure of acidity, mainly of acidity produced by bacterial fermentation where stability of milk at 68% ethanol is taken as the acceptable level. But once this test is considered for milk heat stability testing, stability at higher ethanol percentage is required for acceptance. Thus, at present milk is being tested for ethanol stability at percentages higher than 68%. Different ethanol percentages are being used by different milk collectors, but the majority range between 74% - 80%. So, a considerable amount of milk is being rejected every day in the country due to instability at higher ethanol levels.

The dietary cation-anion difference (DCAD) is determined by the charge balance of the major cations and anions in the diet and studies have shown that, nutritional or metabolic factors which cause ionic imbalance may alter milk protein stability (Marques et al., 2011; Fischer et al., 2012). Potassium is high in forage-based diets and is the major cation that determines the DCAD in this type of feed (Roche et al., 2002).

In a study conducted in the central province of Sri Lanka showed that hybrid Napier (CO3) grown is significantly high in potassium level. Kandy, Matale and Nuwara-eliya districts were showing a DCAD level of 808, 1194 and 1153 (mEq/kg) according to the equation [(Na + K) - (Cl + 0.6 S)] published by Goff et al., 2004 and a potassium percentage of 4.3, 6.3 and 5.7% respectively (Pathirana et al, 2019). Forages having a potassium level of more than 2.5% are considered as high potassium forages and result in cationic diets (Undersander and Kelling, 2001). Our studies revealed that ethanol instability and unfair rejection of milk can also occur with the use of excess potassium fertilization. Hence, fertilization of pasture using manure rich in potassium especially poultry manure or inorganic potassium has to be revisited.

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Bovine viral diarrhea (BVD)

Bovine viral diarrhea (BVD) is an economically important multi systemic disease associated with gastrointestinal, respiratory, and reproductive system of cattle worldwide. It is caused by Bovine viral Diarrhea virus (BVDV), a single-stranded positive-sense RNA virus which belongs to the genus Pestivirus. There are four virus species within this genus, namely BVDV 1, BVDV2, classical swine fever virus and Border disease virus.

This infectious disease mainly causes continuous economic losses to the cattle industry worldwide, primarily due to decreased reproductive performance. In some cases, infected cattle do not exhibit any clinical signs, but they are immunosuppressed so this disease mainly impacts on animal health and it also directly contributes to the economic loss.

The clinical signs of BVD infection are highly variable and most common signs are fever, lethargy, loss of appetite, ocular and nasal discharges, leukopenia, diarrhea, decreased milk production and reproductive problems such as early embryonic death, abortion, still birth, mummification and congenital defects.

A commonly recognized congenital defect is cerebellar hypoplasia which is clinically manifested by ataxia, tremors and stumbling in new born calves. The transmission of BVDV occurs vertically as well as horizontally via excretions and secretions, including nasal discharge, tears, saliva, urine, feces, milk and semen. BVD is usually mild in Transiently infected (TI) animals but it can cause suppression of the immune system and make the animals more susceptible to common infections. Animals excrete relatively low levels of virus for a short period of time and will develop life-long immunity.

The ability of BVDV to cross the placenta during pregnancy is more important and can result in various complications. The transmission of BVDV to the uterus at 30 to 45 days of gestation will decrease the conception rates and the viability of the embryo. If the virus transmission occurs between 30 to 125 days of gestation and survive the result will be the birth of persistently infected (PI) calves. At this stage, the fetus acquires BVDV infection in utero before its immune system becomes functional. So it is not immunocompetent at the time of infection; thus, it cannot protect against BVDV and the infection will persist throughout the lifetime of the calf and result will be the persistently infected calves. Transmission of BVDV to the fetus after 120 to 150 days of gestation may result in abortion, stillbirth, congenital defects, or birth of a live, normal appearing calf. Congenital anomalies are the most frequent outcome of Infections that occur during days 125 to 175 of gestation. Fetuses that become infected after 175 days are more resistant to infection because they are immunocompetent; however, these fetuses are more likely to experience a serious health problem during the first 10 months of life.

PI animals can be born in two ways. The first way is transmission of BVDV from a PI cow to her fetus and most of the time calf will become PI calf. The second way is acute infection of a pregnant cow during the first 120 to 150 days of gestation. PI animals are considered as the primary reservoirs for BVDV. Because they are shedding large quantities of virus throughout their lives with their excretions and secretions. So PI animals are the most efficient transmitters of BVDV than transiently or acutely infected animals.

Sri Lanka has a cattle population of ~1.8 million animals. Animal Virus Laboratory has facilities to diagnose BVDV Antigen and antibodies against BVD using commercial enzyme-linked immunosorbent assay (ELISA) kits. Indirect ELISA is performed to detect antibodies in serum whereas antigen capture ELISA is used to detect BVDV antigen in ear notch of calves.

The calves which are positive for BVDV antigen are tested again after 3-4 weeks. The calves which are positive for BVDV antigen by second testing are considered as persistently infected calves and need to be removed from the herd.

Due to the nature of BVDV infections, there is no treatment. All control programs which are in use in many countries of the world, mainly depend upon the detection of PI animals, eliminating them and preventing their return into the herds. Detection of PI animals at early stage, particularly soon after birth is of significant benefit to save valuable animals from this infection.

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Bovine haemoparasitic diseases cause heavy economic losses to a farm

Common bovine haemoparasitic diseases in Sri Lanka are:

1. Babesiosis
2. Anaplasmosis
3. Theileriosis

They are protozoal diseases transmitted by ticks and cause significant economic losses in the dairy industry. There are four developmental stages in all three species;

1. Shizogony- Asexual development
2. Merogony- Asexual development
3. Gametogony-Sexual development
4. Sporogony- Asexual development

Babesiosis is caused by two blood protozoa species, Babesia bigemina and Babesia bovis. They are intra-erythrocytic organisms with their asexual development (schizogony and merogony) happening in cattle RBCs.

This asexual multiplication causes RBC rupture and pyrexia, anorexia, anemia and haematurea, which are the typical clinical signs of Babesiosis. Out of these two organisms, Babesia bovis is acute and can also penetrate blood brain barrier, causing more fatalities. Comparatively, Babesia bigemina is less fatal, but the clinical signs are the same. The economic losses of the disease come from milk production reduction, abortions through transplacental transmission and fetal infection, mortalities, and the cost for drugs and veterinary care.

Bovine Anaplasmosis is caused by a rickettsia species, Anaplasma marginale which is also transmitted by several species of ticks. Like Babesia, Anaplasma is also an intra-erythrocytic organism having its asexual multiplication in bovine RBC. Also, like with Babesia, asexual multiplication causes anaemia, anorexia, and milk production losses. But Anaplasma does not cause haematurea and jaundice, and it can be differentially diagnosed from Babesiosis.

Anaplasma also causes more production losses by causing infertility in heifers and cows. Anaplasma is transmitted horizontally from dam to progeny and once infected, they are infected for life and cause economic losses throughout the lifetime. The economic losses are contributed by milk production reduction, infertility, deaths, costs for drugs, and veterinary care.

Bovine Theileriosis is different from both these diseases in that it affects not only RBCs but also WBCs too. Schizogony happens in Lymphocytes and merogony happens in RBC whereas sexual development happens in tick midgut cells, like in all three species. In all three species, sporogony happens in the tick salivary glands, and sporozoites are released into the animal when the tick takes a blood meal. Theileriosis can be presented as an acute disease as well as a chronic disease, depending on many factors such as age, breed, sex, immunity of the animal, and abundance of ticks.

Clinical signs vary according to the stage of the disease. In acute cases, pyrexia, anaemia, anorexia, and jaundice happen. In chronic cases, milk production reduction, weight loss, infertility, and late term abortions can happen. The economic losses are from milk production reduction, weight loss, infertility, abortions, deaths, cost of drugs, and veterinary care. Controlling the ticks in the farm and practicing good husbandry is the most effective way to prevent these diseases and the economic losses due to those diseases.

What the farmers can do to control tick borne haemoprotozoal diseases:

- Cultural control- Prevent new infestations

Checking and treating any newly purchased cattle for ticks before introduction into the herd,

a. Quarantine/separation for 2 weeks
b. Observe for signs of disease
c. Check closely for evidence of tick infestation and treat

- Chemical Control-Pesticides

Drugs are distributed on the skin surface. Attached ticks may be killed and a new one might be prevented from attaching

Can be done as either sprays or pour –ons, two weeks of persistent activity

Withdrawal periods must be followed

- Systematic dipping or spraying with Coumaphos- every 7-14 days for 9 months.
- Once cattle are free from ticks, treatment with Doramectine every 25-28 days for the remainder of the 9 months.
- The most valuable time to apply acaricides to adult cattle is through the calving period with at least two treatments three-to-four weeks apart.
- The main aim of these treatments is to prevent infection of cattle during the vulnerable period of calving and early lactation.
- Controlling tick growth in and around the cattle shed by

  a. Regular pruning the shrubs surrounding the cattle shed and allowing direct sunlight.
  b. Maintaining a grass and shrubs free area around the cattle shed and maintaining that area dry.
  c. Cleaning the cattle shed with hot water and with an acaricide.
  d. Spraying acaricide around the cattle shed.

Treatment

- Diminazine Aceturate (Berenil) - **Babesia**
- Buparvoquone -Withdrawal periods should strictly adhere. (Milk-48 hrs, meat-42 days) - **Theileria**
- Imidocarb dipropionate- **Babesia**, **Theileria**, **Anaplasma**
- OTC- **Theileria**, **Anaplasma**

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Is it really anthelmintic resistance...???

The division of Parasitology/ VRI has had several inquiries from field veterinary surgeons regarding anthelmintic resistance. The common problem was frequent worm infestation despite treatment with anthelmintics in the market. In order to investigate the problem, the farms were visited and anthelmintic resistance trials were conducted with Albendazole and Levamisole. A thorough history of the de-worming schedule was obtained. The grazing land was also inspected and herbage was collected to calculate the intensity of larval contamination. The results revealed that the efficacy of Albendazole and Levamisole in controlling nematodes in cattle was 100% and 98.7% respectively and provided very effective control.

Then what was the problem??

The grazing lands were heavily contaminated with nematode larvae and re-infection was the reason for frequent disease outbreaks!!

Advice:

The farmers were advised to pay attention to management practices and rest the pasture for a while and do cut and feeding for a few months or practice rotational grazing.
Whenever possible it is advisable to keep animals grouped according to their age and send them for grazing.

They were advised to contact the range veterinary surgeon if symptoms, such as diarrhea, lethargy or weight loss was observed. Although anthelmintic resistance was not a problem in the farms investigated, it may be a problem in other farms. If you have any doubt please contact the Dr (Ms). N.D.Senasinghe Head, Parasitology Division, VRI and we are ready to help you with all parasitological problems of your animals.

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Leishmaniasis

Leishmaniasis is a vector borne disease caused by a protozoan parasite called Leishmania spp. The disease is seen in canids including domestic dogs and in humans, thus is considered as an important zoonosis disease worldwide. Infected sand fly acts as the mode of transmission in both animals and humans.
In canids, the disease is manifested by cutaneous lesions that are often found in hairless areas. In addition to that, a number of non specific signs including fever, loss of appetite and lethargy can also be seen.
In humans, the disease is presented in three different forms; visceral leishmaniasis, cutaneous leishmaniasis and muco-cutaneous leishmaniasis where the cutaneous form is being the commonest presentation in Sri Lanka. People with malnutrition, concurrent diseases and poor domestic sanitation are more vulnerable to acquire the disease.
The tentative diagnosis of the disease in both humans and canids basically depends on the clinical presentation where laboratory tests can be used to confirm the disease. Timely treatment often results in good prognosis. Early diagnosis and treatment, vector control and control of animal reservoirs are the measures to be taken to control the disease.
Please contact Veterinary Research Institute for further details.

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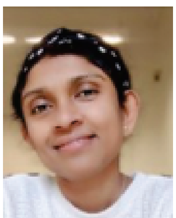
Campylobacter contamination in your kitchen

Foodborne infections are a significant public health problem and are a common cause of illness and death worldwide. In 2010, the "WHO initiative to estimate the global burden of food-borne diseases", reported that the consumption of contaminated food resulted in 600 million cases of illness and 420,000 deaths worldwide (WHO, 2015).
In recent years, campylobacteriosis has been listed as a leading cause of bacterial gastrointestinal infection in humans which commonly occurs by consumption of contaminated undercook chicken meat. Disease symptoms can vary from asymptomatic to severe enteritis and include abdominal cramping, fever, and bloody diarrhea, which last for 5-7 days. Most cases of campylobacteriosis are sporadic and self-limiting, but can occasionally lead to serious sequelae such as Guillain Barre syndrome and Miller-Fisher syndrome in immune-suppressed patients.
C. jejuni and C. coli are the primary causal agents for campylobacteriosis, mainly colonised in the chicken gut and contaminate chicken meat during processing. As the infection dose of Campylobacter in humans is as low as 500-800 cells, reducing of cross - contamination is very important. Studies have reviled that 75-80 % of chicken meat and meat by-products are contaminated, mostly the bacteria found on the surface of the meat, not the inside. Furthermore, Campylobacter in meat juice from thawed or raw poultry packages also represents a significant risk as the meat juice could potentially contaminate ready-to-eat foods or combined with poor hygiene lead to cross-contamination in the kitchen. Cross-contamination has been identified as an important contributory factor in a number of outbreaks.
In Sri Lanka, consuming of frozen meat is the most common eating pattern. But with the changes in the life style, consuming of chill chicken, especially purchasing of parts, such as drumsticks, thigh, breast, and wings from the supermarkets and also curry chicken from the wet markets have increased. Risk of persistent of Campylobacter in chill chicken is significant. Notably, purchasing of chicken from the wet market has increased due to the present economic crisis. The risk of contamination of the food chain is significantly high due to the poor hygienic conditions in the wet market. The use of high temperatures and prolonged time of cooking might reduced the outbreaks in Sri Lanka. That's because except the termotolerents, Campylobacter can not survive over 50°C.

Increased consumption of undercooked chicken meat such as barbecue, meat salads pre cooked meat products would be the future challenge in food safety.
According to the studies, it has been proved the outbreaks are not only due to the consumption of chicken meat, but also the contamination of the kitchen and the refrigerator. Therefore it is very important to reduce cross-contamination in the kitchen. Adhere to food safety rules such as clean, chill, cook, and separate are very important in reducing cross-contamination in your kitchen. Washing chicken before cook is not advised by WHO and CDC, as it disseminate bacteria, and many research have shown it causes significant cross-contamination of the kitchen bench and utensils. In Sri Lanka, usually chicken meat always wash properly before cooking, considering the hygiene. Therefore if you are washing, it is important to avoid washing of the chicken meat directly under the tap in the sink, it could spill the contaminated water over the kitchen bench and increase the risk of cross-contamination. It is very important to wash your hands and utensils using a sanitizer after touching meat before preparing another dish, especially a vegetable salad or a fruit salad. It is highly advised to use separate cutting boards and knives for meat. When you are preparing ready-to-eat foods, it is not advisable to use the same kitchen bench while you are preparing a chicken meat dish.
Many research studies have suggested that, when properly applied, kitchen biocides could be helpful in reducing the public health and food poisoning risks associated with kitchen cross-contamination with. Further storing of meat in the refrigerator also a very important risk factor. Meat has to be stored separately in tightly closed containers to prevent the dropping of meat juices on to other foods. It is highly advisable not to store chilled chicken for a long time in the refrigerator, as the prolonged viability of Campylobacter in chicken meat juice may increase the risk of human infections. So better to purchase chilled chicken which is enough for a dish per meal. Avoid the reuse of meat packages or polythene bags given from the meat store, because the chicken juice can cross contamination the packages and bags, due to the oily texture it won't be removed easily. We, chicken meat lovers, should always be aware of the possibility of getting campylobacteriosis from our kitchen to fork.



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Nutritional quality analysis of under-utilized feed ingredients to be used in animal feed rations

Feed is 70-90% cost of production in animal products. Sri Lanka imported 0.3 MMT feed ingredients at a cost of 31 BLKR (2021). Yet, certain abundant under-utilized feed resources are only used in small scale animal production. Such ingredients can be utilized to reduce importation cost slightly, if the nutrient composition is determined. Thus, six, abundant, under-utilized, local agro-industrial byproducts were selected: corn residue, manioc leaves, manioc roots, sugarcane residue, soya spent grain (sauce production) and beer-pulp. Six replicate samples from each collected and analysed. Manioc leaves and soya spent grain had 20-30% crude protein, therefore, useful as poultry feed but limited inclusion of manioc leaves due to high fiber (18%). Corn residues and sugarcane residues suitable for ruminant feeding after enrichment. Manioc roots has high NFE (87%), indicating high level of sugars and starches, so possible energy supplement.

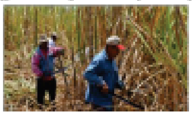

All analysed ingredients had, at least one of macro or micro minerals, at a significant level. If processed properly, the above feed ingredients can be utilized as animal feeds and has an added advantage of mineral supplementation.

Corn (*Zea mays*) residue - : dry tree after harvesting the crop

Mannioc (*Manihot esculenta*) leaves

Mannioc (*Manihot esculenta*) roots -: edible starchy tuber

Sugarcane (*Saccharum officinarum*) residue -:plant top



Soya (*Glycine max*) spent grain-: Soy sauce production

Beer-pulp-: liquid by product of the fermentation process

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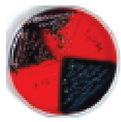
Biofilm Formation and Antimicrobial Resistance in Staphylococci associated with Bovine Mastitis

A biofilm is an assemblage of bacteria which are attached to a surface. It may form on a variety of surfaces including both living tissue and non living devices. Biofilms are enclosed in an extracellular polymeric substance (EPS) matrix. EPS matrix of a biofilm may vary in chemical and physical properties but it is primarily composed of polysaccharides. Different bacterial organisms produce biofilms of different properties depending on the type of EPS.

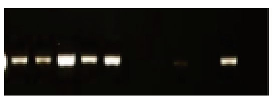
According to the literature biofilms are responsible for antimicrobial resistance in bacterial infections. One suggested mechanism is preventing transport of antimicrobial agents through the biofilm, by binding antimicrobial agents directly to the EPS matrix. Second proposed mechanism is by providing an ideal environment for the exchange of extrachromosomal DNA in plasmids which encode for resistance to antimicrobial agents.

In bovine mastitis, producing biofilms by Staphylococci has been identified as a virulent factor that enables adherence and colonization on the mammary gland epithelium leading to prolonged infections. Since biofilms can contribute to high prevalence of mastitis, a study was conducted to determine the ability of biofilm formation and antimicrobial resistance among Staphylococcus spp. (17 isolates) obtained from subclinical bovine mastitis cases at various farms.

All isolates were subjected to biofilm detection using the Congo Red agar method and susceptibility to six antimicrobial agents was examined by Kirby-Bauer disk diffusion method (CLSI). Fifty nine percent of the isolates were biofilm formers. Biofilm producing isolates showed > 70% resistance to ampicillin, cloxacillin, streptomycin and tetracycline. Lack of susceptibility to ampicillin and streptomycin was statistically significant ($P<0.05$) among biofilm formers compared to non-biofilm producers. All tested isolates were susceptible to gentamicin and had lower resistance to cephalexin (18%) and enrofloxacin (5.8 %).



Biofilm producing bacterial colonies



Antimicrobial resistance (AMR) gene detection by PCR

This study revealed the ability to produce biofilms by Staphylococci in bovine mastitis and increased antimicrobial resistance among biofilm producers. Monitoring biofilm formation, antimicrobial resistance and detection of related bacterial genes is important to design new mastitis control strategies.

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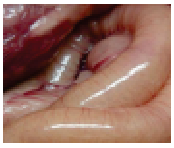
A major reason for growth retardation in broiler chickens

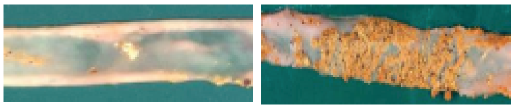
Necrotic enteritis (NE) is a widespread and economically important disease in broiler chickens caused by Clostridium perfringens. Feed quality and management stresses are major factors induce the multiplication of the commensal C. perfringens to become an opportunistic pathogen and induce the pathogenesis of NE. The subclinical form is not usually detected due to the absence of clear clinical signs therefore, it is not treated and prevails unnoticed apart from a poor growth performance, wet litter conditions and the possible contamination of poultry products for human consumption.
The damage in the world's poultry industry due to the disease has been estimated based on liver discards only as $ 20 billion (7200 billion LKR). The estimation has not counted the growth retardation which is the main damage to broiler production industry currently.
The clinical form first reported in 1970 in Sri Lanka is an earliest report of the disease, worldwide (Wijewanta and Senaviratna, 1971). In a small study, 88% prevalence of the sub-clinical NE reported in North Western Province of Sri Lanka. Before the introduction of the prophylactic antibiotics into animal feeds (1960s-1970s) clinical NE was a significant concern. However, the disease was not reported globally after the introduction of antibiotic growth promoters.

The disease re-emerged largely as clinical and subclinical forms, in countries where the feed grade antibiotics were banned from animal feeds. Strict bio-security measures controlled the clinical NE with an acute onset, but the sub-clinical form prevails causing production drop in broilers.



Ballooned Intestines

Liver lesions



Gross lesions of the Intestines

Disease needs to be suspected whenever you notice poor growth performances and unusually wet litter conditions in broiler flocks at the age of 2-5 weeks. For diagnosis birds needed to be sampled randomly from the suspected flocks and the postmortems lesions will leads to a tentative diagnosis.
Small intestinal focal necrotic lesions are initially similar to lesions of coccidiosis. However, the size of the necrotic patches increase with increasing severity. Lesions are commonly observed around 14-30 days of age in broiler chickens. In addition to the necrotic lesions, pseudo membrane formation on the mucosal surface of the small intestine will be observed at the age of 20-35 days. Intestinal damage at the fast growing age leads to poor weight gain. Liver and gallbladder lesions (Cholangio-hepatitis) such as congested, fibrosed or necrotic patches in livers or enlarged gall bladders with thick bile are commonly identified at the latter stage of the disease. Disease confirmation with intestinal or liver histopathology subjected to immune fluorescence or liver cultures confirming C. perfringens. Further, the caecal C. perfringens counts above 10^6 colony forming units are also a confirmatory tool.

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