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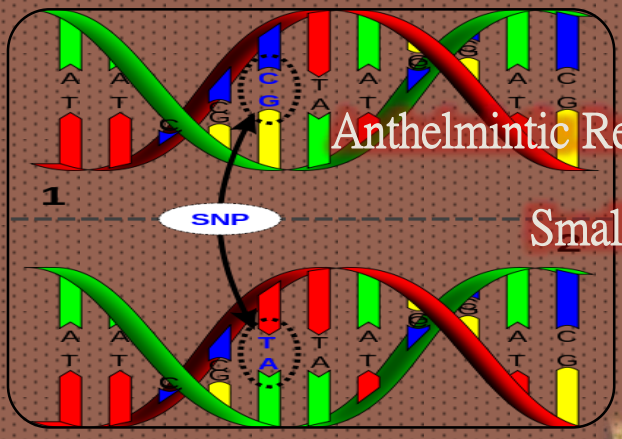

Anthelmintic Resistance - A Serious Set Back To Small Ruminant Production

A1 and A2 Variants of Beta Caseins in Cow Milk and Human Health

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Cholesterol – Good or Bad?

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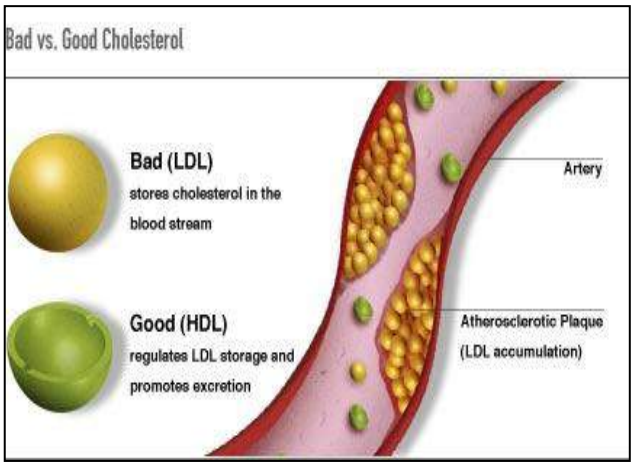
Cholesterol is one of the steroids, formed in cell membranes. Cholesterol - a waxy compound that some have likened to soft candle wax, is a kind of sterol, which is found naturally in the tissues of both plants and animals, though only animals have cholesterol.



Cholesterol is synthesized in our body as well as we take it in our diet also. It is synthesized from acetyl CoA by the liver. Now, cholesterol is transported in blood in lipoprotein particles, which are further of different types e.g. very low density lipoprotein (VLDL) and high density lipoprotein (HDL). VLDL transports lipids including cholesterol, to the tissues of the body and HDL remove cholesterol from tissues. High level of VLDLs in blood may be associated with atherosclerosis caused by buildup of cholesterol rich deposits in the walls of arteries. These make the lumen of artery smaller, reducing blood flow and increasing blood pressure, can also lead to blood clots. The clots may block blood vessels leading to heart attack (myocardial infarction), if coronary arteries supplying the heart muscle are blocked, or stroke, if arteries supplying the brain are affected. We manufacture, though, most of our cholesterol - about 85%, though estimates vary, only about 15% comes from food.

Atherosclerosis

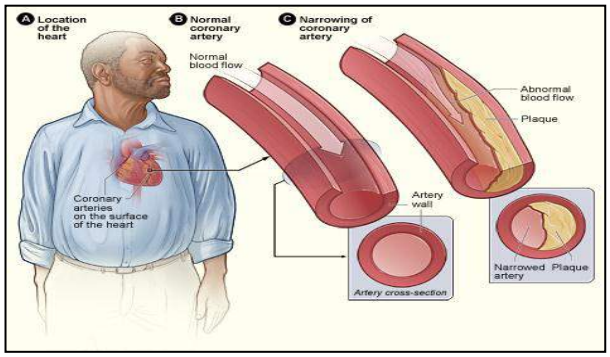
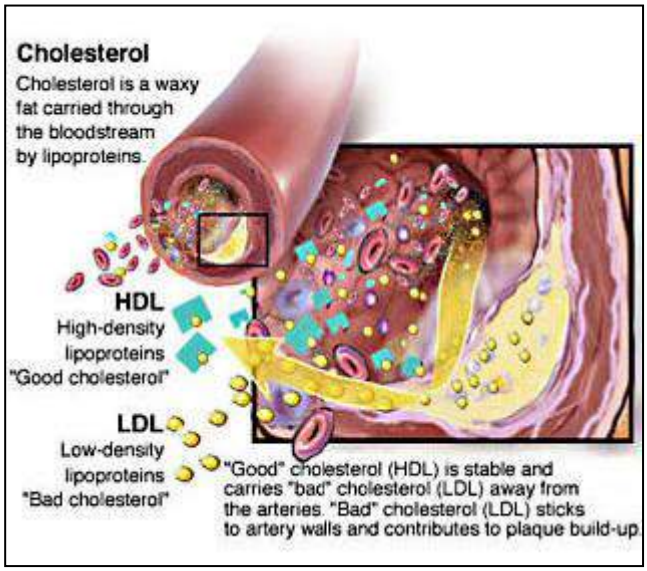


Figure-A. Shows the location of the heart in the body. Figure-B. Shows a normal coronary artery with normal blood flow. The inset image shows a

cross-section of a normal coronary artery. Figure-C. shows a coronary artery narrowed by plaque. The buildup of plaque limits the flow of oxygen-rich blood through the artery. The inset image shows a cross-section of the plaque-narrowed artery.

Cholesterol requirement in body

Cholesterol performs several important functions in the body. Cholesterol is found in every cell in our body and without it our bodies would not function properly. Cholesterol is a structural component of cells. Cholesterol along with polar lipids make-up the structure of each and every cell in our bodies. Cholesterol is there to basically provide a protective barrier. When the amount of cholesterol increases or decreases, the cells are affected. This change can affect our ability to metabolize and produce energy. This can ultimately affect other aspects of our bodies' function such as food intake and digestion. Perhaps the most important of these is its role in forming and maintaining cell walls and structures. Cells also need cholesterol to help them adjust to changes in temperature and it's used by nerve cells for insulation. Additionally, cholesterol is essential for synthesizing a number of critical hormones, including the sex hormones i.e. testosterone, progesterone and estrogen. Cholesterol plays an important role in our body's digestion.



Cholesterol is used to help the liver to create bile, which aids us in digesting the food that we eat. Without the bile our bodies are unable to properly digest foods, especially fats. When the fat goes undigested it can get into the bloodstream and cause additional problems such as blockages of the arteries and cause heart attacks and heart disease. Cholesterol is also needed to make vitamin D; in the presence of sunlight, cholesterol is converted into vitamin D.

Dietary sources

Animal fats are complex mixtures of triglycerides, with lesser amounts of phospholipids and cholesterol. As a consequence, all foods containing animal fat contain cholesterol to varying extents. Major dietary sources of cholesterol include

cheese, egg yolks, beef, pork, poultry, fish, and shrimp. Human breast milk also contains significant quantities of cholesterol. From a dietary perspective, cholesterol is not found in significant amounts in plant sources. In addition, plant products such as flax seeds and peanuts contain cholesterol-like compounds called phytosterols, which are believed to compete with cholesterol for absorption in the intestines.

Biosynthesis

All animal cells manufacture cholesterol for their use, with relative production rates varying by cell type and organ function. About 20–25% of total daily cholesterol production occurs in the liver; other sites of higher synthesis rates include the intestines, adrenal glands, and reproductive organs.

Regulation of cholesterol synthesis

Biosynthesis of cholesterol is directly regulated by the cholesterol levels present, though the homeostatic mechanisms involved are only partly understood. A higher intake from food leads to a net decrease in endogenous production, whereas lower intake from food has the opposite effect. The main regulatory mechanism is the sensing of intracellular cholesterol in the endoplasmic reticulum (cell organell) by the protein SREBP (sterol regulatory element-binding protein 1 and 2). In the presence of cholesterol, SREBP is bound to two other proteins: SCAP (SREBP cleavage activating protein) and Insig1. When cholesterol levels fall, Insig-1 dissociates from the SREBP-SCAP complex, which allows the complex to migrate to the Golgi apparatus. Here SREBP is cleaved by S1P and S2P (site-1 and -2 protease), two enzymes that are activated by SCAP when cholesterol levels are low.

Cholesterol levels for men

A number of different factors contribute to our overall cholesterol level, but the general rule is that that number should always stay below 200. Any level over 200 is borderline high while anything over 240 is considered high and poses a real health risk. While metabolism helps children and young men to maintain a healthy cholesterol level, it is normal for most men to see a dramatic change in their levels over the age of 40. The best way to demonstrate this is by the average cholesterol readings for those two age groups. For example, men under the age of 40 have an average overall cholesterol reading of 185; that jumps to an average of 205 for the ages of 40-49 and to 208 for 50 and over. In other words, the average man over 40 is now at risk of heart disease due to high cholesterol.

Cholesterol levels for women

As with men, the ideal cholesterol levels for women of all ages are under 200, and the lower they go the better. Women have the biggest issues maintaining a healthy cholesterol level following menopause. Women under the age of 40 actually have a lower average cholesterol score than men of the same age (183), but that average jumps to a borderline score of 194 between the ages of 40 and 49. By 50-59 years, cholesterol levels for women overtake those of men, coming in at a dangerous average of 219.

Cholesterol levels in different daily foods:

	Food	Cholesterol (mg/100g)
1.	Egg Yolk	1234
2.	Caviar (Fish Roe)	588
3.	Liver, Pate, Foie Gras	564
4.	Butter	215
5.	Shrimp	195
6.	Fast Foods (Breakfasts)	172
7.	Oil Packed Fish	142
8.	Cheese	123
9.	Processed Meats (Sausage Lamb, Duck)	158
10.	Shellfish (Oysters, Clams, and Mussels)	105
11.	Cookies, Cakes, Pies, and Brownies	117
12.	Fried Chicken (esp. Fast Foods)	112
13.	Toffee	104
14.	Ice Cream	92
15.	Whole Milk (3.25% Milkfat)	10

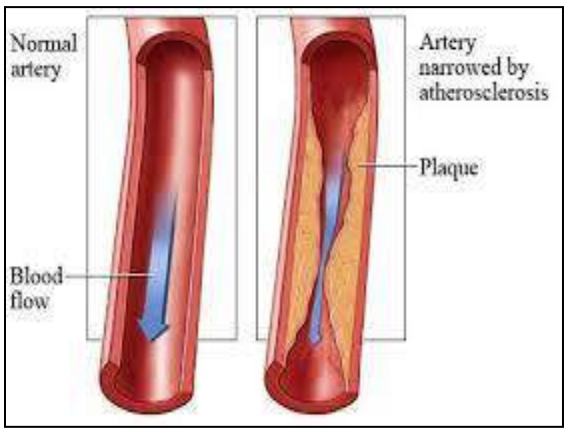
High Risk Groups who need to limit or eliminate cholesterol consumption:

- Individuals with a family history of high cholesterol - Regulation of cholesterol blood levels are hereditary and it is advisable to find out if relatives have high cholesterol levels.
- Older adults - cholesterol levels rise with age, particularly in post-menopausal women.
- Over weight individuals - being over-weight increases risk of heart-disease.
- People with low physical activity levels - Excersize is an effective way to lower bad cholesterol levels (LDLs) and raise good cholesterol levels (HDLs). People who are not physically active are at risk for high cholesterol levels.
- Individuals with high blood pressure - High blood pressure in combination with high cholesterol levels greatly increases the risk of heart disease and heart attacks.
- Smokers - Individuals who smoke cigarettes have a higher risk of heart disease and should avoid high cholesterol foods.

Cholesterol: Top 5 foods to lower your numbers

Diet can play an important role in lowering our cholesterol. Here are five foods that can lower our cholesterol and protect heart. Can a bowl of oatmeal help lower our cholesterol? How about a handful of walnuts or even a baked potato topped with some

heart-healthy margarine? A few simple tweaks to our diet — like these, along with exercise and other heart-healthy habits — may be helpful in lowering our cholesterol.



1. Oatmeal, oat bran and high-fiber foods

Oatmeal contains soluble fiber, which reduces our low-density lipoprotein (LDL), the "bad," cholesterol. Soluble fiber is also found in such foods as kidney beans, apples, pears, barley and prunes. Soluble fiber can reduce the absorption of cholesterol into our bloodstream. Five to ten grams or more of soluble fiber a day decreases our total and LDL cholesterol. Eating 1&1/2 cups of cooked oatmeal provides 6 grams of fiber. If we add fruit, such as bananas, we'll add about 4 more grams of fiber. To mix it up a little, try steel-cut oatmeal or cold cereal made with oatmeal or oat bran.

2. Fish and omega-3 fatty acids

Eating fatty fish can be heart healthy because of its high levels of omega-3 fatty acids, which can reduce your blood pressure and risk of developing blood clots. In people who have already had heart attacks, fish oil — or omega-3 fatty acids — reduces the risk of sudden death. The American Heart Association recommends eating at least two servings of fish a week. The highest levels of omega-3 fatty acids are in: Mackerel, Lake trout, Herring, Sardines, Albacore tuna, Salmon, Halibut. We should bake or grill the fish to avoid adding unhealthy fats. If you don't like fish, you can also get small amounts of omega-3 fatty acids from foods like ground flaxseed or canola oil. You can take an omega-3 or fish oil supplement to get some of the benefits, but you won't get other nutrients in fish, such as selenium. If you decide to take a supplement, just remember to watch your diet and eat lean meat or vegetables in place of fish.

3. Walnuts, almonds and other nuts

Walnuts, almonds and other nuts can reduce blood cholesterol. Rich in polyunsaturated fatty acids, walnuts also help keep blood vessels healthy. Eating about a handfull (1.5 ounces, or 42.5 grams) a day of most nuts, such as almonds, hazelnuts, peanuts, pecans, some pine nuts, pistachio nuts and walnuts, may reduce our risk of heart disease. Just make sure the nuts we eat aren't salted or coated with sugar. All nuts are high in calories, so a handful will do. To avoid eating

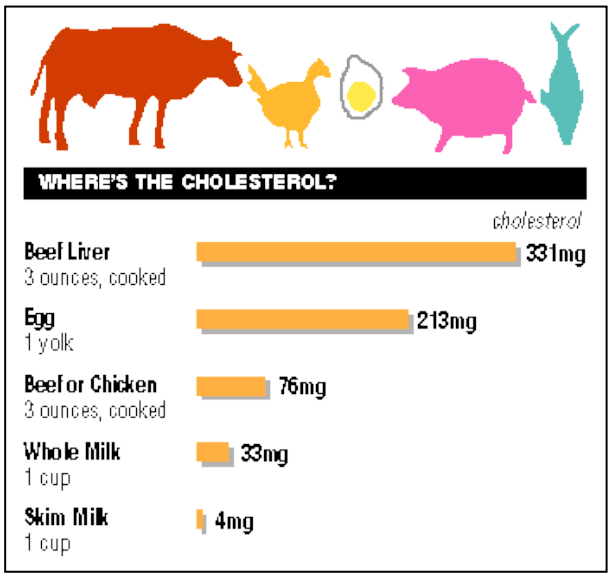
too many nuts and gaining weight, replace foods high in saturated fat with nuts. For example, instead of using cheese, meat or croutons in salad, add a handful of walnuts or almonds.

4. Olive oil

Olive oil contains a potent mix of antioxidants that can lower our "bad" (LDL) cholesterol but leave our "good" (HDL) cholesterol untouched. Try using about 2 tablespoons (23 grams) of olive oil a day in place of other fats in diet to get its heart-healthy benefits. To add olive oil to diet, we can saute vegetables in it, add it to a marinade or mix it with vinegar as a salad dressing. We can also use olive oil as a substitute for butter when basting meat or as a dip for bread. Olive oil is high in calories, so don't eat more than the recommended amount. The cholesterol-lowering effects of olive oil are even greater if choosen extra-virgin olive oil, meaning the oil is less processed and contains more heart-healthy antioxidants. But keep in mind that "light" olive oils are usually more processed than extra-virgin or virgin olive oils and are lighter in color, not fat or calories.

5. Foods with added plant sterols or stanols

Foods are now available that have been fortified with sterols or stanols — substances found in plants that help block the absorption of cholesterol. Margarines, orange juice and yogurt drinks with added plant sterols can help reduce LDL cholesterol by more than 10 percent. The amount of daily plant sterols needed for results is at least two grams — which equals about two 8-ounce (237-milliliter) servings of plant sterol-fortified orange juice a day. Plant sterols or stanols in fortified foods don't appear to affect levels of triglycerides or of high-density lipoprotein (HDL), the "good" cholesterol.



Other changes to our diet

For any of these foods to provide their benefit, we need to make other changes to your diet and lifestyle. Cut back on the cholesterol and total fat — especially saturated and trans fats — that we eat. Saturated fats, like those in meat, full-fat

dairy products and some oils, raise our total cholesterol. Trans fats, which are sometimes found in margarines and store-bought cookies, crackers and cakes, are particularly bad for our cholesterol levels. Trans fats raise low-density lipoprotein (LDL), the "bad," cholesterol, and lower high-density lipoprotein (HDL), the "good," cholesterol. In addition to changing the diet, keep in mind that making additional heart-healthy lifestyle changes are key to lowering cholesterol. Take advice from doctor about exercising, quitting smoking and maintaining a healthy weight to help keep cholesterol level low.

Pharmacogenetics and the Concept of Individualized Medicines

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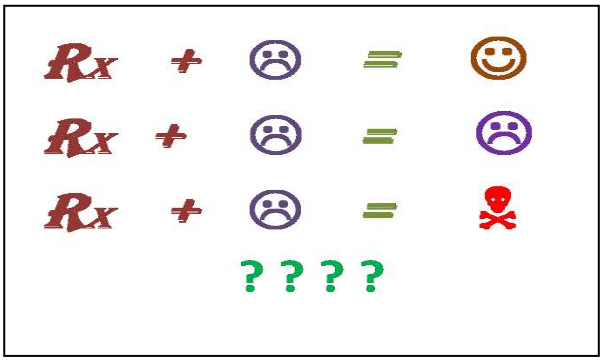
Inter-individual variation in drug response among patients and animals is well known and poses a serious problem in medicine. There are no biomarkers at present that can predict which group of patients or animals responds positively, which are non-responders and who experiences adverse reactions for the same medication and dose. Physicians and veterinarians have to optimize a dosage regimen for an individual patient or animal by a trail- and – error method. This kind of blind approach may cause adverse drug reactions. Adverse drug reaction in patients causes more than 2 million hospitalization including 1,00,000 deaths per year in the United States. But, there is no proper record of adverse reaction available in India both for human and animals. The known sensitivity of collies breeds of dog to p-glycoprotein substrates (macrolytic lactones, loperamide and corticosteroids) in normal doses is most important in veterinary practice. This adverse drug reaction could be due to multiple factors such as disease determinants, environment and particularly genetic factors. It is now clear that much individuality in drug response is inherited; this generally determined variability in drug response defines the research area known as pharmacogenetics. Identification and characterization of a large number of genetic polymorphisms (biomarkers) in drug metabolizing enzymes, drug targets and drug transporters may provide substantial knowledge about the mechanism of inter-individual differences in drug response. Pharmacogenetics and pharmacogenomics are the two recent developments to investigate inter-individual variation and drug response. This knowledge may ultimately allow the



development of personalized medication based on the genotype of each patient.

Availability of high density genomic SNP maps and rapidly expanding known functional polymorphisms, have generated high expectations for applying pharmacogenetics to the optimization of therapies for individual patients. Pharmacogenetics is a harbinger of personalized medicine, a paradigm shift from the mindset of **"one – drug – fits – all" to "the right drug for the right patient at right dose and time"**

Pharmacogenetics

Study of the genetic basis for variation in drug response.

Pharmacogenomics

Tool for surveying the entire genome to assess multigenic determinants of drug response.

Molecular Mechanism of genetic polymorphism

Polymorphism

It is a variation in the DNA sequence occuring with a frequency of 1% or more in the population. There are two types of genetic variants,

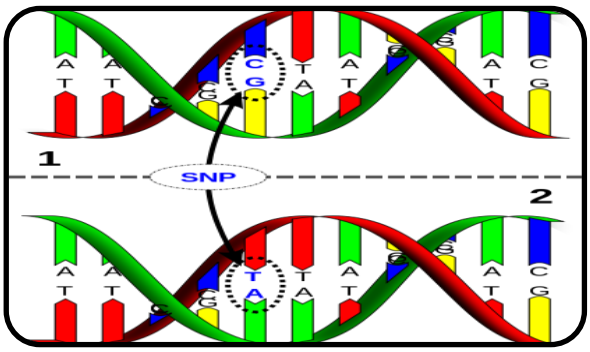
a. Single Nucleotide Polymorphism (SNP)
b. Insertions/ Deletions (Indels)

a. SNP (single nucleotide polymorphism)

It is most frequent type of genetic variant and occurs usually 1/100 to 1000 base pair. SNP occurs in the coding regions and non-coding regions of genes and produces changes in protein structures, stability, substrate affinities, splicing, or introduces stop codon. SNP are associated with significant changes in drug efficacy and drug disposition.

b. Insertions/ Deletions (Indels)

This is the second major type of polymorphism. It involves gene duplications (insertions) and gene deletions that results in the complete lack



of protein production, or inversions of genes that may disrupt gene function.

Genetic polymorphism occurs in

- Drug Target proteins
- Drug Metabolizing Enzymes
- Drug Transporters

Pharmacogenetics in drug development:

Pharmacogenetics can be used to improve drug discovery and drug development in 2 ways,

- Development of new drugs to overcome drug resistance or target new receptor
- Optimization of drug metabolism and pharmacokinetics to minimize variations in drug level.

Ethnic diversity

Pharmacogenetics may be highly useful in the development of drug that works well with certain population groups. To develop drug, we need to consider ethnical difference in different population. There exists inter-ethical difference in polymorphism of genes encoding metabolizing enzymes, transporters and disease associated proteins. Drug treatment may be tailored for greater effect if important genetic variation exists between racial and ethnic groups. By knowing these variants, patients can be classified into low, intermediate and high dose groups. Pharmacogenetics study on race and ethnicity is worthwhile because these are useful indicators of genetic variation. However, this kind of race and ethnicity classification for medical treatment leads to discrimination.

Species differences in drug administration

Veterinarians must be aware of differences between species and also of differences that can occur among the breeds.

1. Ivermectin can cause CNS depression in collies breed of dog at normal doses due to defect in the P-glycoprotein transporter in the brain.
2. Ivermectin should not be used in tortoise and crocodiles because of potential toxic effects.
3. Ruminants have α2D receptors; hence, xylazine is a much more potent sedative in cattle than other species.
4. Great Dane and Irish Setters are more sensitive to bloat following xylazine administration due to aerophagia.
5. Cats have a low level of glucuronyl transferase enzyme; hence, they are highly sensitive to aspirin, paracetamol and phenolic compounds.
6. Morphine is more potent in cats than dogs. In dogs, the dose is 1 mg/kg to produce the analgesia. In cats, the dose for producing analgesia is 0.1mg/kg. Higher doses in cats may produce excitement.
7. Benzimidazoles anthelmintics are administered once to herbivores, but to non-herbivores, daily for 3 to 5 days. The administered anthelmintics drugs stay for longer

period in the GI tract of ruminant-heribivores animals than non-herbivores. Hence, more absorption takes place in the ruminant-herbivores.

8. Xylazine, an alpha2-adrenergic agonist, used widely in several species as a sedative, is also a reliable emetic, particularly in cats, in which it stimulates the chemoreceptor trigger zone (CTZ) in the medulla oblongata, while it does not induce emesis in species such as ruminants and horses lacking the vomiting reflex.
9. The cholinesterase levels in the ruminants are lower than horses and humans and hence, ruminants are highly sensitive for organophosphorous poisoning than horses and humans.
10. The muscle relaxant drug succinyl choline is metabolized by the plasma esterase enzyme. The level of enzyme is much lower in ruminants compared to horses. Hence, ruminants require lower doses (0.02mg/kg body weight) than the horses (0.1mg/kg body weight).
11. The cold blooded animals like fishes and reptiles have much lower metabolic rate compared to mammalian species. Hence, they require less dosage of drugs compared to mammals.
12. Goat metabolizes the benzimidazoles group of anthelmintics quickly than the cattle and sheep. Hence, goat requires benzimidazoles drugs two times of the cattle dose.

HACCP Based Food Safety System in a Typical Fresh Frozen Buffalo Processing Unit

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Fresh frozen buffalo processing unit is committed for implementation of an effective HACCP system. During hazard identification, evaluation, and subsequent operations in designing and applying HACCP system consideration has been given to the impact of raw materials, ingredients, food manufacturing practices, role of manufacturing processes to control hazards, likely end use of the product, categories of consumers of concern, and epidemiological evidence related to food safety. The intent of the HACCP system is to focus control of CCPs. Redesign of the operation is considered if a hazard which must be controlled is identified but no CCPs are found. HACCP is applied to each specific operation separately. HACCP application is reviewed and if necessary the changes are made when any modification is made in the product, process or any step. During HACCP implementation due consideration has been given to the nature and size of the operation. Basically all the activities, being carried out at Fresh frozen buffalo processing unit, inside the premises are included in the scope. The scope for the application of the HACCP based Food Safety System in Fresh frozen buffalo processing unit is as below.

Scope of application

- Receipt and unloading of carcasses
- Washing
- Chilling
- De-boning
- Fresh packing
- Freezing
- Frozen packing
- Container loading
- Dispatch

Product list: Topside, Knuckle, Silverside, Rumpsteak, Cube Roll, Chunck, Tender Loin,Shank, Slice, trimming & Related cuts.

Subcontracted activities:

- Calibration of equipment
- Pest control
- Water analysis
- Internal audit conduction

HACCP SYSTEM REQUIREMENT

Management Responsibility:

The Management of Fresh frozen buffalo processing unit is responsible for the safety (and suitability) of the processed food. Fresh frozen buffalo processing unit has a specific Food Safety Policy.The policy of Fresh frozen buffalo processing unit has been documented, displayed and communicated to all concerned. Periodically, Fresh frozen buffalo processing unit verifies the implementation of the policy and reviews the outcome. The HACCP system enables the management of Fresh frozen buffalo processing unit to demonstrate its commitment and responsibility with respect to the supply of safe products. The HACCP system ensures that all required activities are effectively defined, implemented and maintained.

FOOD SAFETY POLICY

Fresh frozen buffalo processing unit is committed to provide safe and hygienic food (fresh frozen boneless buffalo meat) to our customers.

Fresh frozen buffalo processing unit shall implement and maintain the Food Safety Management System

Fresh frozen buffalo processing unit shall comply with the applicable requirements of these standard as well as statutory and regulatory requirements.

Fresh frozen buffalo processing unit shall continually improve the effectiveness of the implemented food safety management system.

Food Safety Objectives:

FOOD SAFETY OBJECTIVES (from XX-YY-ZZ to XX-YY-ZZ)

1. Aim for at least **XX** % customer satisfaction index based on the questionnaire sent to customers
2. Aim for at least **XX** % cumulative supplier rating figure based on the re-evaluation criteria
3. Aim for at least **XX** % competence index of both management staff and operational staff members
4. Aim for being HACCP and ISO 9001-2008 compliant and certified organization
5. Aim for 0% deviation from the critical limits w.r.t. all CCPs
6. Aim for 0% down-time for all freezers
7. Aim for zero food safety related customer complaints

HACCP team :

Assembling of HACCP team

Fresh frozen buffalo processing unit assures that appropriate product specific knowledge and expertise is available for the development of an effective HACCP plan. This has been accomplished by assembling a multi disciplinary team. Expert advice is obtained from external sources, whenever required.

Criteria to become HACCP Team Leader/ member, Validation Team Leader/Member, Emergency Team Leader/Member,

Sr.No.	Criteria	Stream 1
1	Minimum qualification	Food Technologist
2	Minimum years of experience	--
3	Minimum training undergone	a. HACCP basis b. FSS basis
4	Minimum skill required	a. Communicating in local language b. Motivating others c. Basic process knowledge d. d.) Basic product knowledge

Scope of the HACCP plan is as follows.

Receipt and unloading of carcasses, Washing, Chilling, De-boning, Fresh packing, Freezing, Frozen packing, Container loading till Dispatch.

HACCP Team

Sr.No	Name	Team Design.	Organizational designation
1	X	Team Leader HACCP	Q.C. officer
2	Y	Team member	Manager, production and maintenance
3	Z	Team Member	Asst Manager Production
4	W	Team Member	Stores / Dispatch officer

Resources:

The management examines the requests and provide, in a timely manner, all the resources needed by the HACCP team(s) to develop, implement and maintain the HACCP system. When corrective actions, verification procedures or customers indicate that operational improvements are necessary, the management shall examine the issues and provide appropriate resources to ensure food safety.

Management Review

Plan for MR

Fresh frozen buffalo processing unit reviews the HACCP system at planned intervals of every 12 months, to ensure continuing suitability, adequacy and effectiveness. The review evaluates the need for changes to the HACCP system, including product safety, policy and objectives. The review provides evidence of the commitment to improve the HACCP system and its performance. The minutes of MRM are recorded and maintained As an evidence of the commitment to improve the HACCP system and its performance. Fresh frozen buffalo processing unit reviews and evaluates the results of the entire verification process at planned intervals, of no more than 12 months. Frequency of verification and internal audits shall be such that organization can ensure continuing suitability; adequacy

and effectiveness of the HACCP- based Food Safety System. For instance, the effective control of CCP is evaluated monthly, where as frequency of once a year is used to verify the actuality of process lines and layout. Fresh frozen buffalo processing unit collects and analyze the resulting data to evaluate where improvement is needed Fresh frozen buffalo processing unit ensure that preventive action are taken without undue delay to eliminate the causes of (potential) non conformities in order to prevent recurrence(occurrence). The preventive actions are appropriate to the effects of the (potential) non conformities encountered. The effectiveness of the preventive action is taken is validated. Follow–up action the verification and review of actions taken.

Product Information

Product Characteristics and Intended use

A full description of the product has been given below including relevant safety information such as composition, physical / chemical structure, microbial treatment,(Primary and secondary) packaging, durability (shelf life), storage conditions and method of transport.The intended use has been described. Vulnerable groups of the population have been stated.

Sr No	Aspect	Details
1.	Product name	Boneless frozen fresh buffalo meat (Topside, Knuckle, Silverside, Rumpsteak, Cube Roll, Chunck, Tender Loin, Shank, Slice, trimming & Related cuts)
2.	Raw Material	**Fresh Buffalo carcasses** **1**.Colour :- Reddish pink. 2.Odour :- Beefy 3.Temperature:-4-7^{o}c 4. Appearance:-
3.	Product characteristics Reference : A.34.07 Page No. 449-450 PFA 27 TH Edition 2009	**Raw Frozen (blast/ plate freezing)** **Physical :-** 1. Core temperature -18° C. 2. Colour :- Reddish pink. 3. Odour :- Beefy . Microbiological:- 1. TPC/gm: < 1 X 10^5 ; 2. E. coli/gm: < X 10^2 3. Staphyloccocus aureus/gm : < 1 X10^2 4. Salmonella/25gm : Absent 5.yeast and mould/gm : 1 X10^3 6. CL. Perfringes/gm : 30 7. CL. Botulinum/gm : 30 8. L. Monocytogenes/25gm : Absent **Chemical:-chlorine 30ppm:** **1. pH less than or equal to 6.0**

Process Information

Flow Diagram :

The flow diagram has been constructed by the HACCP team as follows. The flow diagr covers all steps in operation. When applying HACCP to a given operation consideration has been given to steps preceding and following the specific operation. The HACCP team confirms the processing operation against the flow diagram during all stages and hours of operation and amends the flow diagram where appropriate.

Hazard Analysis

The HACCP team has listed all hazards that reasonably expected to occur at each step from primary production, processing, manufacture and distribution till the point of consumption.

Hazard Identification and Risk Analysis &Control Measures

Parameters and Critical limits

a. Critical process and product parameters

For each specific control measure related to a CCP the process and/or product parameters are identified which are meant to demonstrate that control at the step is being maintained.

There may be more than 1 CCP at which control is applied to address the same hazard. For determination of CCP, a decision tree has been used (below), which indicates a logical reasoning approach.

List of CCPs

Step No. and name	Likely hazards and reason for the same	Control measure	**CCP (Y/N) and CCP no.**
(blast / plate) Freezing	M..not following required time and temperature combination	a)following of SOP for freezing	**Y** **CCP #1**
storage at -20°C	M..not maintaining required temperature	a)preventive maintenance of temperature controlling unit b)monitoring that stored volume / weight does not exceed designed specifications of storage space	**Y** **CCP # 2**
Metal Detector	P : presence of metal particles due to rusted hooks, knives and operators ornaments	a.) periodic verification of hoks and knives b.)well instructed operators c.) periodic monitoring of operators	**Y** **CCP # 3**

MONITORING AND MEASURING

HACCP Plan

The CCPs are monitored. Monitoring is the scheduled measurement or observation of a CCP relative to its critical limits. The monitoring method is able to detect the loss of control at the CCP.

The monitoring method also provides information in time to make adjustments to ensure control of the process to prevent violation of the critical limits. Where possible,

process adjustments are made when monitoring results indicate a trend towards loss of control at a CCP. The adjustments are done before a deviation occurs. Data derived from monitoring is evaluated by HACCP team to carry out corrective actions when indicated. When monitoring is not continuous, it is ensured that the amount or frequency of monitoring is sufficient to guarantee that the CCP is in control. All records and documents associated with monitoring are signed by the personnel doing the monitoring and by a responsible reviewing official. Specific corrective actions have been developed for each CCP in the HACCP system in order to deal with deviation when they occur. The action ensures that the CCP has been brought under control. Actions taken also includes proper disposition of the affected product. Verification and auditing methods, procedures and tests, including random sampling and analysis is used to determine if the HACCP system is working correctly. Frequency of verification is designed to confirm that the HACCP system is working effectively. Verification includes review of HACCP system and its records, review of deviations and product dispositions and confirmation that CCPs are kept under control. Validation (based on scientific literature and customer complaints) confirms the efficacy of all elements of the HACCP plan. Appropriate records pertaining to the HACCP system are maintained. MLOR lists the records maintained in the HACCP system.

Product release

Products can only be released when non conformities of products are absent and no corrective actions are necessary.

Corrective Actions

For each Critical Control Point, the documented corrective actions to be taken, is available, including the responsibilities and authorities of the personnel which is involved, in case an action-limit value or critical limit is exceeded. The procedure shall include the process to investigate the cause of the deviation. The actions to be taken are established in advance. This could also involve the formation of a so-called 'emergency team'. This team shall evaluate the causes of the deviation and shall decide which additional preventive actions are to be taken All corrective actions taken, the causes and consequences, and the individuals involved in the corrective actions shall be recorded. The effectiveness of the corrective actions, for both the process and the product, shall be evaluated. Products resulting from the process while the critical limit has been exceeded shall be treated as nonconforming products. The corrective actions may include:

With respect to the product:

- Actions ranging from blockades to product recall;
- Temporary hold of the product/batch;
- Identification of non-conforming products;
- Re-work of the product;
- Disposal/destruction of the product/batch.

With respect to the process:

- Adjusting the process;
- Adjustment/correction of process conditions.

Product Recall

The management shall establish arrangements that provide procedures for recall of the products from the market place and/or from end consumers.

Validation

Validation is not a part of verification, but a separate activity prior to authorizing the HACCP plan. The objective of validation is to ensure that the hazards originally identified by the HACCP team are complete and correct and that they will be effectively controlled under the proposed plan. To meet the objectives of validation it is necessary to review the effectiveness of the supporting evidence used in the HACCP study as well as the general and specific control measures, the monitoring system and corrective actions. Each time when the food business operation changes in a manner that could adversely affect food safety this review shall be up-dated.

- Validation is performed by demonstrating that:
- The established list of potential hazards is based on sound scientific data and has included all hazards;
- The questions used to assess the significance are answered using sound scientific and technical knowledge;
- The control measures (general or specific) are appropriate to control the hazards, i.e. to prevent or eliminate, to reduce or maintain at an acceptable level;
- Fluctuations of the control parameters (equivalent to a process criterion) within the defined critical limits will not affect the safety of the product. The parameters and methods used to monitor the control measures are appropriate;
- Corrective actions are appropriate and shall prevent the release of unsafe products and provide evidence that the situation can be corrected immediately.

Verification

The procedures for verification of the HACCP system is established, documented and implemented. The main purpose of verification is to determine compliance with the specifications of the HACCP system and to confirm that the HACCP system is working

effectively through the application of (auditing) methods, procedures, tests (including random sampling and analysis) and other evaluations, in addition to monitoring .

Procedures for verification are documented and shall include as a minimum:

a. Purpose;
b. Methods, standard operating procedures or tests applied;
c. Tasks and responsibilities;
d. Frequency;
e. Records.

The verification procedure shall address, as a minimum, the following topics:

a. Review of the HACCP system and its corresponding records;
b. Analysis of (near) recalls and product dispositions;
c. c .Assessment of all specific control measures, non conformities and corrective actions taken to seek confirmation of implementation and effective control of CCP's;
d. Assessment of all general control measures to seek confirmation of implementation and to demonstrate an effective control of associated hazards;
e. Compliance of the actual flow diagrams and layout with the documented situation;
f. Compliance of the PRP documents with the operational situation;
g. Analysis of customer and consumer complaints related to hygiene and food safety;
h. Review of analytical outcome of random sampling and analysis of products;
i. Evaluation of conformity with applicable legislation and regulations (as well as conformity to foreseeable changes in legislation and regulations) and identification of changes in legislation and regulations concerning food safety;
j. Review of gaps between current and desired level of knowledge, awareness and training of staff with respect to hygiene and food safety, resulting in effective (on-the-job) training sessions;
k. Consistency of the current documentation.

Documentation and records

Documentation

Documented HACCP system has been established and maintained in order to ensure conformity with the requirements of HACCP standard and the applicable legislation and regulations. Documentation is appropriate to the nature and size of organization operation. HACCP manual includes policy of the food business operator wrt food safety, scope of the HACCP-based Food Safety System, the

documented specifications, procedures and instructions established or reference to them.

Documents required by the HACCP-based Food Safety System shall be controlled.

A documented procedure has been established to define the controls needed:

a) to approve documents for adequacy prior to issue
b) to review and update as necessary and re-approve documents
c) to ensure that changes and the current revision status of documents are identified
d) to ensure that relevant versions of applicable documents are available at points of use
e) to ensure that documents remain legible and readily identifiable
f) to ensure that documents of external origin are identified and their distribution controlled
g) to prevent the unintended use of obsolete documents, and to suitably identify them if they are retained for any purpose

Two separate manuals are prepared

i) Food Safety System Manual. (Apex Manual) and named as FSMS manual.
ii) PRP Manual (Pre Requisite Manual)

Work instructions are also prepared.

All WI's are controlled by HACCP Team Leader.

Records

Records are established and maintained to provide evidence of conformity with the requirements and with the effective operation of the HACCP-based Food Safety System. It is ensured that records remain legible, readily identifiable and retrievable.A documented procedure has been established to define the controls needed for identification, storage, protection, retrieval, retention time and disposal of records.

Records cover the following :

a) Demonstration that the members of the HACCP team have adequate knowledge, expertise and different disciplines available
b) Management reviews and related actions
c) Hazard analysis and information sources (legislation, standards, literature, hygiene codes, GMP, Codex) used by the HACCP teams to identify and evaluate the hazards and risks
d) Assessment of every step in the process and the reasons for establishing the Specific Control Measures (CCP related) and General Control Measures
e) Monitoring reports (dated and signed) of the Specific Control Measures to demonstrate the control of the related CCPs
f) Non conformities occurred (exceeded action limits and critical action limits) of the Specific Control Measures and the corrective actions taken
g) Verification program (including internal audits) and their evaluation
h) Traceability of foodstuffs

Crimean Congo Hemorrhagic Fever

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Crimean Congo Haemorrhagic Fever (CCHF) is a widespread disease caused by a tick-borne virus (*Nairovirus*) of the *Bunyaviridae* family. The disease was first characterized in the Crimea in 1944 and given the name Crimean hemorrhagic fever. It was then later recognized in 1969 as the cause of illness in the Congo, thus resulting in the current name of the disease.The CCHF virus causes severe viral haemorrhagic fever outbreaks, with a case fatality rate of 10-40%. CCHF is found in Eastern Europe, particularly in the former Soviet Union, throughout the Mediterranean, in northwestern China, central Asia, southern Europe, Africa, the Middle East, and the Indian subcontinent.

ETIOLOGY

Crimean-Congo hemorrhagic fever is caused by Crimean-Congo hemorrhagic fever virus (CCHFV). This virus is a member of the genus Nairovirus in the family Bunyaviridae. All of the 32 members of the *Nairovirus* genus are transmitted by ixodid ticks (Hyalomma tick) in animals, but only three have been implicated as causes of human disease and CCHF virus is the most important human pathogen amongst them.

EPIDEMIOLOGY

Crimean congo hemorrhagic fever occurs most frequently among agricultural workers following the bite of an infected tick, and to a lesser extent among slaughterhouse workers exposed to the blood and tissues of infected livestock and medical personnel through contact with the body fluids of infected patients. During the summers of 1944 and 1945 over 200 cases of an acute, hemorrhagic, febrile illness occurred in Soviet troops. On July 28, 2005 authorities reported 41 cases of CCHF in Turkey's Yozgat Province, with one death. As of August 2008, a total of 50 people were reported to have lost their lives in various cities in Turkey due to CCHF. 3128 crimean congo hemorrhagic fever cases with 5% of case-fatality rate have been reported by the Ministry of Health of Turkey Between 2002 to 2008. On May 27, 2010 hospitals

reported 70 cases of CCHF in Kosovo's Kosovo Polje, with 4 deaths reported so far. The Authorities are not able to deal with the disease because of the lack of advanced medication. In September 2010 an outbreak has been reported in Pakistan's Khyber Pakhtunkhwa province. Poor diagnosis and record keeping has caused the extent of the outbreak to be uncertain, though some reports indicate over 100 cases, with a case-fatality rate above 10%. In January 2011, the disease has been reported in Gujarat, India, with 4 reported deaths, which consisted of the patient along with the doctor and the nurse who treated the patient. As of May 2012, 71 people are reported to have contracted the disease in Iran, resulting in 8 fatalities. In October 2012, a British man died from the disease at the royal free hospital in London. He had earlier been admitted to gartnavel general hospital in Glasgow after returning on a flight from Kabul in Afghanistan. In July 2013, in kariyana village in babra taluka, Amreli district, India the virology report of four persons who died last week tested positive for CCHF. On August 16, 2013, a farmer from Agago, Uganda was treated at Kalongo Hospital for a confirmed CCHF infection. Additionally, the deaths of three other people in the northern region were suspected to have been caused by the virus. Six people who had come in contact with the Agago man were placed under observation, and released after showing no symptoms in two weeks. Another unrelated suspected CCHF patient as admitted to Mulago Hospital on the same day. The Ministry of Health announced on the 19th that the outbreak was under control, but the second patient, a 27-year old woman from Nansana, died on the 21st. She is believed to have contracted the virus from her husband, who returned to Kampala after being treated for CCHF in Juba, South Sudan.

MODE OF TRANSMISSION

Humans who become infected with CCHF acquire the virus from direct contact with blood or other infected tissues from livestock having viraemia, or they may become infected from a tick bite. The majority of cases have occurred in those involved with the livestock industry, such as agricultural workers, slaughter house workers and veterinarians. CCHF can be transmitted from one infected human to another by contact with infectious blood or body fluids. Documented spread of CCHF has also occurred in hospitals due to improper sterilization of medical equipment, reuse of injection needles, and

contamination of medical supplies. Nosocomial infections were documented in Albania, Bulgaria, Turkey, Russia, Iran and Pakistan.

At-risk populations

Animal herders, livestock workers, and slaughter houses in endemic areas are at risk of CCHF. Healthcare workers in endemic areas are at risk of infection through unprotected contact with infectious blood and body fluids. Individuals and international travelers with contact to livestock in endemic regions may also be exposed.

SIGNS AND SYMPTOMS

The length of the incubation period depends on the mode of acquisition of the virus. Following infection by a tick bite, the incubation period is usually one to three days, with a maximum of nine days. The incubation period following contact with infected blood or tissues is usually five to six days, with a documented maximum of 13 days. Onset of symptoms is sudden, with fever, myalgia (muscle ache), dizziness, neck pain and stiffness, backache, headache, sore eyes and photophobia (sensitivity to light). There may be nausea, vomiting, diarrhoea, abdominal pain and sore throat early on, followed by sharp mood swings and confusion. After two to four days, the agitation may be replaced by sleepiness, depression and lassitude, and the abdominal pain may localize to the upper right quadrant, with detectable hepatomegaly (liver enlargement). Other clinical signs include tachycardia (fast heart rate), lymphadenopathy (enlarged lymph nodes), and a petechial rash (a rash caused by bleeding into the skin) on internal mucosal surfaces, such as in the mouth and throat, and on the skin. The petechiae may give way to larger rashes called ecchymosis, and other haemorrhagic phenomena. There is usually evidence of hepatitis, and severely ill patients may experience rapid kidney deterioration, sudden liver failure or pulmonary failure after the fifth day of illness. The mortality rate from CCHF is approximately 30%, with death occurring in the second week of illness. In patients who recover, improvement generally begins on the ninth or tenth day after the onset of illness.

DIAGNOSIS

CCHF virus infection can be diagnosed by several different laboratory tests:

- Enzyme Linked Immuno Sorbent Assay (ELISA)
- Antigen detection
- Serum neutralization

- Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) assay and
- Virus isolation by cell culture.

Patients with fatal disease, as well as in patients in the first few days of illness, do not usually develop a measurable antibody response and so diagnosis in these individuals is achieved by virus or RNA detection in blood or tissue samples. Tests on patient samples present an extreme biohazard risk and should only be conducted under maximum biological containment conditions. However, if samples have been inactivated (e.g. with virucides, gamma rays, formaldehyde, heat, etc.), they can be manipulated in a basic biosafety environment.

TREATMENT

Treatment is primarily symptomatic and supportive, as there is no established specific treatment. Ribavirin is effective *in vitro* and has been used during outbreaks, but there is no trial evidence to support its use. A Turkish research team led by Refik Saydam Health Institute has developed treatment-serum derived from blood of several CCHF-patients, which have been proven to be 90% effective in CCHF-patients.

Vaccine

The major hindrance in developing vaccine against CCHF virus is the great genetic variation noted in different strains. Despite this genetic variability, Ahmed et al. have shown that some epitopes are conserved, and CCHFV vaccines may have to be either immunogens derived from several CCHFV strains, or can target the immune response on conserved neutralizing epitopes. An inactivated vaccine derived from mouse brain has been used in the former Soviet Union and Bulgaria. However, in most of the countries vaccine is not available.

PREVENTION AND CONTROL

Controlling CCHF in animals and ticks

It is difficult to prevent or control CCHF infection in animals and ticks as the tick-animal-tick cycle usually goes unnoticed and the infection in domestic animals is usually not apparent. Furthermore, the tick vectors are numerous and widespread, so tick control with acaricides (chemicals intended to kill ticks) is only a realistic option for well-managed livestock production facilities. For example, following an outbreak at an ostrich abattoir in South Africa, measures was taken to ensure that ostriches remained tick free for 14 days in a quarantine station before slaughter. This decreased the risk for the animal to be infected during its slaughtering and

prevented human infection for those in contact with the livestock.

Reducing the risk of infection in people

Although an inactivated, mouse brain-derived vaccine against CCHF has been developed and used on a small scale in Eastern Europe, there is currently no safe and effective vaccine widely available for human use. In the absence of a vaccine, the only way to reduce infection in people is by raising awareness of the risk factors and educating people about the measures they can take to reduce exposure to the virus.

Public health advice should focus on several aspects:

- Reducing the risk of tick-to-human transmission:
  - Wear protective clothing (long sleeves, long trousers).
  - Wear light coloured clothing to allow easy detection of ticks on the clothes.
  - Use approved acaricides (chemicals intended to kill ticks) on clothing.
  - Use approved repellent on the skin and clothing.
  - Regularly examine clothing and skin for ticks; if found, remove them safely.
  - Seek to eliminate or control tick infestations on animals or in stables and barns.
  - Avoid areas where ticks are abundant and seasons when they are most active.
- Reducing the risk of animal-to-human transmission:
  - Wear gloves and other protective clothing while handling animals or their tissues in endemic areas, notably during slaughtering, butchering and culling procedures in slaughterhouses or at home.
  - Quarantine animals before they enter slaughterhouses or routinely treat animals with pesticides two weeks prior to slaughter.
- Reducing the risk of human-to-human transmission in the community:
  - Avoid close physical contact with CCHF-infected people.
  - Wear gloves and protective equipment when taking care of ill people.
  - Wash hands regularly after caring for or visiting ill people.

Controlling infection in healthcare settings

Health-care workers caring for patients with suspected or confirmed CCHF, or handling specimens from them, should implement standard infection control precautions. These include basic hand hygiene, use of personal protective equipment, safe injection practices and safe

burial practices. As a precautionary measure, health-care workers caring for patients immediately outside the CCHF outbreak area should also implement standard infection control precautions.

Samples taken from people with suspected CCHF should be handled by trained staff working in suitably equipped laboratories.

CONCLUSION

CCHF was always an impending threat to India, which has now become a reality with the current outbreak in Gujarat. The vector and reservoir animals were already present. The related species of the genus Nairovirus, eg. Ganjam virus of Nairobi sheep disease is also transmitted by same vector as CCHF virus and has been reported previously. The emergence of this deadly viral infection in a huge country like India having all ecological suitability for the virus is a challenge for the entire medical fraternity. This emphasizes the need for active surveillance not only for existing pathogens in any geographic location but also for those that pose future threat. The use of molecular techniques even for surveillance is of paramount importance for preventing further spread of this highly pathogenic virus.

An Overview on Chromosomal Fragile Sites in Domestic Animals

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The term '*Chromosomal fragile sites*' (CFSs) was coined in 1970 to describe recurrent chromosome breaks on the long arm of chromosome 16 (Magenis *et al*., 1970). A chromosomal fragile site is a specific heritable point on a chromosome that tends to form gaps and breaks on metaphase chromosomes following partial inhibition of DNA synthesis. They are chromosomal sites showing susceptibility to breakages and discontinuities in specific conditions of cell culture and also following induction with chemical substances.

Fragile sites and reproduction

Chromosomal fragility is considered to play a key role in karyotype evolution, chromosomal rearrangements and disease etiology related to productive and reproductive efficiency of farm animals. The fragility of chromosomes and their relation with chromosome rearrangements were carried out in many livestock species. Extensive studies have been undertaken on the fragile sites (non-random chromosomal breaks/gaps) in several species of Bovidae regarding different methods of induction, and their clinical and biological significance (Riggs and Ronne, 2009). In result, bovine chromosome fragility (mainly chromosome X) was revealed to be associated with pathologies (baldy calf syndrome, dwarfism) and fertility impairment (repeat breeders, long calving interval and abortions).

Reports of CFSs in Livestock:

- Orthologs of human CFSs have been found in the syntenic regions of a number of other mammalian species, including other primates, cat, dog, pig, horse, cow, Indian mole rat, deer mouse, and laboratory mouse.
- Ali *et al.* (2008) studied the dynamics of spontaneous and FUdR-inducible fragile sites in sheep (*Ovis aries*).
- Prasanthi *et al.* (2006) showed that fragile sites can lead to various kinds of

chromosomal rearrangements, which can reduce fertility.

- Llambi and Nunez (2007) described fragile sites associated with mental retardation syndrome, parakeratosis, baldy calf syndrome, low fertility and other hereditary defects affecting human and domestic animals.

Inductions of fragile sites

Fragile sites are specific loci that appear as constrictions, gaps, or breaks on chromosomes from cells exposed to partial inhibition of DNA replication.

Agents involved in the CFSs induction

- Carcinogens,
- Antibiotics,
- Genotoxins,
- Iinsecticides,
- Irradiation
- Inhibitors of DNA replication
- Cytotoxic inducing agents such as FUdR, methotrexate or thymidine
- Hoechst 33258
- Aphidicolin (APH) inducible fragile sites have been detected in the chromosomes of cattle, buffalo, horse and pigs.

Nicolae *et al.* (2009) reported significant increases of Sister Chromatid Exchanges (SCEs) in the females of river buffalo with chromosome fragility expressed by many gaps, breaks and fragments. The common fragile sites were only weakly induced by conditions of thymidylate stress, which induced the fragile X and other folate-sensitive fragile sites. Conversely, aphidicolin, a specific inhibitor of DNA polymerase, strongly induces the common fragile sites but only weakly induces the fragile X. The expression of all fragile sites is enhanced in particular cell types by post treatment with caffeine or theophylline. Thus, the common and rare fragile sites share some similarities but also exhibit some differences in their modes of induction. All fragile sites appear similar at the cytological level. Aside from the fragile X, which is associated with one form of mental retardation, the biological role or significance of fragile sites is unknown. However, on the basis of correlation of breakpoints, suggestions have been made that fragile sites might be playing a role in generation of non-random chromosome rearrangements. Recent advancements in sequencing and mapping of domestic animal genomes provide tools for molecular characterization of fragile sites in animal chromosomes.

CONCLUSION

The domestic animals with reduced fertility or infertility, particularly those not

exhibiting any type of chromosomal aberrations should be monitored for the fragility of chromosomes. The data generated in the future will help to validate the association of fragile sites with reproductive failure in different breeds and species of domestic animals.

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A1 and A2 Variants of Beta Caseins in Cow Milk and Human Health

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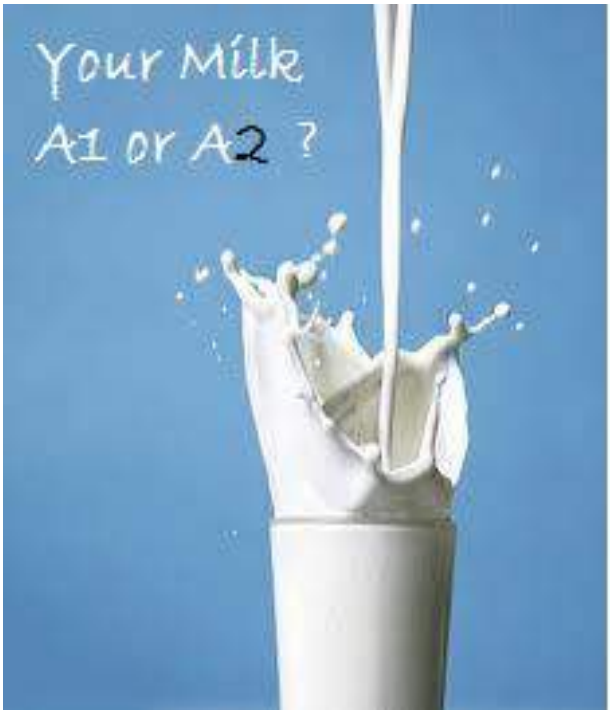
Milk is a common source of animal protein and associated micro elements for vegetarians. It has body-building proteins, bone-forming minerals and health-giving vitamins and energy-giving lactose and fat. Milk is about 85% water. The remaining 15% is the milk sugar lactose, protein, fat, and minerals. The protein portion is 80% casein and 20% whey. The proteins present in milk are considered as complete proteins of high quality, i.e. they contain all essential amino acids in fairly large quantities. Recently, a relationship between disease risk and consumption of a specific bovine β-casein fraction with either A1 or A2 genetic variants has been identified.

Milk proteins

Six different types of proteins are present in milk out of which four are casein proteins (α, β, γ and K-casein) and two are whey proteins (β-lactoglobulin and α-lactalbumin). Casein protein is peculiar because it is present only in milk and exists in the form of calcium caseinate-phosphate complex. β-casein is 30% of the total protein content in cow's milk. It is the second most abundant protein in cow's milk that contains 209 amino acids.

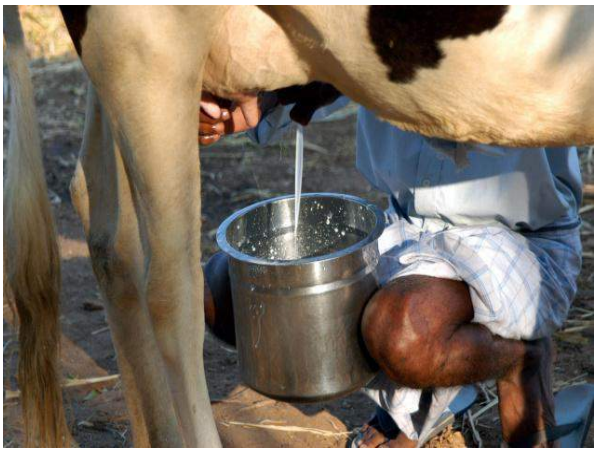


There are 12 genetic variants of β-CN: A1, A2, A3, B, C, D, F, H1, H2, I and G out of which A1 and A2 are the most common. Milk high in β-casein Al is referred to as 'Al milk' while milk high in 3-casein A2 is called 'A2 milk'. A1 variant has histidine at position 67

of the amino acid sequence while A2 possess proline at this position. A2 beta-casein is the beta-casein form cows have produced since before they were first domesticated, over 10,000 years ago. It is considered safe and nutritious and has no known negative effects on human health. Sometime in the past few thousand years, a natural mutation occurred in some European dairy herds that changed the beta-casein they produced. The gene encoding beta-casein was changed such that the 67th amino acid in the 209 amino acid chain that is the beta-casein protein was switched from proline to histidine. This new kind of β-casein that was created is known as A1 β-casein, and is generally more common in many of the big black-and-white cow breeds of European descent such as the Holstein and Friesian.

Human health hazards

Gastrointestinal proteolytic digestion of A1 variant of β-casein (raw/processed milk) leads to generation of bioactive peptide, beta casomorphin 7 (BCM7). Infants may absorb BCM-7 due to an immature gastrointestinal tract whereas adults gather the biological activity locally on the intestinal brush boarder. In hydrolysed milk with variant A1 of beta-casein, BCM-7 level is 4-fold higher than in A2 milk.



Recently, a relationship between disease risk and consumption of a specific bovine β-casein fraction with either A1 or A2 genetic variants has been identified. BCM7 is suggested to be associated as a risk factor for human health hazards as it can potentially affect numerous opioid receptors in the nervous, endocrine and immune system. It is also known to be an oxidant of low dietary lipoproteins (LDL) and oxidation of LDL is believed to be important in formation of arterial plaque. Epidemiological evidences claim that consumption of beta-casein A1 milk is associated as a risk factor for type-1 diabetes, coronary heart disease, arteriosclerosis, sudden infant death syndrome, autism, schizophrenia etc. A broad range of studies from American and European investigations has shown reduction in autistic and schizophrenic symptoms with decrease in A1 milk intake.

Further, animal trials have also supported the linking of type-1 diabetes to milk exposure in general and A1 beta-casein in particular. A2 milk has not been associated with these diseases. Symptoms of Al milk protein intolerance can be similar to those of lactose intolerance, including digestive issues such as bloating, abdominal pain, nausea, diarrhea and constipation. Human trials are needed before it can be said with confidence that the All A2 composition of milk is important in human health.

Status of Indian cows

Recent research has shown that Bos indicus cows (Indian cows) are potential sources of BCM 7 (Beta Casomorphine 7)-free A2 milk which is considered good milk, compared to Bos taurus cows (exotic cows), which produce Al milk. Initial studies on indigenous cow (Zebu type), buffalo and exotic cows (taurine type) have revealed that A1 allele is more frequent in exotic cattle while Indian native dairy cow and buffalo have only A2 allele, and hence are a source for safe milk.

Detection of A1 milk

A genetic test developed by the A2 Milk Company based in Australia determines whether a cow produces the A2 or Al type protein in its milk. This is done through a DNA test of a cow's tail hair. The test allows the A2 Milk Company to give licenses to milk producers once these producers prove their cows produce A2 β-casein protein in their milk. Now, more and more Al companies are beginning to test their bulls to establish whether they carry the desirable A2 gene. Al milk has been implicated as a potential etiological factor in Type 1 Changing the dairy herds to more A2 producing cows on commercial basis may significantly improve public health. The Government of India has already taken steps to identify the breeds having A2 gene.

Changing the dairy herds to more A2 producing cows on commercial basis may significantly improve public health. Selection for increasing milk yield may contribute for the higher proportion of undesirable A1 alleles in the population. Considering the public health implication, adequate weightage should be given to select bulls with A2A2 genotype while making selection for increasing milk yield of crossbreds. The Government of India has already taken steps to identify the breeds having A2 gene. Preliminary studies have confirmed the hypothesis that Indian breeds of cows produce the safe A2 type milk. The National Dairy Research Institute, Karnal recently fixed A2 casein allele in Deoni cattle and also reported that Malnad Gidda

predominantly (151 out of 154) have casein allele. Research is already going on for fast DNA detection tools to identify Al/A2 milk producing cows in India.

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Rabies: The rage of death

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Ever since the domestication of animals, we are continually living under the threat of diseases which are transmitted from animals. Historically, rabies is the single most fearsome disease ever known to mankind. The name rabies is synonymous with death. That's why in Western as well as Indian mythologies, the God of death is often depicted accompanied by a dog. Anubis, the God of netherworld in Egyptian mythology was dog faced. The word rabies itself means rage. The origin of this word is believed to be from the Sanskrit word 'rabhas' which also means rage. It was thought that rabies is caused by a worm called lyssa which lives under the tongue. Lyssa is also the name for Greek Goddess of madness. In 1857, Louis Pasture proved that rabies is caused by an RNA virus belonging to Lyssa virus genus of the Rhabdoviridae family. There are 15 different members in the Lyssavirus group, all which causes rabies like diseases in humans and other animals. The typical rabies virus, like all other members of Rhabdoviridae, has a characteristic 'bullet shape'. This particular morphology helps the virus to attach itself firmly to host cells. The RNA gives rise to its corresponding DNA which has four important genes. These genes are designated by the letters G,L,P and psi. The G and P genes codes for the proteins which give rise to viral structural proteins and L gene codes for enzyme reverse transcriptase which converts RNA into DNA during the multiplication of the virus. People or animals get rabies when they are bitten by a rabid animal. Licking, scratching and grooming can also potentially transmit rabies from affected animals. There are a wide range of animals which can act as natural hosts for rabies. Certain animals like bats and wild carnivores of dog family can harbour the virus without any ill effects to themselves. Such animal hosts are known as reservoir hosts, which play a main role in maintenance of the virus in nature. Other animals including man and herbivores get rabies and eventually die hence they are

known as dead end hosts. Birds are naturally resistant to rabies because of their higher body temperature which is unfit for the virus to live and multiply. In India, bite from rabid dog is the most important cause of rabies in humans. In USA, raccoons are the common agents and in Europe, vampire bats are the common culprits. It is estimated that every year, some 65,000 human deaths occur in India alone due to rabies. Drinking raw milk from rabies infected livestock is another important means of getting rabies infection. Rabid livestock, especially cows can attack the owners. They can spray saliva while raging which may contain virus particles. Man to man transmission rarely occurs, however, there are reports of transmission of rabies accidently to a man who received cornea from another person died of rabies, which was confirmed later. Visiting caves where bats dwell can also be dangerous, as the atmosphere inside caves might be saturated with droplets from breath of bats, some of which may be rabid. The bats do not show any symptoms of rabies.

The virus, after entering in the body through bites, scratches or licks, multiplies in the muscles, after which they enter the nerves which supplies the muscles (neuromuscular junction). After entry, the rabies virus travels along the nerve using the nerve's own transport mechanism at the rate of 2 mm/hr towards the brain. Within the brain, the rabies virus enters the large neurons, especially, the neurons of hippocampus region in man and other carnivores and in cells of Purkinje in cerebellum – little brain- in herbivores. In these neurons, the replication of virus induces programmed cell death (apoptosis) leading to death of brain cells. This is the reason for neurological symptoms - madness - and consequent death associated with rabies. From the brain, the virus again moves towards the periphery passing along the branches of trigeminal nerve and reaches salivary glands. That's how the virus appears in saliva of rabies infected animals. This is the smart way developed by the virus during its evolution for its spread from the one animal to other using two basic tools like madness and bite. In madness infected animal will lose its control and bite to other animal and at the same time saliva will enter in the body during the bite which is fully loaded with deadly virus particles. In bats and other reservoir hosts, the body has somehow reached a compromise with the virus, which is the reason for their survival. In these animals body's defence system does not overreact to the virus infection. In fact, the death of neurons is triggered by

the body's defence mechanism, which ironically is trying to protect the cells from further invasion by inducing death of virus affected cells. This is the most complicated part of the disease mechanism in rabies.

Historically, the most attributed clinical sign of rabies is hydrophobia or fear on seeing water. This behaviour is seen only, if any, in animals. Humans show difficulty in drinking or eating. Animals, on the other hand show violent behaviour at the sight of water. This is a psychological manifestation coupled by thirst and paralysis of muscles of pharynx which prevents drinking. In animals, two different manifestations of rabies occur. In furious form, which is the most explicit form, the animals become violent and go out of control. They tend to attack other animals or even nonliving objects such as rock, trees or vehicle tyres. The pitch of their sound or barking becomes deeper and there will be drooling of saliva. The animal fails to recognize even it's owner. In cats, only furious form is present. In livestock, cows become violent and tend to chase whoever approaches them. They bellow continuously, yawn and tend to mount over other cows of the herd. There will be difficulty in drinking and eating due to paralysis of muscles of pharynx. Ultimately, paralysis sets in and the animal is succumbed. In dumb or paralytic form, the animals become paralysed, without eating or drinking. They become unresponsive to owners and may not listen to their commands. Wild animals lose fear and shyness to humans and other animals. Dumb form of rabies is very difficult to diagnose clinically.

Diagnosis of rabies before death is very difficult from clinical signs alone. Antigens of rabies virus are not found anywhere else other than nervous system. Impression smear from cornea of eye and saliva are commonly used for diagnosis. From dead animals, impression smears collected from hippocampus and cerebellum are used for diagnosis. The most common test employed for impression smears is direct Fluorescent Antibody Test (dFAT). Here, the impression smear is made to react with anti-rabies antibody with a fluorescent marker (usually, fluorescein isothiocyanate FITC). If rabies antigen is present in the smear, it will bind with the antibody, which can be seen under a fluorescent light microscope. Positive smears will show bright apple green dots in a dark background, while in negative smears, only dark background will be visible. Other tests like enzyme linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) are also

used. Previously, a simple staining technique of impression smears using Seller's stain was employed for diagnosis of rabies. Positive smears will show magenta coloured particles or inclusion bodies within the cytoplasm of neurons. These bodies are known as Negri bodies, which are characteristically seen in rabies. Unfortunately, only 70 % of rabies affected animals are known to show Negri bodies. Therefore, absence of Negri bodies do not guarantee absence of rabies. Furthermore, brains of cats and other felids such as leopard and tiger also show Negri like bodies known as Lyssa bodies, which are normal in their brains.

The animal bites or exposure to rabies virus are classified into 3 categories, depending on the severity. Type 1 contact involves licking by rabid animal to other animal or man which is not having any visible wound or feeding or touching of the rabid animal. Type 2 contact involves nibbling or mild scratching by the rabid animal. Type 3 contact, which is the most severe form of exposure involves deep biting or scratching with bleeding or licking on abraded skin or mucous membranes or eyes by rabid animal. Any attack from wild animals should also be considered as Type 3 exposure.

There is no treatment for rabies, once the clinical signs have started. The period between virus entry and appearance of clinical signs (incubation period) vary greatly between different species of animals. In dogs, the usual period is 10 days, where as in cows, it is 14 - 21 days. In humans, the period is highly variable from a few days even up to one year. The usual incubation period for humans is taken as 10 -15 days. There is no treatment for rabies as of today once the patient has shown clinical signs. Start of clinical signs marks the beginning of a fatal vicious sequence of brain damage, which culminates in death of the patient. Despite its deadly nature, rabies is 100 % preventable by vaccination. All livestock and pet animals should be ideally vaccinated annually. People who are closely associated with animals such as veterinarians, animal handlers etc should get vaccinated. This is called pre-bite vaccination. Post exposure prophylaxis (PEP) also called post-bite vaccination can save the life of the bite victim, if initiated immediately. The vaccine used for the pre-bite therapy can neutralize the virus before they enter into brain. The bite wounds should be immediately washed with luke warm water and strong soap (soap containing carbolic acid is ideal). PEP should be initialized as soon as possible to arrest the infection in very initial stage. Usually, five doses of vaccine

are given in the muscle, on the day of bite (day 0) followed by 3, 7, 14 and 28 days of bite (known as Essen's schedule). This schedule is proven to be helpful to prevent the disease by stimulating the body's defence system. Type 1 contacts do not require any PEP, if animal is known and healthy. However, first aid procedures such as washing of the exposed area with soap and water are recommended. Type 2 contacts require PEP by vaccination. The wounds should be aseptically cleaned and dressed. Type 3 wounds require the most severe attention. Wound management, PEP and anti rabies immunoglobulin therapy should be promptly initiated.

Effect of Climate Change on Animal Reproduction

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India's human population (about 70%) is dependent on agriculture for their livelihood. Livestock sector is the integral part of Indian agriculture. This sector provides sustainability and stability to the national economy by contributing to farm energy and food security. It also provides economic stability to farmers due to uncertainties in the crop production and yield. Livestock sector is affected by climate change both directly and indirectly. Many environmental parameters like air temperature, humidity, wind speed and other climatic factors influence animal performance viz. growth, milk production, wool production and reproduction. The effect of climate change on animal production has been identified from(a) feed grain availability, (b) pasture and forage crop production and quality, (c) health, growth and reproduction and, (d) disease and their spread. Studies have revealed that milk yield of crossbred cows in India are negatively correlated with temperature-humidity index. The influence of climatic conditions on milk production has been also observed for local cows which are more adapted to the tropical climate of India. Heat stress has detrimental effects on the reproduction of buffaloes, although buffaloes are well adapted morphologically and anatomically to hot and humid climate. Thermal stress on Indian livestock particularly cattle and buffaloes has been reported to decrease oestrus expression and conception rate. The length of service period and dry period of all dairy animals was increased from normal during drought.

Heat stress affects the reproductivefunctions of dairy animals mainly by two general mechanisms (i) Increase in body temperature which can compromise reproductive function mainly due toredistribution of blood flow from the bodycore to the periphery and therebyincreasing sensible heat loss. (ii) Reduced feed intake which reduces metabolic heatproduction, affects energy balance andavailability of nutrients for

productive functions such as cyclicity, establishment of pregnancy and foetal development, functions of germ cells, the earlydeveloping embryo and other cellsinvolved in reproduction.

Climate Change Scenario

One of the environmental threats that our earth faces today is the potential changes in earth's climate and temperature patterns. An estimate indicated that the earth's average temperature has increased between 0.3°C-0.6°C, the sea level between 10-25 cm, atmospheric carbon dioxide concentration by more than 20 percent and methane by 145 percent over pre-industrial levels.

TheIntergovernmental Panel on Climate Change (IPCC) indicated that many of the developing countries tend to be especially vulnerable to extreme climatic events as they largely depend on climate sensitive sectors like agriculture and forestry. Therefore, climate change is one of the most serious long-term challenges facing farmers and livestock owners around the world. Rise in temperature due to climate change is likely to have impact on livestock production and health. The temperate cattle breeds and their crossbred will be affected more compared to zebu cattle because Indian breeds have more capacity to withstand the stress of thermal stress, feed and water scarcity, diseases and parasite load.

Heat stress assessment

A simple and most practical method to measure the heat stress in cattle and buffaloes is temperature–humidity index (THI).It plays an important role in the reproductive functions of cattle and buffaloes and it is suggested that THI has negative effect on reproductive performances of buffaloes.The test is based on atmospheric temperature and relative humidity (RH %). Equations for calculation of THI and their interpretation based on ambient temperature is measured in °F (LPHSI, 1990), When temperature is expressed in °C and Body temperature and respiratory frequency can also be used to determine heat stress in cattle and buffaloes.

Adaptation of buffaloes and crossbred cattle

Effect of temperature and humidity on cattle and buffaloes has been investigated with emphasis on their thermal stability and adaptability.Metabolism of animals has been affected by increased environmental temperature and magnitude of the response depended upon species, breed and physiological stage of the animal. Heat production study on adult cattle and buffaloes indicated that the heat produced by Zebu

cattle was 62.0 Kcal/hr/100 Kg body weight against 96.3 Kcal/hr/ 100Kg for buffaloes during summer season. The heat production was more during hot-humid and winter season than summer season in both cattle (80 Kcal/hr/ 100Kg) and buffaloes (107 Kcal/hr/100 Kg). This study clearly indicates higher energy needs during winter and rainy season than summer due to extra energy expenditure during rainy season and winter season. In maintaining body temperature heat dissipation by radiation, conduction, convection and evaporation plays significant role. The distribution of sweat gland, the capacity of skin vascular blood dispersion and the effective adrenergic governing the sweating rate are the mechanism responsible for efficient distribution of heat from animal's surface.

As the environmental temperature increases heat loss by conduction, convection and radiation decreases and heat loss by evaporation increases. The Zebu cattle have higher number of sweat glands and produce more sweat than Taurus cattle and the crossbred. This mechanism helps the Zebu cattle to maintain low body temperature compared to Taurus cattle. The necessity of heat dissipation to maintain thermal balance particularly during hot humid conditions force animals to employ open mouth panting mechanism with protruding tongue to complement heat elimination process.

Effect of Climate on Reproductive functions

The livestock species which are more vulnerable to climatic changes are cattle and buffaloesand both female and males are adverselyaffected. During hot dry (March- June) and hot humid (July-September) season, the THI values exceeds 80 in most parts of India. The pattern of estrus varies among cattle and buffaloes. Most of the buffaloes exhibit sexual activity during cooler parts of the year (October- Feb), when the THI generally remains < 72.

The thermal stress depressed estrus activity from April to June in temperate cattle. The heat stress (>32 °C) was aggravated by high humidity (vapour pressure >24 mmHg) from July to September and it had harmful effects on the ovarian activity, resulting in depressed estrus frequency during rainy season.The dairy cows that become pregnant during the warm months are less than the cool month of the year.

The pattern of estrus in buffalo is different from that of cattle since majority of buffaloes exhibits estrus from October to March when ambient temperature is

low and THI value is less than 70. In addition to ambient temperature, humidity and solar radiation profoundly affect expression of reproductive rhythm in buffaloes and cattle. The incidence of calving is also predominant from October to March, facilitating upbringing of off-springs due to availability of good quality fodders during this period. The climate change scenario due to rise in temperature and higher intensity of radiant heat load will affect reproductive rhythm via hypothalamo- hypophyseal–ovarian axis.

The main factor regulating ovarian activity is GnRH from hypothalamus and the gonadotropins i.e. FSH and LH from anterior pituitary gland. There is decrease inLH pulse amplitude and frequency in heat stressed cattle. Plasma inhibin content was lower in heat stress cows and cyclic buffaloes. The effects are more pronounced in buffaloes than cattle which may be due to high thermal load in this species as a result of difficulty in heat dissipation due to unavailability of place for wallowing and lesser number of sweat gland. The higher thermal loads, if persisted for longer periods due to either non dissipation of heat or uncomfortable environment conditions, will affect production, reproduction and health on long term basis. Therefore, heat mitigation measures and strategies need to be adopted to reduce thermal stress, fertility losses and health consequences on animals.

Reproductive functions and Seasonal trends

There is adverse effect of heat stress on many reproductive functions like gamete formation, embryonic development, foetal growth and development. The potential impact of heat stress on a mammalian population can be seen by examining seasonal trends in reproductive functions of livestock species. The effects of summer in lowering fertility are much more in high yielding cows. Domestic animals with high yielding genetic potential have direct impact of global warming on reproductive performance. In addition, the existence of allelic variation in genes regulating body temperature and cellular resistance to heat shock will be responsible and may be tool to manipulate genetic adaptation to increasing global temperature in various species of domestic animals.

The maximum occurrence of estrus was seen during winter months and the lowest during summer months. Due to high incidence of silent heat, large numbers of buffaloes are left un-bred during summer. Season of calving had a

profound influence on the service period in this species. The longer service period of buffaloes in summer may be due to the high incidence of silent estrus. Severe heat stress days with temperature humidity index > 85 were from May to August. After onset of monsoon in June /July difference between morning and evening THI is reduced and buffaloes got some opportunity for relief from thermal stress. From May to June, THI with a value of > 80 increased by approximately 450% than March. Low temperature and THI during nights in summer provide an opportunity to buffaloes to dissipate heat during night hours compared to day hours. This may be the reason that buffaloes experienced less stress during hot dry season compared with hot humid season.

The peak of reproductive activity in Zebu and crossbred cows was observed in March which coincided with the start of increase in sun shine days and subsequently through in reproductive behaviour was observed during peak solar radiation and hot days. Reproductive activity in buffaloes started increasing from July and reached at the peak during October. The expression of estrus and conception rate was recorded low during summer in crossbred cattle and buffaloes.The levels of estradiol 17ß on the day of estrus were significantly low during this period in both species. Low estradiol level on the day of estrus during summer period in buffaloes may be the likely factor for poor expression of estrus in this species.

During heat stress, motor activity and other manifestations of estrus reduced and the incidence of anestrus and silent ovulation are increased. Due to these effects a reduction in the number of mounts during heat stress compared to cold weather, leading to poor detection of estrus. The effects of heat stress can be directly related to the increase in rectal temperature of heat-stressed cows/ buffaloes during summer/ hot humid season. A small increase in maternal rectal temperature would cause decreased pregnancy rates in cattle. The increase in body temperature affects the reproductive tract and the early embryonic development.

Reproductive disorders and Season/Climate

The incidences of reproductive disorders were higher from April-September, when ambient temperature and humidity were higher.The prevalence of retained placenta was highest during July-September. The overall reproductive disorders were maximum in September and minimum in November. Seasonal

effects on reproductive disorder associated with calving in cattle have been observed by Verma et al. (1986). The author observed that the reproductive problems were highest during rainy season (14.87%) and lowest during winter-season (7.44%). Occurrence of retained placenta, dystocia and urethra prolapse was 11.27, 2.38 and 1.88%, respectively in unfavourable summer/rainy season. The increased intervention of man in regulating behaviour and environment of livestock so as to exploit the best of their genetic potentials has led to an increase in the incidence of the reproductive disorders. Underfeeding coupled with high environmental temperature stress was also incriminated for long anestrous and anovulatory periods. Inadequate nutrient intake has been found to deplete body energy reserves resulting in extended interval from calving to first estrus. Season of calving had influence on the reproductive performance.

Climate change impact on reproductive functions

A rise in temperature by >4°C due to global warming is likely to impact cattle and buffaloes negatively. The increase in thermal stress days by 260% will negatively impact estrus expression/ ovarian activity and conception rate in cattle and buffaloes. The effect may be much more pronounced in buffaloes, temperate and crossbred breeds compared to indigenous breed of cattle due to poor adaptability of these species to tropical climatic conditions. Changes in climate would lead to decrease in milk yield and conception rate in dairy cows. Hahn (1995) further reported that conception rates in dairy cows were reduced 4.6% for each unit change in THI, when the THI reaches above 70.

Increase in temperature and/or humidity have the potential to affect the conception rates of domestic animals which were not adapted to these conditions. The number of changes in reproductive performance due to further global warming will include:

- Decreased duration and intensity of the estrus period.
- Decreased conception (fertility) rate.
- Decreased size and development of ovarian follicles.
- Decreased fetal growth and calf weight at calving.
- Increased risk of early embryonic losses.
- Increased number of artificial insemination per conception.

- Increased incidence of silent heat in buffaloes.

CONCLUSION

The high temperature adversely affected production and reproduction in buffaloes and cattle. The reproductive efficiency in these species decreased considerably. The frequency, intensity and duration of estrus decreased due to adverse climatic conditions in the summer season. Conception rate falls between 20-30% in summer compared to winter. Reproductive disorders viz. Dystocia, retained placenta and uterine prolapsed were higher in crossbreds compared to native breeds of cattle. Incidences of anestrus, silent estrus were more in buffaloes during summer period. Increase in temperature due to global warming is likely to further reduce the reproductive efficiency and milk production of livestock species.

Therefore, it will be important to modify the managemental practices of cattle and buffaloes as per the climatic conditions. It requires development of suitable breeds by selection of cattle and buffaloes species which are more tolerant to heat stress and can sustain the productivity in the changing climatic scenario. Climatic conditions will ultimately be the limiting factors for livestock production system. Therefore, changing of micro environmental conditions in the tropics is very important particularly for exotic and crossbred breeds of cattle and buffaloes compared to heat tolerant breeds (Zebu).

Strategies for Reducing Methane Emission from Ruminant Animals

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Greenhouse gases such as CO_2, CH_4, NO_2 and O_3 contribute to climate change and global warming through their absorption of infrared radiation in the atmosphere. Among these gases CO_2, contributes 76.7 % while CH_4 14.3 % respectively to the total GHG gases. Methane is especially potent traces gas due to its global warming potential, 25 times that of carbon dioxide, and its 12-year atmospheric lifetime; it is the second largest anthropogenic greenhouse gas, behind carbon dioxide. Globally, livestock produces about 80 million tonnes of enteric CH_4 annually which is about 18% of total methane emissions (Moss et al. 2000; IPCC 2007). The CH_4 produced in a cattle production system will mostly be by enteric fermentation (85–90%) and the rest is produced by the manure. Methane is produced in the rumen as a product of normal fermentation of feedstuffs. Although methane production can also occur in the lower gastrointestinal tract, as in non-ruminants, 89% of methane emitted from ruminants is produced in the rumen and exhaled through the mouth and nose remaining 11% through the anus (Murray *et al*., 1976). The rising concentration of CH_4 is strongly correlated with increasing populations, and currently about 70% of its production arises from anthropogenic sources. Its concentration has more than doubled during 1750 to 2013 (CDIAC, 2013). Methane represents a significant energy loss to the animal ranging from 2% to 12%. Methanogens living on and within rumen ciliate protozoa may be responsible for up to 37% of the rumen CH_4 emission. It avoids hydrogen accumulation, which would lead to inhibition of dehydrogenase activity involved in the oxidation. India emerged as the largest contributor to the livestock methane budget, simply because of its enormous livestock population,

although the emission rate per animal in the country was much lower than in the developed countries. In Indian conditions the animals are mostly fed on poor quality roughages of low digestibility and emit less methane than exotic cattle of developed countries fed with highly digestible good quality feed.

Table 1: Methane produced (%) due to enteric fermentation and % energy loss through CH_4 in livestock Tubiello *et al*, 2013 and E Johnson and Ward, 1996

Species	Non - dairy cattle	Dairy cattle	Buffalo	Sheep	Goat	Others
% CH_4 contribution	55	19	11	7	5	3
Species	Feed lot cattle	Range cattle	Dairy cattle	Camels	Buffalo	
% energy lossnthrough CH_4	3.5-6.5	6.0-7.5	5.5-9	7.0-9	7.5-9	

Table 2: Methane production in livestock per annum (Tg) McMichael *et al.*, 2007

Species	Dairy cattle	Beef cattle	Sheep and Goat	Buffalo	Camels	Pigs	Horses
CH_4, production / anum in(Tg)	18.9	55.9	9.5	6.2-8.1	0.9-1.1	0.9-1.0	1.7

Table 3: Normal Ruminal microbial population (Janssen and Kirs, 2008

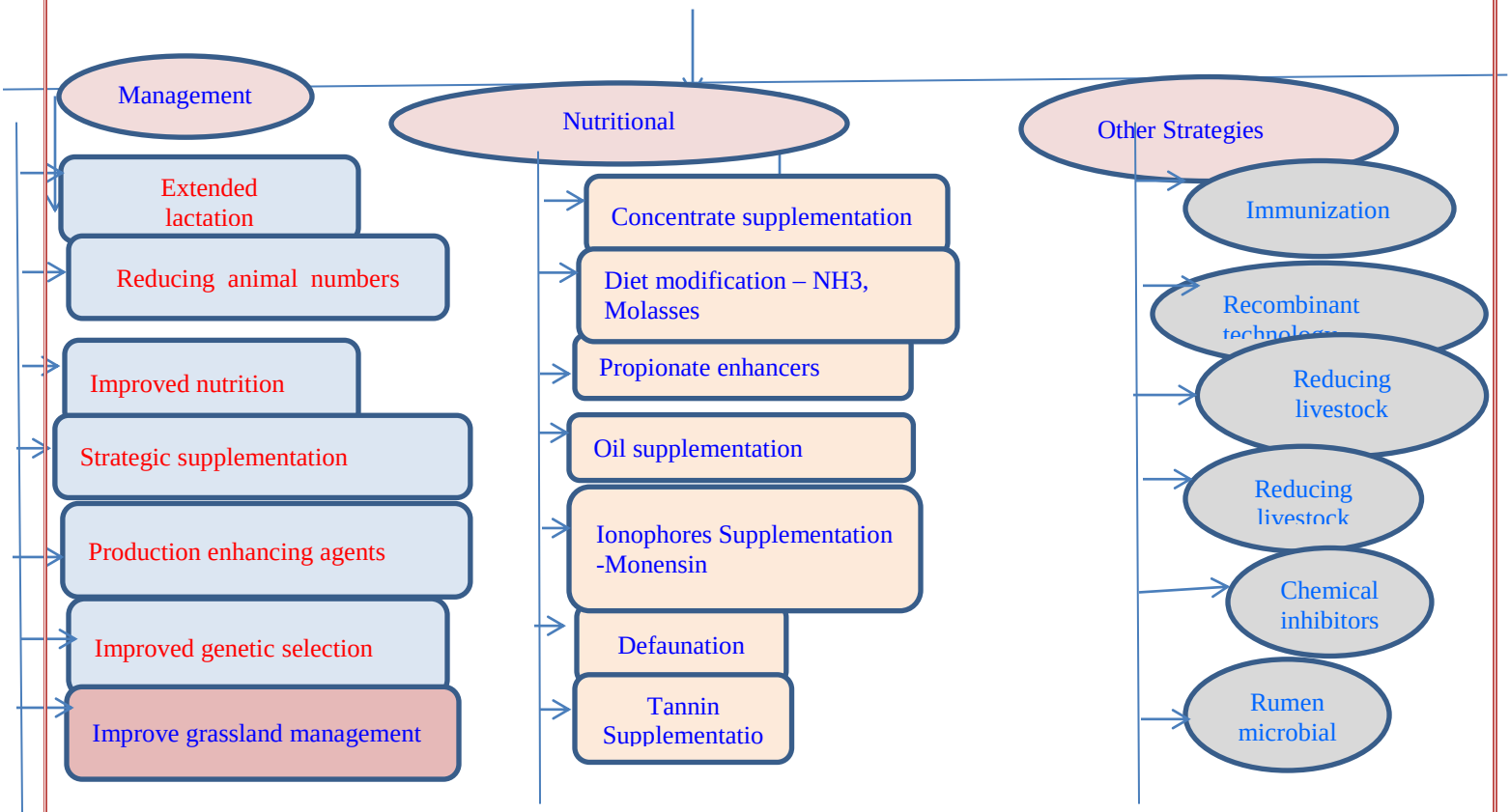
Microbes	Methanogens	Bacteria	Protozoa	Fungi
No. present in rumen/ml	10^8 X10^{10}	10^{10} X10^{12}	10^5X10^6	10^4X10^5

Mechanism of Methane formation:

CH_4 is produced by two types of methanogens, the slow-growing methanogens 130 h produces CH_4 from acetate (e.g. *Methanosarcina*) and fast growing methanogens (generation time 4–12 h) that reduce CO_2 with H_2. Acetate and butyrate production results in a net release of hydrogen while the propionate formation is a competitive pathway for hydrogen use in the rumen.

Abatement strategies are often limited by the diet fed, the management conditions, physiological state and use of the animal, as well as government regulations; resulting in difficulties applying a one size fits all approach to the problem of enteric methane mitigation

Strategies to reduce methane emission from livestock:



Methane Reduction Strategies: Methane mitigation is effective in one of two ways: either a direct effect on the methanogens or an indirect effect caused by the impact of the strategy on substrate availability for methanogenesis, usually through an effect on the other microbes of the rumen. The metabolic pathways involved in hydrogen production and utilization, as well as the methanogenic community are important factors that should be considered when developing strategies to control CH_4, emissions by ruminants. Any given strategy has to address one or more of the following goals:

- a reduction of hydrogen production that should be achieved without impairing feed digestion;
- a stimulation of hydrogen utilisation towards pathways producing alternative end products beneficial for the animal; and/or
- an inhibition of the methanogenic archaea (numbers and/or activity).

This should ideally be done with a concomitant stimulation of pathways that consume hydrogen in order to avoid an increase in the hydrogen partial pressure

in the rumen and its negative effect on fermentation as described above.

Dietary Composition

Type of Carbohydrates

Increasing the concentrate in the diet of animals reduced methane by 15–32% depending on the ratio of concentrate in diet. Relationship between concentrate proportion in the diet and CH_4, production is curvilinear. Replacing structural Carbohydrates from forages in the diet with Non-structural carbohydrates shift of VFA production from acetate towards propionate occurs with the development of starch-fermenting microbes. The low ruminal pH might also inhibit the growth and/or activity of methanogens and of cellulolytic bacteria.The nature and rate of fermentation of carbohydrates influence the proportion of individual VFA formed and thus the amount of CH_4, produced. Fermentation of cell wall carbohydrates produces more CH_4, than fermentation of soluble sugars, which produce more CH_4, than fermentation of starch therefore diets rich in starch that favor propionate production will decrease CH_4, production per unit of fermentable organic matter in the rumen. Conversely, a roughage based diet will favor acetate production and increase CH_4, production per unit of fermentable organic matter.

Level of Intake: An increase in feeding level induces lower CH_4, losses as % of gross energy intake (GEI). The CH_4, loss as % of GEI declined by 1.6 percentage units for each multiple increase of intake. This is caused mainly by the rapid passage of feed out of the rumen and as a result of the increased passage rate; the extent of microbial access to organic matter is decreased, which in turn reduces the extent and rate of ruminal dietary fermentation. About 28% of the variation in CH_4, production was attributed to the mean retention time. Also, a rapid passage rate favors propionate production, which is a competitive pathway for the use of H_2.

Forage Species and Maturity: Digestion of cell wall fibres increases methane production, by increasing the amount of acetate produced in relation to propionate. Dietary manipulation through increased green fodder decreased methane production by nearly 5-6%. CH_4, production in ruminants tends to increase with maturity of forage fed, and CH_4, yield from the ruminal fermentation of legume forages is generally lower than the yield from grass forages

Feeding Frequency*:* Low meal frequencies are tending to increased propionate production; reduce acetic acid production and lower CH_4, production in dairy cows. This effect is associated with the lowering of methanogens as a result of high fluctuations in ruminal pH, since low meal frequencies increase diurnal fluctuations in ruminal pH that can be inhibitory to methanogens.

On the other hand, more frequent feeding was shown to increase the acetate: propionate ratio which is beneficial for methane production.

Forage Preservation: There is limited information with regard to the effects of forage preservation on CH_4, production. Methane production (% of GEI) was shown to be lower when forages were ensiled than when dried. This is because digestion is reduced in the rumen with ensiled forages due to the extensive fermentation that occurs during silage making. Methanogenesis tends to be lower when forages are ensiled than when they are dried, and when they are finely ground or pelleted than when coarsely chopped. The treatment to the roughages will definitely increases digestibility and hence the fermentation reduces due to the passage of the digesta along retention time in the rumen.

Grazing Management: Implementing proper grazing management practices to improve the quality of pastures will increase animal productivity and lower CH_4, per unit of product.

Manipulation of Rumen Fermentation

Addition of Fats or Lipids: Increased lipid content in the feed is thought to decrease methanogenesis. This is due to inhibition of protozoa, increased production of propionic acid, biohydrogenation of unsaturated fatty acid. Unsaturated fatty acids - used as hydrogen acceptors as an alternative to the reduction of carbon dioxide. Fats are added to dairy cattle diets to increase the energy density of diets, enhance milk yield and modify the fatty acid composition of milk fat. It has been shown that the medium chain fatty acids (C8–C16) cause the greatest reduction in CH_4, production. So therefore addition of this fatty acid essentially lowered down the emission of methane from the ruminants.

Ionophores: Ionophores are highly lipophilic substances, which are able to shield and delocalize the charge of ions and facilitate their movement across membranes. Monensin is the most

commonly used and studied ionophore, with others such as lasalocid, tetronasin, lysocellin, narasin, salinomycin and laidomycin also being used commercially. Ionophores, which are added to ruminant diets to improve the efficiency of feed utilization, have been shown to decrease CH_4, production

Defaunation: Defaunation, which is the elimination of protozoa from the rumen by dietary or chemical agents, has been shown to reduce ruminal CH_4, production by about 20 to 50% depending on the diet composition. Protozoa in the rumen are associated with a high proportion of H_2 production, and are closely associated with methanogens by providing a habitat for up to 20% of rumen methanogens. Removal of protozoa (defaunation) from the rumen is often associated with an increased microbial protein supply and improvement of animal productivity.

Hence, defaunation has been suggested as a way to reduce CH_4, production with little or minimal effect on rumen digestion. The reduced ruminal methanogenesis observed with defaunation can be attributed to factors such as a shift of digestion from the rumen to the hind gut or the loss of methanogens associated with protozoa during defaunation.

NEW POTENTIAL MITIGATION OPTIONS

Probiotics: There is very little information on the effects of probiotics on CH_4, production in dairy cattle. The effects of the most widely used microbial feed additives, *Saccharomyces cerevisiae* and *Aspergillus oryzae,* on rumen fermentation were earlier studied in vitro. *Aspergillus oryzae* was shown to reduce CH_4, by 50% as a result of a reduction in the protozoal population. It has been shown that yeast culture influenced microbial metabolism and improved DMI, fiber digestion, and milk production in lactating cattle. However, the specific mode of action is still unknown. It has been proposed that probiotics provide nutrients, including metabolic intermediates and vitamins that stimulate the growth of ruminal bacteria, resulting in increased bacterial

Bacteriocins: Direct suppression of methanogens may be possible through stimulation of natural or introduced ruminal organisms to produce bacteriocins as a means of biological control. Bacteriocins are bactericidal compounds that are peptide or protein in nature, and are produced by bacteria. However, little information is available concerning their effect on methanogenesis. They often display a high degree of target organism

specificity, although many have a very wide spectrum of activity. Nisin, an exogenous bacteriocin produced by *Lactococcus lactis*, is the best studied and understood bacteriocin.

Archaeal Viruses: Another possible method of biological control of methanogens is the use of archaeal viruses (bacteriophages). Bacteriophages are obligate pathogens that can infect and lyse bacteria and methanogens. They are highly host-specific. Although the presence of bacteriophages in the rumen is well known knowledge of archaeal viruses is still limited.

Immunization: In the past 3 year, researchers in Australia have vaccinated sheep with a number of experimental vaccine preparations against methanogens, so that the animals produce antibodies to methanogens. Methane production was reduced between 11 and 23% in vaccinated animals and productivity was improved. No long- or short-term adverse effects on sheep were found. Researchers anticipate that commercial vaccines will allow a 3% gain in animal productivity and a 20% reduction in CH_4, production. It is important to note that the vaccines currently under development are based on cultivable methanogens.

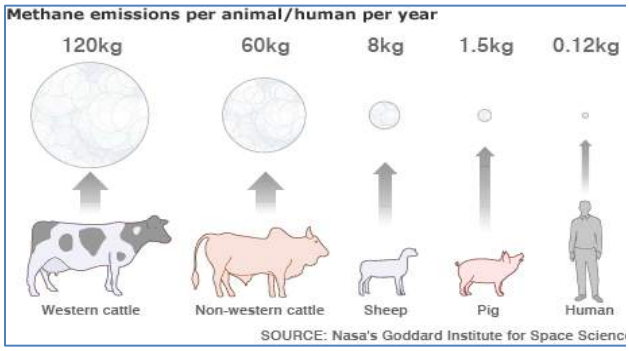
Plant extracts (condensed tannins, saponins, essential oils): There is growing interest in the use of plant secondary compounds as a CH_4, mitigation strategy. For tannin-containing plants, the antimethanogenic activity has been attributed mainly to the group of condensed tannins. Hydrolysable tannins, although they also affect methanogens, are usually considered more toxic to the animal and have not been extensively tested. Two modes of action of tannins on methanogenesis have been proposed in vitro by a direct effect on ruminal methanogens and an indirect effect on hydrogen production due to lower feed degradation. However, the antiprotozoal effect of saponins may be transient and is not always accompanied by a decrease in CH_4, production indicating that other modes of actions are also important. Similar to tannins, the source of saponins is important. Many biologically active molecules present in essential oils have antimicrobial properties that are capable to affect rumen fermentations. Among them, it has recently been shown that garlic oil and some of its components decreased CH_4, production. This was attributed to the toxicity of organosulphur

compounds such as diallyl sulphide and allicin on

methanogens. This effect was corroborated for allicin by quantitative PCR (McAllister and Newbold, 2008).

Number and productivity of animals*:* Livestock reduction through culling is also decrease the load of methane producing unproductive animal which leads to decrease the emission of methane. Proper livestock management especially in developing countries such as reducing the incidence of disease and reproductive problems can decrease CH_4 emission in a herd for each unit of production.

Variations in total GHG emission: Change in production system



From a forage-based to a concentrate-based system and low-producing animals to high-producing animals results in simultaneous variation of all GHG. Winter feeding system based on concentrates with high-yielding cows produced 37% less enteric CH_4, compared to grass system with low-producing cows, but this difference was compensated by a much higher CH_4 emission from slurry, compared to the very low emission from urine and faeces on pasture. Grass-based system in New Zealand has a lower global warming potential than in European more intensive systems.

Manure management: it refers to capture, storage, treatment, and utilization of animal manure in an environmentally sustainable manner. The sharply different manure management practices in India, as compared to the western countries, lead to much lower methane emissions from manure.

(a) Better manure management and methane recovery techniques. The flaring process decreases up to 95% of harmful atmospheric effect of methane.

(b) To create a condition unfavourable for methane generation.

Time budget for reducing methane through various strategies:

Timeline for development	Mitigation practice for the dairy industry	Expected reduction in methane
Immediate	Feeding oils and oilseeds	5 - 20%
	Higher grain diets	5 - 10%
	Using legumes rather than grasses	5 - 15%
	Using corn silage or small grain silage rather than grass silage or grass hay	5 - 10%
	Ionophores	5 - 10%
	Herd management to reduce animal numbers	5 - 20%
	Best management practices that increase milk production per cow	5 - 20%
5 years	Rumen modifiers (yeast, enzymes, directly fed microbials)	5 - 15%
	Plant extracts (tannins, saponins, oils)	5 - 20%
	Animal selection for increased feed conversion efficiency	10 - 20%
10 years	Vaccines	10 - 20%
	Strategies that alter rumen microbial populations	30 - 60%

CONCLUSION

There are a number of nutritional technologies for improvement in rumen efficiency like, diet manipulation, direct inhibitors, feed additives, propionate enhancers, methane oxidisers, probiotics, plant secondary metabolites and defaunation. Keep in mind to reduced methane from ruminants we can have best option such as genetic selection for low residual feed, animal population and their productivity. Change in production system from forage based to concentrate based help to reduced methane emission from livestock along with efficient utilisation and disposal of manure definitely reduced methane from ruminants.

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Anthelmintic Resistance – A Serious Set Back To Small Ruminant Production

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"Anthelmintics are used traditionally as an integral part of helminthic control strategies for grazing livestock to prevent production losses from parasitic infections. The continuous and indiscriminate use of the same anthelmintics over years together as the sole means of control are now failing due to the emergence of resistance strains of helminths. Resistance to the commonly used anthelmintics in gastrointestinal nematodes of sheep has become an increasingly wide spread problem throughout the world. Diagnosis of Anthelmintic resistance can be diagnosed by the history and a failure to respond to treatment, with confirmatory testing such as an on-farm trial using a faecal worm egg count reduction test (WECRT). This paper summarizes the ways and means to avert and delay this anthelmintic resistance."

In India, sheep and goat production plays a vital role in augmenting socio - economic status of the rural masses, particularly the small land holders and landless farmers, who rely on these animals for their animal protein source and income for their livelihood (Lateef, 2003). Sheep and goats are susceptible to many dreadful diseases causing heavy economic losses in terms of reduction in weight gain, delay in maturity, reduction in lambing percentage, poor hide quality and mortality in extreme cases. Among the diseases of small ruminants, gastrointestinal helminthic problems always come to the fore because majority of species reach their host directly through vegetation, without involvement of intermediate host. This factor, together with ideal climatic conditions for survivability of pre parasitic stage, favour high prevalence of gastrointestinal nematodes in tropical countries and hence, they are recognized as a major constraint to livestock production throughout the tropics and elsewhere (Githiori *et al.,* 2004). Direct and indirect losses due to nematode infections are estimated to be high and control of these parasites is therefore considered important. Winrock International (1992) indicated that over $4 billion loss in animal productivity as a result of animal diseases and half of this loss was due to internal parasites such as helminths.

Effective parasite control has become heavily dependent on anthelmintics. The compulsory and often excessive use of these anthelmintics in combination with poor managemental practices has resulted in resistance to most of the available anthelmintics. The development of anthelmintic resistance by roundworms to these anthelmintics, poses a potential crisis for sheep producers and measures to avert and delay this are highly essential.

What is resistance?

Resistance (AR) is the heritable change in the ability of some nematode parasites to survive treatment with a therapeutic dose of anthelmintic drugs. The genes responsible for resistance are present in many of the important pathogenic round worms of animals. The first report of anthelmintic resistance has been described in 1957 against phenothiazine. Since then, it has become a world wide problem especially in sheep and has been documented to almost all classes of anthelmintics. The parasite once developed resistance to one anthelmintic, will also become resistant to other drugs which have similar mode of action (Side resistance). Some parasites develop resistance against different classes of anthelmintics at the same time (cross resistance).

How resistance occurs?

- Indiscriminate use of different classes of anthelmintics at short intervals.
- Prolonged use of a single class of anthelmintic drug.
- Improper calculation of dose of anthelmintics leading to under or excess dosing.
- Improper dosing due to spillage or faulty equipments.
- Drenching of anthelmintics without knowing the type of worms present in animals.

How can diagnose the resistance?

Anthelmintic resistance can be suspected when there is poor response to anthelmintic treatment. A number of *in vitro* and *in vivo* tests can be used for detecting the resistance in gastrointestinal nematodes. Egg hatch assay, larval migration inhibition assay and larval development test are the most commonly used *in vitro* tests. These assays involve examining the development of eggs and larvae, in various concentrations of the anthelmintic. The larval development test can be used to detect resistance to both benzimidazole and levamisole. The *in vivo* technique, faecal egg count reduction test (FECRT) is the most practical method of determining resistance by nematodes in sheep and other animals. It is a simple

and field friendly test that does not require highly trained personnel, extensive resources, sophisticated equipment or facilities. It can be applied to detect the development of resistance to all class of anthelmintics and in all species of animals. The susceptibility or resistance of worms to anthelmintics is determined by comparing the faecal egg output (epg) in animals prior to and 15 days after anthelmintic medication. When there is reduction of egg per gram (epg) by more than 95 per cent after 15 days of treatment, the drug is susceptible. A reduction of epg less than 90 per cent is indicative of resistance. If the anthelmintic kills 90 percent or more of the worm eggs, it is considered to be effective. If it kills 60 to 90 percent of worm eggs, it is considered to have a moderate level of resistance. Anthelmintics reducing less than 60 percent of worm eggs are considered to as severe resistance (Coles *et al*. 1992).

Current and future strategies for combating anthelmintic resistance

The challenge to veterinarians and producers is to utilize known and emerging technologies to control anthelmintic resistance of gastrointestinal nematodes of livestock especially small ruminants such as sheep and goats. The below mentioned are some of the techniques commonly employed to control or delay the development of resistance.

- Use the most appropriate anthelmintic of reputed companies.
- Check the drenching gun is delivering the correct volume or dose of drug.
- Animals should be weighed or measured with a tape to determine the proper dosage since under dosing is a leading cause of development of anthelmintic resistance. When deworming a group of animals, the dose should be set for the heaviest animals in the group, not the average.
- Anthelmintic treatments should be targeted to the most susceptible animals in the herd such as lambs/kids, lactating ewes/does, and high producers. Leaving some animals untreated will help to slow anthelmintic resistance by maintaining anthelmintic susceptible population of worms (Refugia - not exposed to anthelmintics to reduce the intensity of selection for drench resistance in environments where there is a high risk (Van Whk, 2001).
- Frequent deworming is costly. It accelerates the development of anthelmintic resistant worms and leads to a false sense of security,

which may result in unnecessary production losses and animal deaths.

- Double the cattle/sheep dose when deworming goats for all dewormers, except levamisole which should be dosed at 1.5 times the cattle/sheep dose in goats since goats metabolize anthelmintics differently (it clears their system faster) than sheep and cattle and require higher doses.
- Maintain the animal on dry fodder or fasting prior to dosing.
- Anthelmintics should be administered orally, over the tongue of the animal.

Research has shown that benzimidazoles are more effective when the animals are fasted 12 to 24 hours before treatment or when two treatments are given 12 hours apart (repeating the drench 12 hrs after the first dose).

- Anthelmintics should not be used indiscriminately.
- Avoid using the same class of anthelmintics years together; rotate the anthelmintics annually with different mode of action
- Test for anthelmintic resistance regularly and also after deworming by monitoring the egg count
- Avoid the introduction of resistance onto a farm by treating purchased stock on arrival followed by a quarantine period.
- Provide safe pasture for grazing animals.
- Minimize the stocking rate of animals in farm/pasture land.

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Prognosis of Equine Colic by Clinico-Pathological Tests

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Laboratory tests are more valuable in deciding the eventual prognosis of the colic case than in the diagnosis decision for the surgery. Tests that can be helpful include complete blood count, total protein and acid-base status, blood lactate, blood urea nitrogen and blood glucose. However, they may be better utilised in the assessment of the degree of cardiovascular compromise and in determining the appropriate medical therapy prior to surgery (Mark Johnston 1992). Jeremy Davies (1985) reported Blood in EDTA, serum or plasma and peritoneal fluid samples might be collected for laboratory evaluation. However haematocrit PCV (packed cell volume), total serum protein and total peritoneal fluid protein can easily be assessed in the field.

Field Hematology

PCV is probably the most frequent used laboratory test to suggest the ultimate prognosis and need for surgery. Normal packed cell volume is 32% to53%. Values of 60% indicate immediate fluid therapy and, if rising, suggest a serious colic which is producing hypovolaemic shock. If as high as 70% euthanasia is indicated. In general a PCV above 60%, carries poor prognosis. It is better to consider a raised PCV in conjunction with elevated serum total protein rather than a PCV value. Normal value for total serum protein is 6-8.4 ± 0-9 g/dl (as read on refractometer) and normal total peritoneal fluid. protein is 3-5 ± 1-0 g/dl.

Bio chemical analysis

In body, 5-HT is stored in blood platelets and in the enterochromaffin cells of the GI tract. These cells are found in the mucosal epithelium of the whole GI tract of many vertebrate, including horse. It is know that free circulating plsma 5-HT influence GI motility. This was predominantly found in colic horse predisposed to develop ileus, namely intestinal necrosis and endotoxaemia, both platelets and EC cells of necrotizing bowl segments, could serve as a source of 5-HT overload in colic horses (Koenig and Cote 2006). Delesalle *et al*

(2008) concluded that Lactic acid values were increased both in blood and peritoneal fluid of the strangulating small intestinal colic cases, with exceeding 200nmol/l. In endotoxaemia leading to an increased platelet activity, DIC, coagulopathy have been demonstrated in colic horses. The determination of plasma 5-HT concentration could be a useful prognostic parameter for disseminated intravascular coagulation (DIC). Raditional clinical variables as heart rate and presence of abnormal mucus membrane and PCV in surgical and medical colic cases were the significant predictors for outcome. The other variable like Albumin, Anion Gap, Na+ , K+, CL-, total calcium, magnesium, Lactate, etc were ,however important in establishing supportive treatment of the horses.

Acid- Base balance

Moore, *et.al* (1967) evaluated that the normal plasma L-lactate concentration in horses is generally considered to be < 1.5 mmol/L. Increases in plasma L-lactate concentration have been categorized as mild (2.5 to 4.9 mmolIL), moderate (5.0 to 9.9 mmol/L), and severe (> 10 mmol/L), with a low probability of survival in horses affected with colic when the blood lactic acid is > 6.72 mmol/L. An increased amount of plasma L-lactic acid in 14 horses with intestinal disorders was held responsible, in part, for a high anion gap. A high anion gap has also been associated with an unfavorable prognosis in horses affected with colic. However, the high anion gap in horses with intestinal disorders has never been completely explained by an increase in whole blood lactate, pyruvate, hydroxybutyrate, or acetoacetate concentrations. Significant, decreases in HCO3-, TCO2, and base excess were found in most horses with colic, suggesting an underlying metabolic acidotic process, although the pH was not affected (Autran 1994). Hypokalemia in horses with colic was most likely associated with altered intake and absorption or with excessive K+ losses from the gastrointestinal tract caused by diarrhea (Carlson 1996). Most of the horses with colic having a higher level of L-lactate. The L isomer of lactate predominates in mammalian metabolism and is of particular importance in tissue hypoxia. In order to meet the continuing needs for adenosine triphosphate during anoxia, anaerobic glycolysis predominates over the aerobic tricarboxylic acid cycle, resulting in the increased production of L-lactate from pyruvate by the action of L-

lactate dehydrogenase (Nappert and Johnson 2001).

Peritoneal fluid Analysis

Classification of peritoneal fluid

Type of fluid	Total nucleated cell count (X 10 litre)	Total protein (g/litre	Major underlying process
Normal	<10	<25	-
Transudate	<5 usually <1.5	<25 usually <15	Decreased COP Increased CHP
Modified transudate	1.5-10	25-35	Increasde CHP
Exudate	>10	>30-35	Incresed CP

From DeHeer et al., (2002)

COP- colloidal osmotic pressure, CHP- Capillary hydroststic pressure

CP- Capillary permeability.

The appearance, and biochemical and cellular constituents of peritoneal fluid reflect the state of the mesothelial surfaces and the organs they cover. The smaller constituents, such as electrolytes, creatinine and urea, reflect local and/or systemic events. In the field, only the gross appearance and, possibly, the protein content and/or specific gravity of peritoneal fluid can be quickly assessed.

GROSS EXAMINATION

Volume

A subjective assessment of peritoneal fluid volume can be made at the time of sample collection based on ease of collection and flow rate. However, the fluid may be pocketed within the abdominal cavity, and a low flow rate does not always correlate with a low volume.

Appearance

Normal fluid is clear, some time with a few small white flocculi and pale yellow in color. Peritoneal fluid finding in cases of colic below in table.

Smell

The presence of gut contents, bacteria or urine may be suspected from the smell.

Protein content and specific gravity

Increased in protein and specific gravity values are expected in horses with peritonitis and are accompanied by significant inflammation or vascular compromise of the gut. These values are normal in medical colic. Such as a colonic impaction.

Total nucleated cell count

The total nucleated cell count is of value if time and facilities allow and is required for the correct interpretation of cytology.

Peritoneal fluid changes

There have been numerous studies on the predictive value of peritoneal fluid analysis in cases of medical and surgical colic and these have produced differing results.

Type of colic	Appearance	Total nucleated cell count (X 10 litre)	Total protein g/litre	Specific gravity
Normal	Pale yellow	0.5-5.0	5-15	1.000 - 1.015
Medical	Yellow, clear to slightly turbid	5-15	16-25	1.016 - 1.020
Surgical	Yellow/orange to pink/red/brown, turbid	>15	>26	>1.021
Acute grass sickness	Deep yellow, clear to slightly turbid	1.1-40	14-62	1.015 - 1.041

Elespeth milne (2004) reported that the Peritoneal fluid analysis is a useful ancillary diagnostic procedure to help determine the need for surgery in cases of acute equine colic. However, that, of the readily available tests on peritoneal fluid, gross appearance is the single most valuable indicator of the need for surgery. A combination of gross appearance, protein concentration and/or specific gravity improves the predictive value.

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