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Outlook on Leishmaniasis

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Abstract

Leishmaniasis is a neglected tropical disease with socio economic impact caused by intracellular protozoan parasite i.e. *Leishmania* transmitted to vertebrate during blood feeding by sand fly i.e. *Phlebotomus* and *Lutzomyia* sp. This disease is present in almost all continents such as Asia, America, and Mediterranean area. According to World Health Organisation (WHO), it is one of the seven important tropical diseases in the world. It can occur in many forms such as visceral, cutaneous and muco-cutaneous forms and can also lead to formation of Post-kala-azar dermal leishmanoid for whole life of an individual. Public awareness and knowledge about the disease transmission and zoonotic potential of the disease would be of immense help. Current popular article provides a brief information about some crucial aspects of *Leishmania* including life cycle, different forms of disease, clinical signs, diagnosis treatment and control.

Introduction

Environmental changes due to global warming, is providing more conducive atmosphere year by year for various insects and as a result chance of vector borne diseases such as malaria, *Trypanosomiasis*, Dengue fever, West Nile Virus, Leishmaniosis, Lyme disease etc. is also increasing. Among many vector borne diseases, Leishmaniosis (caused by protozoa i.e. *Leishmania* sp) still remains a serious concern as it is the most crucial neglected diseases worldwide (Bailey et al 2019; Bilgic-Temel et al 2019) with a socio-economic impact.

Total burden of this disease across world is still unknown, but it is estimated that 1.3 million new cases, 12 million prevalence and 65000 (approx) mortality in 101 countries occur due to Leishmaniosis annually and the figure could be much higher (WHO, 2020, Alvar et al 2012). At present there is no vaccine which is 100% efficient against Leishmaniosis and at the same time use of conventional drugs are also not sufficient (Jaafari et al 2019). People specially living in rural area should be made aware of this zoonotic disease so that they can understand the disease transmission, can adapt best possible preventive measures and hence the chances of disease may be reduced. . Current popular article deals with various aspect of *Leishmania* sp. infection such as life cycle, different forms of disease, clinical sign, diagnosis and treatment in summarised form.

Genus- Leishmania

Various species of *Leishmania* occur in the mammals, lizards and vertebrates. In vertebrate hosts like man and animals, the amastigote form is seen, whereas in intermediate host i.e. insects they occur in the promastigote form. Majority of species are morphologically identical and are classified based on geographical distribution and pathogenicity. Ultra-structural studies and by serotyping of metabolites released in culture media by promastigote forms, immunology and biochemistry, it is now easy to identify the species and sub-species. Almost there are 21 (<https://www.cdc.gov/dpdx/leishmaniasis/index.html>) *Leishmania* sp. are known to infect human population and more than 30 *Phlebotomus* sp. (Sandflies) act as vectors for propagation of disease (<https://www.cdc.gov/parasites/leishmaniasis/epi.html>).

Mainly three forms of diseases are known clinically:

- 1) Visceral leishmaniosis or kala azar caused by *Leishmania donovani donovani*, *L. d. infantum* and *L.d. chagasi*
- 2) Cutaneous leishmaniosis caused by *L. tropica*. Other species are *L. aethiopica*, *L. peruviana*, *L. mexicana* etc.
- 3) Muco-cutaneous Leishmaniosis: caused by *L. braziliensis*

The amastigote stages in vertebrate host are ovoid or round in structure with 2.5- 5.0µm x 1.5-2.0µm size. Nucleus and kinetoplast are visible after staining. On electron microscopy, a tract of flagellum and basal granule are observed. On staining (Giemsa stain) the cytoplasm appears blue. The nucleus appears red and kinetoplast is purple. Promastigotes (in intermediate host) are spindle shaped 14-20µm x 1.5- 3.5µm.

Life cycle

Leishmania infection is directly related with poverty, mal nutrition (<https://www.who.int/news-room/fact-sheets/detail/leishmaniasis>), organ transplant and immune suppressive condition (Oryan and Akbari 2016). People working as construction workers, or people working nearby forests due to more chances of fly exposure.

During biting for blood feeding, the vector injects the promastigotes form of parasites which are engulfed by the macrophages on the skin in vertebrate hosts and are transformed to amastigote in 1-4 hrs (2-4µm). These forms divide and multiply by binary fission inside a parasitiphorous vacuole and the macrophages burst and free the amastigotes which may enter other macrophages in the skin leading to cutaneous leishmaniosis.

Amastigotes of the *L. donovani* group are then carried to internal organs and the enter various host cells mainly reticuloendothelial system in the skin, spleen, liver, bone marrow, lymph nodes, mucosa etc. and even in mononuclear cells of the blood, where they reproduce by repeated binary fission and cause visceral leishmaniosis in 4-6 months. In the vectors (sandfly) either *Phlebotomus* or *Lutzomyia*, acquires infection by biting the infected hosts and ingests amastigotes form of parasite along with blood, which later get transformed to slender promastigotes (10-20µm) in the midgut of sand fly and then they multiply by repeated binary fission. These promastigote forms move from gut of the vector toward the pharynx and buccal cavity and block the proboscis of fly, then these organisms are the injected to a new host during blood feeding.

History and regional impact

Leishmania donovani donovani causes visceral leishmaniasis or kala azar (black coloration of skin due to organisms) also known by names such as kala- dukh (Bihar), sarkari beemari (Assam), Dum Dum fever (Calcutta), Burdwan fever (West Bengal). *L. donovani* was discovered at the same time by Leishman and Major C. Donovan. The organisms bears Donovan's name second because the ship carrying the news of his discovery took 3 months to reach England. This parasite was discovered in India by Leishman and Donovan in 1903 from splenic tissue of a child at Madras medical college Hospital, Madras (now Chennai). The vector was also identified here by Swaminathan and others in 1942. Its treatment was formulated by Sir U.N. Brahmanchari in 1992 in India. Man is mainly affected. Dogs and foxes are reservoir hosts

Different types of disease occur such as Indian, Chinese, Mediterranean, African, Russian and American kala azars.

Kala azar is a zoonotic disease in other regions of the world. 500000 people are affected globally, 50% cases are in Indian sub-continent, Nepal and Bangladesh is the places where this disease is anthroponotic (disease spreading from man to animals). It occurs in India since 1824 (Jessore, now in Bangladesh). Epidemics outbreaks have occurred in Assam. Now 95% of the cases are form Bihar followed by West Bengal and Eastern Uttar Pradesh. It is also endemic in Northern Eastern state, Tamil Nadu, Jammu and Kashmir, Gujarat, Punjab and Puducherry. From Bikaner, Rajasthan, in 2001-2011, 1379 patient having 2730 lesions of cutaneous leishmaniasis was reported, majority of patient were from lower middle class background. Positivity by skin smear and biopsy was 69.5 and 45.8% respectively (Aara et al 2013). From January to June 2018, 14 patients with 25 lesions were again reported in Rajasthan from poor socio-economical group. Antigen test, microscopy, PCR, and histology positivity rate was 7, 71.4, 72.7 and 38.5% respectively (Rajni et al 2019).

The vectors in India are *Phlebotomus argentipes* which are anthropomorphic-nocturnal flies and usually young adults and children of 5-15 yrs are affected by this disease and incubation period ranges from 10 days to 1 yr (max 9 yrs). Symptoms include an irregular fever, malaise, headache, gastro intestinal disorders, bleeding from mucous membrane of mouth, nostrils, darkening of skin, hair becomes brittle. Splenomegaly and hepatomegaly are marked. Ulceration of digestive tract in advanced cases, emaciation and mortality ranges from 7-90%. In recovered and treated cases, small spots and nodules occur on skin especially of face and neck containing organisms and is known as Post Kala Azar Dermal Leishmanoid (PKADL). In chronic cases eczema, alopecia, ulceration on eyelid, lips, and nostrils is seen. Serious leishmanial infections are characterized by a complex host parasite interaction. Host immune system responds extensively and the parasite evades the immune response.

L. donovani infantum causes infantile visceral leishmaniasis. Dogs acts as reservoirs. It occurs in Mediterranean countries and vector is *Phlebotomus* species.



PKDL from Sudan (Zijlstra 2019)

Anthroponotic cutaneous leishmaniasis (a-g localized skin lesions; h- ulcoid leishmaniasis (Karimi et al 2021)

Cutaneous leishmaniasis

L. tropica minor causes by cutaneous wet oriental sore diseases in man. Rodents and dogs are reservoir hosts. Disease known as Delhi boil or old world cutaneous leishmaniasis. It occurs in desert region as in Rajasthan, previously in Delhi and Haryana. Vectors are *P. papatasi* and *P. sergenti*. It occurs in man, dogs, gerbils, (*Meriones hurriane*) and the other wild rodents. It is found in hot and dry climatic areas. The parasites are restricted to the skin- multiply and rupture infected cells and 3-4 weeks post infection reddish papules occur on skin, crust forms, ulceration occurs and ulcer coalesce and spread 2-12 months later they heal and leave a heavily pigmented scar. It runs a chronic course, healing occurs and immunity is solid.

Diagnosis

1. History and clinical sign
2. Demonstration of *Leishmania donovani* bodies in peripheral blood smears, skin scraping, biopsy material and symptoms.
3. Culture of aspirates in Novy, MacNeal, Nicolle medium or NM medium.
4. Sero-diagnosis by direct agglutination tests, IFAT and ELISA.
5. Chemical tests:
 - a) Napier's aldehyde test (In cases > 3 months old) - Drop of formalin + 1-2 ml of suspected serum. Milky white gel in positive cases.
 - b) Chopra's antimony test- Tricking down of 4% urea stibamine in a tube containing 1-2 ml diluted serum (1:10). Formation of white precipitate in 15 mins indicates positivity.

Treatment

1. Sodium stibogluconate or meglumine antimonite or sodium antimony gluconate – 20mg/ kg I/m for 20-30 days (Max 850 mg/ day).
2. Pentamidine- 4mg/kg I/M- 5 week 15 injection has side effects as diabetes, renal impairment.

3. Miltefosine- oral 50 mg capsule- 95% effective. 100-200mg/ day (upto 2800-5600 mg/ adult).
4. Amphotericin B- alternate drug. 0.5- 1mg/kg slow infusion daily or every 2 days for 8 weeks.

Control measure

1. Wear full cloth and cover as much skin as possible
2. Exposed area such as hand etc must be applied with insect repellent
3. Surrounding area should also be sprayed with disinfectant
4. Use fans and screens in home
5. Avoid playing in dusk and dawn

Conclusion

Proper knowledge about vector and protozoan parasite-borne zoonotic disease like leishmania will provide benefit to rural people and in-turn reduce the incidence of disease in rural belt of India.

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Applications of CRISPR/Cas9 Genome Editing Technology in Veterinary Medicine

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Abstract

Genome editing technology is a technique for targeted genetic modifications, enabling the knockout and knock in of specific DNA fragments in the selected genome. This technology has been widely used in various types of biomedical, clinical and agricultural research. Three gene editing techniques including Zinc-finger nucleases (ZFNs), Transcription activator-like effector nucleases (TALENs), and CRISPR/Cas9 are commonly used in biomedical and life science research, with CRISPR/Cas9 now being the most widely used. In this review, we discuss the applications of CRISPR/ Cas9 genome editing in the fields of veterinary medicine and animal husbandry.

Introduction

CRISPR is an acronym for “Clustered Regularly Interspaced Short Palindromic Repeats.” CRISPR genome engineering technology allows researchers to easily edit the DNA of any genome. Naturally, the CRISPR/Cas system plays an important role in microbial immunity. It acts as self-defence system in a sequence specific manner against exogenous virus or plasmid in bacteria by cleaving their DNA or RNA. When a virus or bacteria infects a microbial cell, the microbe employs a special CRISPR-associated nuclease (Cas9) to chop off a piece of the foreign DNA. The short RNA fragment known as a guide RNA (gRNA) directs the nuclease to its target sequence. The chopped off DNA fragment maybe then stored between the palindromic CRISPR sequences to retain a genetic memory for disabling future infections from the same viral strain (Hille and Charpentier, 2016).

Applications of CRISPR/Cas9 genome editing technology in veterinary medicine and animal husbandry

The use of CRISPR/Cas9 technology in the field of veterinary research is greatly revolutionizing the ability to manipulate the animal genome to create appropriate disease models and disease resistant animals and also improves the quality meat production and animal welfare.

Transgenic animal models development

Developing appropriate animal models is necessary to understand the pathobiology and molecular mechanisms of human and animal diseases and it also plays an important role in drug development and organ transplantation. Many animal disease models have been generated for basic and clinical research by combining reproductive technologies like micro injection with genome editing. In addition, these animal models play an important role in the field of pre-clinical gene therapy and stem cell therapy research for rare genetic diseases.

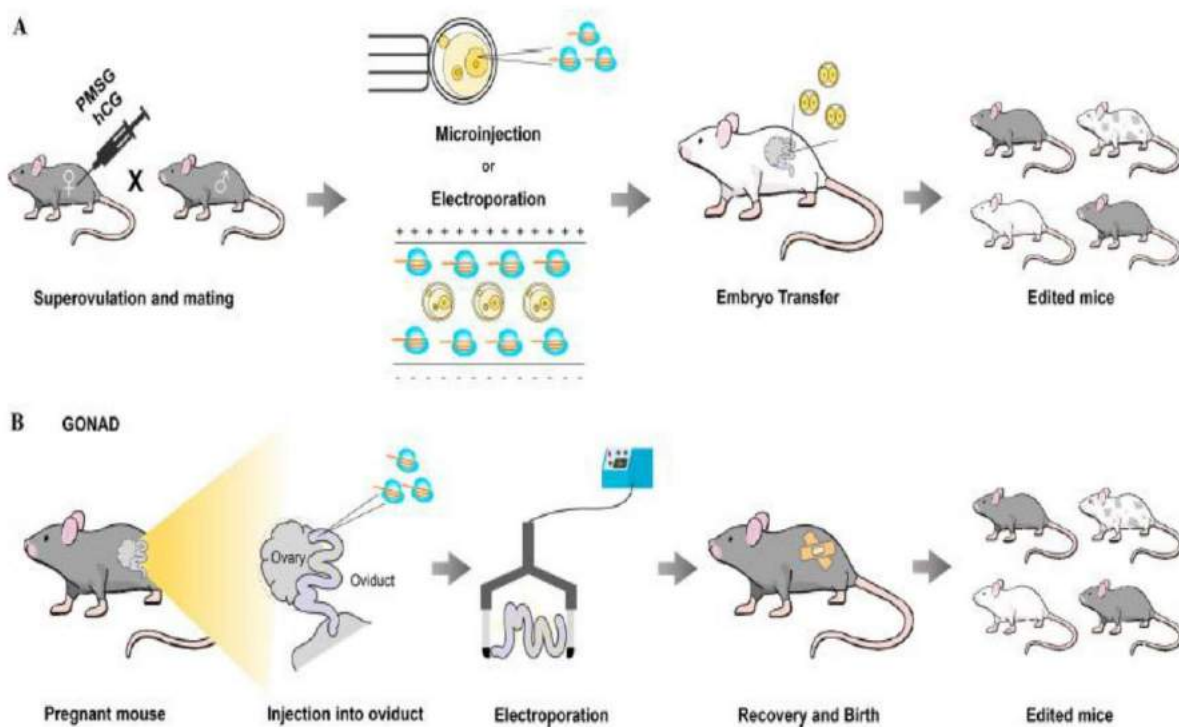


Figure 1. A: Transgenic mice production using CRISPR system. A. CRISPR delivery to embryos using microinjection or electroporation. Edited 2-cell-stage embryos are transplanted into a surrogate mother and the genome edited offspring are obtained. **B:** Direct injection of the Cas9/gRNA complex into oviduct of pregnant mouse, followed by an electrical impulse produce genome edited pups (Adopted from Lee et al. 2020).

Table 1. List of animal models developed by using CRISPR/Cas9 technique (Lee et al. 2020)

Species	Disease model	Targeted gene	Technique
Mice	Tyrosinemia	Fah	Microinjection
	Hemophilia A	F8	iPSC correction & IV injection
	Hemophilia B	F9	Intravenous injection
	Duchenne Muscular Dystrophy	Dystropin	Intramuscular injection
Rat	Retinal dystrophy	Rho	Subretinal injection
	Parikinson’s disease	TH	Intracranial injection
Pig	Liver failure, traumatic shock	Alb	Microinjection
	Huntington’s disease	Huntingtin	Somatic cell nuclear transfer
Dog	Muscular Dystrophy	Dystropin	Intramuscular injection

Cancer biology

Since the cancer genome is highly complex, with hundreds of point mutations, translocations, and chromosomal aberrations per tumour, suitable animal models are needed to understand the effects and mechanisms of these alterations. Traditional methods to develop mouse models for oncological studies are time-consuming and laborious. The recent development of the CRISPR-Cas9 system is improving the generation of mouse models to study cancer biology.

Table 2. List of mice cancer models developed by using CRISPR/Cas9 (Mou et al. 2015)

Cancer model	Target tissue	Delivery	Genes targeted
Rett syndrome	Embryo	One-cell embryo injection	Mecp2 Cre-LoxP
Colon cancer	ES cell	Intra peritoneal	p53, Apc, Pten
Hepatocellular cancer	Liver	Hydrodynamic injection	Pten, p53, β -catenin

Acute Myeloid Leukemia	Fetal-liver HSCs ex vivo	Intravenous injection of Cas9-edited HSCs	Mll3
lung cancer	Lung	Intranasal/intra-tracheal	p53 and Lkb1, Kras
Dox-inducible Burkitt lymphoma model	Fetal-liver HSCs ex vivo	Intravenous injection of Cas9-edited HSCs	Mcl-1, p53

Swine production and research

Pigs are important domestic animals reared for food and pharmaceutical applications; they also served as ideal animal models for various human diseases such as diabetes, obesity, atherosclerosis and other cardiovascular diseases. Pork is an important meat source in the western countries. Worldwide, pig production accounted for 42% of total livestock production in 2018, and this percentage is expected to increase in the coming years. However, the existing breeding methods are not enough to meet the developing needs of pig production. The use of CRISPR-Cas9 technique has greatly promoted the advancement of pig production and research (Lin et al. 2019).

Following are the applications of CRISPR/Cas9 genome editing technique in the field of pig breeding and research:

1. In the development of rapid viral vaccines against pig pathogens such as pseudorabies virus, porcine reproductive and respiratory syndrome virus, classical swine fever virus and African swine fever virus.
2. In fast and reliable breeding and reproduction of disease resistant pigs. In 2017, Whitworth et al. used CRISPR/Cas9 technique to generate CD163-knockout pigs to protect pig from porcine reproductive and respiratory syndrome virus (PRRSV).
3. In the field of transplant immunology studies which use pigs as an animal model. Sato et al. in 2013 and Petersen et al. in 2016 created piglets with biallelic knockouts of GGTA1 gene by using the CRISPR/Cas9 system. It was proved to be a best model to study xenotransplantation.
4. In the development of swine disease models used in the translational medical research. Swine models of human type I and III *von Willebrand* disease, Huntington’s disease; insulin-deficient pigs for diabetes research, RUNX3-associated stomach cancer have been developed so far by using CRISPR/Cas9 technology.

5. CRISPR/Cas9 technology also improved the quality of pork. Amount of fat and lean meat contents are important factors determines the palatability of pork. Myostatin (MSTN) knockout cloned pigs without selectable marker gene (SMG) developed by combined use of CRISPR/Cas9 and Cre/LoxP showed more pronounced skeletal muscles and decreased back fat thickness.

Farm animal production and research

Genome editing in the farm animals (bovine) is majorly focused on the production of disease resistant animals (e.g., tuberculosis, brucellosis), improved generation of meat and dairy products, animal sexing, introduction of desirable phenotypes (e.g., stress tolerance, disease resistance) and animal welfare (e.g., polled or hornless).

Following are the applications of CRISPR/Cas9 genome editing technique in the field of farm animal breeding and research:

1. Production of disease resistant animals

Tuberculosis-resistant genetically modified cattle (NRAMP1 knock-in), bovine spongiform encephalopathy, and chronic wasting disease resistant cattle (PRNP knock-out), Jhone's disease resistant cattle (IL10RA knock-out), and brucellosis resistant cow (virB10 or Rpo1A transduction) were generated by CRISPR/Cas9 mediated genomic editing of bovine genome (Singh and Ali, 2021).

2. Improving Animal Welfare

A horned phenotype of bovine (plethora) increases the risk of injury or damage to the animal and handler. A polled (hornless) phenotype is preferred in this case. Usually, polled phenotypes are essentially used in Angus meat breeds. In different meat breeds, the polled Celtic (Pc) variation, within the polled locus, induces a polled phenotype. Schuster et al. in 2020 produced polled HF bulls by incorporating the Pc variety into its genome by using CRISPR/Cas12a framework which eliminated the need for dehorning.

3. Improving semen sexing

Semen sexing to find out the sex of developing embryos before foetus transition in animals was improved by knock-in eGFP (green fluorescent protein) gene within the Y-chromosome of bovine fetal fibroblast (BFF) cell lines with the assistance of CRISPR/Cas9 (Zhao et al. 2020).

Conclusion

Over the past few years, CRISPR/Ca9 genome-editing technology improved the development of genetically engineered animals which could be served as animal models for various human diseases in translational research. The use of the CRISPR/Ca9 technique in veterinary and animal husbandry research showed promising results in the production of disease resistant animals, quality meat and also improved the animal welfare. This novel technology will continue to revolutionize veterinary medicine. Precision animal models will pave the way for precision drug discovery.

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Dehorning in cattle

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Horns of animals serve as weapons of defense against predators. Basically horns are hardened corneal projections of several types and there is a need to remove these projections to gain benefits for animal as well as for farmer. Process of removal of such projections is called dehorning which can be either physical or chemical. In this review we can discuss about dehorning; its methods advantage and disadvantages.

Why there is a need of dehorning?

There are various reasons for dehorning It reduces the chances of injury to other animals along with handler. Dehorned animal covered less space as compared to horned animals. During auction dehorned animals sell at good price as compared to horned animals.. Dehorned animals are easy to handle and become docile.

Age of Dehorning

Dehorning is done at early stage of life of calves because it easier for the operator to perform dehorning and less stressful as compared to adult one. Normally the ideal time for dehorning is 2 to 6 week of age. According to Canadian Veterinary Medical Association (CVMA) dehorning can be performed at 1 week of age of calf.

Timing of Operations

Best time for dehorning is winter as well as spring season. In case of summer and autumn season there are more chances of infection that's why in these seasons dehorning is avoid to perform. In every dairy farm, the timing of the dehorning will depend on an effective management system. Dehorning at early age should go hand in hand with many management plans.

Points of concern during and after dehorning.

1. Animals showed pain and discomfort in all types of dehorning method.
2. Young calves recover faster and have fewer problems than adults one.
3. Local anesthesia before dehorning eliminates severe pain within short duration after 4.

Local anesthesia, along with analgesic can be serves as good pain relief.

5. Without uses of anesthesia, Dehorning is impersonal and wrong.
6. The use of painkillers is extra expense for manufacturers. Pain relief can be reduced by the availability of medicines for farmers to use.
7. The use of polled bulls is a more environmentally friendly method of dehorning. Canadian cattle producers increase their use of polled bulls.

Methods of Dehorning

1. Chemical Dehorning

Chemicals like sodium hydroxide and potassium hydroxide are properly applied to the horn bud of calves it will retard raising of horn. The horn secreting cells are destroyed by these chemicals. Chemical are readily available in market in the form paste and sticks. Operator protect from these chemical by wearing gloves, to avoid the chemical placed near to the calves eyes or nose. When the rainy season, chemical dehorning will not use.

Techniques

Injected local anesthesia, analgesia along with sedatives. Examine horn buds (about five percent) by pressing the hair back. Placed a thin layer chemical on horn buds with the use of wooden applicator / stick. Re-place the hair on top of the attachment & horn bud - that is, close the horned bud. Moreover, packaging may instruct operators to reduce hair loss at horn buds, experienced operators have shown that hair is best, as hair retains its natural value, alter the chances of irritation in cow's breasts and sides & low irritation of face of calf skin. Protect calf and cow from accidental burns. One way is to put a tape measure on each horn buds. Duct tape usually falls in a few days. For milk calves, store in individual pens. In some countries, the procedure is only allowed for calves less than eight days of age.

Advantages and Disadvantages

- It is done at early age under less pressure than other methods
- Without blood
- For use in any period
- Pain without anesthesia
- avoid eye contact; the operator must wear gloves
- When it is rainy weather, then don't used
- it is not allowed in few countries
- Horns or scurs follow the wrong procedure
- requires pain management

Hot Iron Dehorning

Hot iron dehorning are available in types that are heated by a furnace or fireplace, 12-volt battery, 120-volt electric, electric packs. The head of the metal is an empty circle and penetrates over the horn buds.

Proper use of hot metal will eliminate horn secreting cells. This method works best for calves' age up to 12 weeks of age. There are different size dehorner metals. The optimum size is when the burner makes a complete ring around the base of the horn. For electrical equipment, use

a short extension cord as the voltage drops through the long cable, reducing the amount of heat generated by the dehorner.

Techniques

Injected local anesthesia with analgesia. Preheat dehorning iron to a red color. Both electric and electric appliances work best when they are "red" hot. Operators or workers wear gloves to protect own hands. Hold the calf's ear off the away so that it does not burn. Placed the tip of the dehorner rods on the horn bud and apply a little pressure. When burning hair begins to smoke, slowly circulate the scent of the iron rod by twisting your wrist. Continue to use heat for 10-15 seconds. Do not leave the dehorner in place for too long, especially for calves. Dehorning is complete when there is a copper ring all around the base of the horn. The horn bud will shrink in about 4 to 6 weeks.

Benefits and Limitations:

Without blood

- It can be used at any time of the year
- Young calves up to 12 weeks old
- Dishonesty when done wrong, leads to scurs (small horn growth)
- Requires expertise - pain management and techniques

Dehorning Spoon or Tube

Dehorning spoons or tubes provide a quick and efficient technique for removing horn buds in calves less than eight weeks of age. With this method, a sharpened metal tube cuts through and removes the horn-producing skin at the base of the horn bud. Use the proper size tube to remove the horn plus about 1/8 inch of skin around the entire horn bud. Dehorning spoons or tubes provides a quick and effective procedure to remove horn bud from calves less than eight weeks old.

In this way, a sharpened steel tube cuts and removes the horn-producing skin at the base of the horn. Use a suitable size tube to remove the horn and 1/8 inch of skin around the entire horn bud.

Techniques

Administer local anesthesia with analgesia. Select the correct size tube (4 sizes available) to fit over the horn buds, and cover about 1/8 inch of skin around the base of the horn. Place the cutting edge straight down on the horn. Press the tube; press and twist the tube until the skin is cut. Cut under the horn bud and remove it, using movement. Apply antiseptic to the wound. Other bleeding may occur. Clean and disinfect the cutting edge of the tube between calves.

Advantage & disadvantages

- Not without blood
- Useful for young calves
- More prone to infection due to open wounds
- avoid use during flies
- Dishonesty when done wrong, leads to scurs
- requires expertise - pain control, techniques, bleeding control

Aftercare

Dehorning and disbudding are surgical procedures. Calves need post-surgery care and attention.

- Carefully monitor bleeding for 30-60 minutes after soaking.
- If bleeding is occurs, go to cauterize the blood vessel with the help of iron rod to stop bleeding.
- Wounds heal without treatment.
- Proper dressing and fly repellent are done at site of wound.
- 10 to 14 days after the dehorning, see any abnormality and given treatment if necessary.
- Get veterinarians opinion for calves that show severe pain or infection.

Disinfection of Equipment

Diseases can spread from animal to animal into blood-borne pathogens contaminate the dehorning equipment. Enzootic bovine leucosis and wart virus are two examples. It is important to eliminate virus from the tube type dehorning after every use.

Techniques

Rinse the blood with chilled water after every calf has its horns removed. Apply the disinfectant after the calf has been dehorned. Change antiseptic lotion regularly to preserve its strength. Prepare an antiseptic lotion by adding four ounces of creosol to one liter of water. All equipment which is used kept in store room after proper cleaning and disinfection. The heat from the electric horns kills the germs effectively in each calf. Occasionally hot iron dehorning need to be cleaned with a wire saw.

Conclusion

It is concluded that the dehorning is an important managemental tools in dairy farm. The main aim of dehorning is prevent the injury to other animals as well as handler and also required less space as compared horn animals. Dehorning will be done at early age of animals. Dehorning is done with suitable method which is creating less painful and discomfort to animals.

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Quail farming in India

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Abstract

The quail also known as Bater, belong to family pheasaniidae. In India there are two species, one is Japanese quail (*Coturnix Coturnix Japonica*), which is reared for meat purpose and other is black breasted quail (*Coturnix Coromandelica*) mostly found in jungle. It is one of smallest in bird's species among all the avians. The broiler quail can be sold at the five weeks of age. Quail begin laying eggs in about six weeks to continue producing high-quality eggs up to 24 weeks of age. Weight of adult Japanese quail reaches 250 - 300 gm and lays 250-280 eggs in a laying cycle. Meat is used as a delicacy for cooked meat, minced meat and tandoori quail. The egg of quail can make a variety of recipes such as boiled eggs and egg pickles. The size of an egg can be up to 10 g. It requires a small house to raise. About 10 quails that need space are equal to the need for one chicken.

Keywords: Quail, small, weeks, meat, egg.

Introduction



Quail are one of the smallest bird species in the Pheasant family. This species was first bred by Japan in 1595. Quail are fast-growing birds with a short generation gap. Quail were first introduced to India in 1974 from California. Broiler quail can be sold in 5 weeks of age. Quail start laying eggs at the age of 6 weeks, and then they continue to lay eggs until they are 24 weeks old. Quail meat is considered a delicacy. From quail meat makes various recipes for chopped meat, tandoori quail and use them

as a ready to cook meat. And eggs were eaten as boiled or made into egg pickles. Quail need a smaller house than chickens. In particular, commercial chicks are housed in multitier cages, thus increasing labor efficiency and better utilization of land space. Japanese quails (*Coturnix coturnix japonica*) are small flying birds weighing around 150 to 200 g. They make a peculiar sound and are considered a delicacy as a meat bird. Due to their small size, this bird is also used as a laboratory model of chicken and other birds. So, cost of conducting experiments can be greatly reduced because they eat less, need less space and reproduce faster than chicken. In addition, they are also easy to handle. With growing consumer awareness and appreciation for the quail's tender and tasty meat quality, the commercial quail production industry has gradually captured a sizeable section of the poultry meat. Another type of quail, the Japanese quail (*coturnix japonica*) has made a huge impact in recent years and many quail farms have been established around the country to produce eggs and meat. This is because increasing consumer awareness of high quality meat, necessitated the production of high quality quail meat. It is very important to choose stocks, which have high reproductive capacity to produce high quality meat and eggs.

Pre-Requirements for Quail Farming

To start a quail farm, it is important that you have the following preferences: Farms should be no more than 5 km from the forest if uninhabited. Farms should not be more than 3 km from the forest in the event of human settlement.

Birds should always be protected in enclosed areas with appropriate safety measures. In other words, they should not be allowed to roam in the open. Appropriate sanitation and hygiene measures must be followed on the farm. Adequate fencing should be created to protect stray animals or visitors to the farm as this can cause bird disturbance. It can also cause noise between birds.

Benefits of quail farming

Need less space to expand. Need less money. Quail are powerful birds. Birds can sell as young as five weeks of age. It matures when we are six to seven weeks old and begins to lay eggs. High clutch level of up to 280. Quail meat tastes great as compared to other chicken and has a low fat content. It promotes physical and mental growth in young people. In terms of a healthy diet, quail eggs are much better than chicken eggs. It has a low percentage of cholesterol. Quail eggs & meat are ideal for pregnant women

and nursing mothers.

Employment: Growing quail is a cheap business compared to poultry farming. It helps as much as food choices. Quail are an important bird in scientific research. This species can be grown in indoor areas. It does not need to be vaccinated with medication. Quail litter has a high amount of fertilizer and can be used to increase crop yields. Quail weigh up to 100 gm and lay 100 eggs a year, Japanese quails weigh up to 250 gm and lay 250 eggs a year.

Future of the Quail farming

Today the market quails meat for more demand. There is a great deal of hypermarket hotels, etc. The Indian government has encouraged people to start quail and try to provide infrastructure to help people. However, a government license is required to sell Japanese quail. The Department of Environment and Forestry issues a license from the Department of Animal Husbandry. The Central Avian Research Institute (CARI) in Izzatnagar, Bareilly Uttar Pradesh is keen to distribute quail for commercial purposes and has shown a willingness to supply entrepreneurs who wish to incubate eggs.

Housing System

To ensure product efficiency, housing is of paramount importance. Quail can be raised in both deep litter and cage system.

Deep Litter

Since quail are the smallest birds, about six birds can easily land on one square foot [1 sq m]. However, as they grow older it is advisable to introduce them to the cage system. This can prevent unwanted wandering and help them gain weight.

Cage System

Plastic cages are widely used to grow quail farming. There are different types of cage systems depending on the purpose of the birds. About 100 quail can be placed in the 3ft X 2.5ft X 1.5ft in the first two weeks. Old quail need more space. The whole unit is 6 meters by 1 foot thick and divided into six parts. There should be a removable wooden plate under the cage. This is to clean the faeces that could fall on the plate. The cages must have adequate ventilation system and adequate provision for drinking water and feed.

Quail have a natural habitat. Therefore, when introducing two groups of birds they should be raised separately for a few days before introducing themselves in the same

cage to avoid fighting in cages. Additional light should be provided in the barn or sheds during the rainy season and winter. While they are being raised for commercial purposes, they should be given light for 24 hours. This increases their growth and thus increases their market value. If they are raised in cages for reproductive purposes, the male ratio: for females should be 1: 3.

Feeding Management in Quail

The quail feed should be well balanced and economical. About 70% of total farming costs are spent on feed alone. In the case of quail farming in the background, the feed may contain agricultural products and household residues. This will ensure maximum profit. In the event that a feed is sold, the price will vary. Feed is usually well digested. Three-week-old quail should be fed with ground corn. Older ones can be given rice polish, sunflower extract, soybean extract, corn, peanut extract, etc. Commercial feeds have low protein content. Therefore, proteinaceous extracts should be added such as soy extracts. Feed can be formulated as shown in Table. 01

The following points should be taken into account when eating quail

Feed particles should be small in size. A six-month-old quail could eat up to 30 grams of feed. For egg-laying purposes, quail need about 450 grams of feed. It requires a 400 gram feed to produce ten eggs. The broiler starter mash can be used by adding 5 kg of oil cakes to a 75 gram feed. The particle should be well grounded.

Table 01: Feed can be formulated as follows:

INGREDIENT	Chick mash (%)	Grower mash (%)
Maize	27	31
Sorghum	15	14
Deoiled Rice Bran	8	8
Sunflower Cake	12.5	12.5
Soya meal	8	-----
Fishmeal	10	10
Mineral Mixture	2.5	2.5
Shell grit	5	---

Reproduction

Quail are first clutch when they are seven weeks old. They receive 50% of egg production at eight weeks of age. In the production of fertile eggs, male quail should be

raised with females for 8-10 weeks. The average male, female ratio is 1: 5. The incubation period is 18 days. The maximum breeding season for quail is between 2-8 months of age. However they start laying eggs from the 7th week. Male and female quails eight weeks of age and older are raised together to produce sound, fertile eggs. Eggs are smoked and incubated at 37.5°C with a humidity of 70-75%. Quail eggs are incubated at 60% humidity for 2 weeks after which the humidity is maintained at 70% until hatching. After hatching the first week is the most important time in the quail's life. Newborn chicks weigh about 8 grams and are therefore less sensitive. Lighting should be provided throughout the hour when the chicks can gather together. This is likely to create a stamp-like situation. Farmers often continue to cut themselves for the first three weeks. Some also extend it to 5 weeks to make a better product.

Quail egg

There is an increasing need for quail eggs for health benefits. The quail egg is about one-fifth the size of a chicken egg and weighs about 10gm. Egg shells are visible, varying in color from white to brown. In terms of healthy eating, the quality of these eggs is much better than that of chicken eggs; instead they contain low cholesterol. The size of the yolk (yellow inside) to albumen (white part), 39:61, is higher than chicken eggs (Table 2). They can produce 1500 quail chicks per week from the 500 quail laid.

Table2: Components of egg

Nutrient	Quantity
Water	74 %
Protein	13 %
Lipid	11%
Carbohydrates	1%
Total Ash	1%
Calorific value	649 k J/100g liquid

Quail meat

From healthy quail, we wear meat up to 70- 73% of their body weight. The quail weigh 140 grams and provide 100 grams of meat.

Health problems in quail farming

When quail parents suffer from a deficiency of vitamins and minerals, resulting in the chicks found in their fertilized eggs often leaning on weak legs. To control this problem

female breeder should be supplemented with nutrients and good vitamins and minerals. Quail are stronger immune system against from infectious diseases than chickens.

Therefore no vaccine is needed to prevent infections. Proper management of quail chicks, disinfection on farm premises, provision of clean quail drinking water and nutritious food will prevent outbreaks on quail farms.

Barriers Male quail often make a distinct noise that often disturbs a man. When male and female quail are raised together, male quail bark at other quail and blind them.

Disease Management

Quail like strong birds, the incidence of disease is less compared to other poultry birds. However, improper care and management can lead to lower productivity and higher mortality. Other diseases that occur between quail are ulcerative enteritis and coccidiosis. Both of these diseases can be controlled with a combination of dietary supplements. In the event of ulcerative enteritis, a gram of streptomycin mixed with a liter of water should be given to the birds for a period of three days. 2 grams of coxial 20 to one liter of water for three days can treat coccidiosis.

The best way to prevent diseases between quail is to ensure good hygiene. Cages should be cleaned regularly. Supporters should be disinfected and water changed from time to time. The bottom of the cage should be dry and there should be a proper air conditioning system in place. Good air circulation ensures that diseases exist. It is best to group the birds according to their age to ensure healthy growth. Sick birds should be separated from healthy ones as soon as infection is detected. In addition, dead birds should be burned immediately.

Licensing in Quail Farming

Quail birds fall under the category of protected species. Therefore, it is compulsory to obtain a quail farming license from the Government of India. The local Department of Animal Husbandry should be contacted for this purpose. The license is usually issued by the Department of Animal Husbandry, Dairy and Fisheries for a period of one year after which it can be renewed. The licensee can only obtain quails for commercial farming from another licensed owner. In addition, a license is granted only if the original requirements

of the farm are met and the required documents are produced as proof of original ownership.

Conclusion

It is concluded that the quail farming in India not common but their management is easy as compared to other poultry farming. They required less space requirement and feed intake as compared to chicken. It start laid egg at 7th weeks of age and egg is enriched all essential nutrients. Quail are stronger as compared to chicken so that less medicinal cost or disease management required.

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On request only

Oxidative stress in pregnancy and lactation: A review

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Abstract

Pregnancy and early lactation are physiological states accompanied by high energy and oxygen demand that might cause increased level of oxidative stress and development of disorders in all living beings. Per parturient period is the most stressful in the life cycle of dairy animals. Numerous physiological and environmental factors (high AT and RH) during the transition period compromise the defense mechanism of the animals and generate more free radicals; due to which the antioxidant activity is reduced. Differentiation of secretory parenchyma, growth of the mammary gland, onset of milk synthesis and secretion are all stressors which are accompanied by high energy demand and an increased requirement for oxygen. Thus increases the production of ROS.

Introduction

Reactive oxygen species (ROS) like superoxide anion radicals, hydrogen peroxide and hydroxyl radical are continuously generated in living cells in normal metabolic reactions leading to oxidative stress. The body produces several antioxidants which neutralize or “clear up” free radicals that harm the cells against oxidative damage.

Under physiological condition, there is a balance between endogenous oxidants and antioxidants. Sometimes internal production of ROS may be too high that antioxidants may not be enough to neutralize all the free radicals produced which leads to oxidative stress (Rejitha, 2013). Various metabolic processes in the body generate free radicals, the effect of which is usually nullified by

the antioxidants such as glutathione in reactions catalyzed by enzymes like glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD) etc., which are the natural oxidative defense mechanism of the body.

Reactive oxygen species (ROS)

ROS are important signaling molecules that might influence cell proliferation, cell death (apoptosis or necrosis) and the expression of genes. They are also involved in several signaling pathways for protein synthesis. However, they played a role in enhancing lipid peroxidation, DNA damage, and altered calcium homeostasis when produced in higher quantity (Genestra, 2007). The protection to the cells against oxidative stress is usually by an interaction among the antioxidant enzymes. The superoxide released by oxidative phosphorylation is first converted to hydrogen peroxide and further reduced to water. This detoxification pathway is the result of multiple enzymes. Superoxide dismutase catalyzes the first step followed by catalases and various peroxidases removing hydrogen peroxide (Lobo *et al.*, 2010).

Mechanism of action of antioxidants

Antioxidants have two different mechanisms of action. A chain breaking mechanism is the first one by which the primary antioxidant donates an electron to the free radical present in the system. Secondary antioxidants remove ROS by quenching chain initiating catalyst forms the second mechanism (Rice Evans and Diplock, 1993). Antioxidants act as radical scavenger, electron donor, peroxide decomposer, hydrogen donor, and enzyme inhibitor, metal ion chelating agent, singlet oxygen quencher, co- antioxidants, gene expression regulators and synergist. Detoxification of free radicals is done both by enzymatic and non-enzymatic antioxidants, which exist in the intracellular and extracellular environment (Lobo *et al.*, 2010).

The antioxidants act through different levels of defense i.e. preventive, radical scavenging, repair and de novo and adaptation. The first line of defense is the preventive antioxidants, which suppress the formation of free radicals by reducing hydro peroxides and hydrogen peroxide to alcohols and water, without generation of free radicals. The major antioxidant enzymes directly involved in the defense mechanism are: SOD, CAT, GPx and glutathione reductase (Pacher *et al.*, 2007 and Pham Huy *et al.*, 2008). Antioxidants like Vitamin C, E form the second line of defense which scavenges the active radicals to suppress initiation of chain and/or break the chain propagation reactions. The third line of defense is the repair and de

novo antioxidants. The cytosolic and mitochondrial proteolytic enzymes, proteinases, proteases, and peptidases remove oxidatively modified proteins and prevent their accumulation. The fourth line of defense is adaptation; where the free radicals generated induce the appropriate antioxidant formation and transportation (Lobo *et al.*, 2010).

Superoxide Dismutase (SOD)

This forms the first line of defense and is a preventive antioxidant. It is present in all aerobic cells and in extracellular fluid (Johnson and Giulivi, 2005). Stralin and Marklund (1994) stated that there were three forms of SOD, mitochondrial Manganese-SOD, cytosolic Copper/Zinc-SOD and extra cellular SOD. They also reported that the control of expression of mammalian SOD isoenzymes was not by oxidants but by cytokines. The SOD catalyzes the dismutation of superoxide anion radical ($O_2 \cdot^-$) into hydrogen peroxide (H_2O_2) by reduction. The oxidant formed (H_2O_2) is further converted into water and oxygen (O_2) by catalase (CAT) or glutathione peroxidase (GPx). The enzyme GPx removes H_2O_2 , by using the same to oxidize reduced glutathione (GSH) into oxidized glutathione (GSSG). A flavoprotein enzyme, Glutathione reductase, regenerates GSH from GSSG, with NADPH as a reducing power source. Besides hydrogen peroxide, GPx also reduces lipid or non-lipid hydroperoxides while oxidizing glutathione (GSH) (Willcox *et al.*, 2004; Bahorun *et al.*, 2006; Pham-Huy *et al.*, 2008).



Reduced Glutathione (GSH)

GSH is considered to be an important non enzymatic naturally occurring antioxidant. It prevents free radical damage and is central to the antioxidant defenses at cellular level (Rejitha, 2013). It is not required in the diet; instead synthesized in cells from its constituent amino acids. Its antioxidant properties are due to the thiol group in its cysteine moiety; which is a reducing agent and thereby reversibly oxidized and reduced (Meister, 1988 and Lobo *et al.*, 2010). It acts as an essential cofactor for antioxidant enzymes GPx, glutathione reductase and glutathione-S-transferase. Under oxidative stress, GSH is consumed by GSH related enzymes and they are detoxified to peroxides produced due to increased lipid peroxidation (Rejitha, 2013).

Lipid peroxidation (LPO)

Lipid peroxidation (LPO) is a non-enzymatic chain reaction based on oxidation of unsaturated fatty acids and important consequence of oxidative stress (Konvicna *et al.*, 2015). The LPO occurs in polysaturated fatty acids located on the cell membranes and proceeds with radical chain reaction. Hydroxyl radicals removes hydrogen atom and produces lipid radical and this is further converted into diene conjugate. Further addition of oxygen to this conjugate, results in peroxy radical, which is highly reactive and attacks another fatty acid forming lipid hydro peroxide and a new radical. Due to LPO, a number of compounds like alkanes, malondialdehyde (MDA) and isoprotanes are formed and used as markers in LPO assay (Lovell *et al.*, 1995 and Lobo *et al.*, 2010). Determination of MDA levels in plasma formed the basis of assessing the oxidative stress in per parturient dairy animals (Rejitha, 2013). Increased MDA after calving indicate imbalance between oxidants and antioxidants (Castillo *et al.*, 2005 and Konvicna *et al.*, 2015).

Conclusion

In late pregnancy and early lactation negative energy balance has been the main reason for development of oxidative stress. Hence it is very important to overcome the oxidative stress through supplementation of anti-oxidants through the feed and also follow standards of ICAR in feeding regimen of the animals. There by the animal health could be improved without any adverse effects on the production and reproduction.

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Hirudotherapy in Veterinary Practice: A Modern Twist to Ancient Science

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Abstract

Leech therapy also known as Hirudotherapy, is an alternative medical practice that has been used since the dawn of civilizations. All credit goes to the saliva of medicinal leeches blessed with more than 100 bioactive compounds (e.g., hirudin, calin, hyaluronidase, destabilase, eglins, bdellins, trypsin inhibitors, and complement inhibitors) that have specific biological functions such as anti-inflammatory, anticoagulant, antitumor, vasodilatory, and antibacterial activity. Like in humans, veterinary patients are treated in the same way for several medical ailments like polycythemia, osteoarthritis, venous congestion, dermatological disorders, hematomas, edemas, mastitis, and ear eczema. To minimize transmission of infection in human and animal patients, it is prudent to obtain leeches solely from a commercial leech bio farm. There are minor contraindications to leech therapy such as acute infections, severe anaemia, allergies, haemophilia, immunosuppression, skin malignancy, hypotension, and pregnancy. This article will provide veterinary and medical professionals with a better understanding of leech therapy based on its medicinal usage in human and animal subjects, which will motivate more scientific investigation into this traditional medical technique.

Introduction

Hirudotherapy is a traditional medical practice that involves the use of medicinal leeches for the treatment of a plethora of medical insults in human and veterinary patients (Whitaker et al., 2004). In human and veterinary clinical practice some of the common medicinal leeches used are *Hirudo medicinalis*, *Hirudinaria manillensis*, *Hirudo orientalis*, *Hirudinaria granulosa*, and *Macrobdella decora* (Abdisa, 2018). Since the beginning

of civilizations, the use of leeches for numerous medical ailments in humans and animals was a common trend. In the current scenario, it's now being practiced in different fields like dermatology, neurology, gynaecology, and reconstructive surgeries (Mory et al., 2000). The medicinal properties of leeches have been explored in their saliva rich in a cocktail of more than 100 bioactive compounds, hirudin being the most common bioactive compound (Abdullah et al., 2012). The therapeutic properties established in their saliva include anti-inflammatory, anticoagulant, thrombolytic, antimicrobial, antitumor, vasodilatory, anesthetic-like properties, and many more (Sobczak and Kantyka 2014; Lu et al., 2018). Knowing the myriad benefits of Hirudotherapy in human patients, it is now achieving a good place in veterinary medicine with the surfeit of indications in animal diseases (Sobczak and Kantyka 2014).

There are some minor contraindications where leech therapy is restricted, like in case of anemia, hemophilia, cancer, hypotonia, and pregnancy (Mory et al., 2000; Glyova, 2005). Hence clinicians must evaluate their human as well as animal patients for the presence of any co-morbid condition before application of leeches. Based on its marvellous results, in 2004, the FDA approved leech therapy as a medical tool in various surgeries (Whitaker et al., 2004). Keeping in view all the above-cited facts, we have compiled this article to re-expose the veterinary professionals about the encouraging remedial effects of medicinal leeches in veterinary patients. Therefore, accumulation, assimilation, and dispersal of information and skill in this innovative therapy will encourage the experts to progress their efforts towards animal and human well-being.

Leech Biology

Leeches are classified as hermaphrodite annelids and out of 600 species, only 15 species are categorized as medicinal leeches, such as *Hirudo medicinalis*, *H. verbena*, *H. orientalis*, *H. granulosa*, and *H. manillensis* (Wollina et al., 2016; Abdualkader et al., 2013). The physical characteristics of leeches reveal the presence of two suckers, one anterior and one posterior (Fig 1). The front sucker has three sharp jaws (each with 100 teeth) and salivary glands that produce a plethora of more than 100 bioactive chemicals when biting the patient's skin, and the wound resembles a Mercedes-Benz emblem (Wollina et al., 2016). The leech's posterior sucker is used for movement and attachment (Fig 1). Surprisingly, leeches may survive for a year without a blood meal after consuming a single blood meal 10 times their body weight (Hildebrandt and Lemke 2011). For feeding, leeches typically stay for 30-45 minutes and get puffed-up with blood, and it may drink approximately 5 to 15 ml of blood, but the blood continues to leak for 4 to 24

hours, necessitating antiseptic treatment (Fort, 2001). Leeches live in pure, clean waters with temperatures ranging from 0°C to 30°C; however, abrupt temperature changes kill leeches.

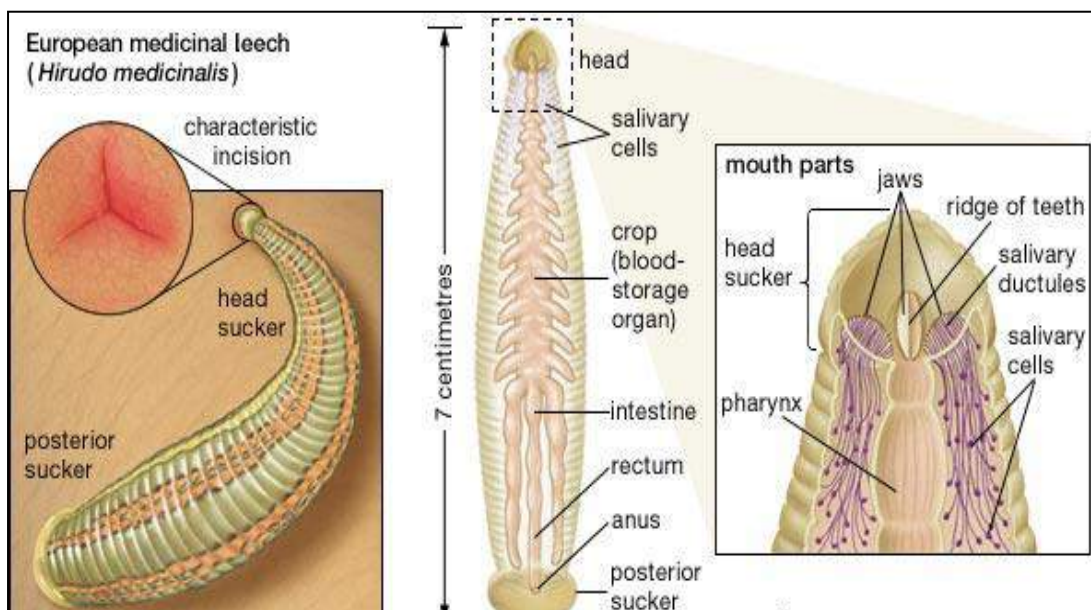


Fig 1: Anatomy of medicinal leech

Technique of therapy

The therapist must utilize commercially obtained medical leeches to perform leech therapy, and only healthy leeches are used for treatment sessions in both animal and human practice (Kantyka and Sobczak, 2014). According to several types of research, it is preferable to do this therapy in the morning, when leeches are fresh and unagitated (Kumar et al., 2012, Haq et al., 2021). Veterinarians must employ one leech every 10 kg body weight of an animal, however, in bulkier animals such as horses, up to 15 leeches are utilized (Sobczak and Kantyka 2014). Figures 2 and 3 show the corresponding author (*Abrar Ul Haq*) of this article using two medicinal leeches for the first time in India to treat mastitis in a goat weighing about 20 kgs. The treatment region must be thoroughly shaved before applying leech (either by hand or with a five-cc plastic syringe) to any animal, followed by the application of a warm sponge or warm dechlorinated water (Abdisa, 2018). Because leeches are highly sensitive to strong aromas and hence avoid biting, the skin should not be treated with an antiseptic solution (Abdisa, 2018). While performing therapy, the veterinarian must allow the leech to be fully satiated, which takes around 30-60 minutes in dogs and cats and 120 minutes in horses. general, the operation takes between 30 and 120 minutes, depending on the condition/disease being treated and the size of the animal (Sobczak and Kantyka 2014). After the leech has finished feeding, it falls off, and blood flow continues from the bite location, which is considered part of the therapeutic method

in both humans and animals. Once the bleeding has stopped, the hirudotherapist will apply an antiseptic solution to the wound and cover it with an appropriate bandage (Kantyka and Sobczak 2013) Following the completion of the treatment method, the therapeutic leeches are placed in a 70% alcohol container for 4-5 minutes, which kills the leech. Finally, leeches are disposed of as a biologically contagious agent (Ben-Yakir, 2005).



Fig 2: Use of medicinal leech in goat



Fig 3: Magnified view of Fig 2

Medicinal properties

The medicinal action of leeches is found in their saliva, which contains a cocktail of therapeutic chemicals such as hirudin, eglins, destabilase, bdellins, chloromycetin, hyaluronidase, histamine-like molecules, and some neurotransmitters (Zavalova et al., 2003; Baskova et al., 2008). Hirudin is a major protein known for its potent antithrombotic effects (Alaama et al., 2014). Aside from its anticoagulant effects, leech saliva also has anti-inflammatory, antibacterial, antioxidative, and analgesic properties (Abdisa, 2018; Malik et al., 2019). Bdelein is a chemical found in leech saliva that inhibits the synthesis of protease, which is implicated in inflammatory processes (Sobczak and Kantyka 2014). The antibiotic action of leech saliva is connected to the enzyme's hyaluronidase (capable of digesting hyaluronic acid), destabilase, and chloromycetin, which cause bacterial cellular components to be obliterated (Abdisa 2018). There have been reports of destabilase's bacteriostatic activity against *E. coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, however, this knowledge is limited and needs to be expanded. (Sig et al., 2014). According to research, leech saliva includes a mix of

neurotransmitters such as serotonin, dopamine, acetylcholine, and enkephalin, which aids in pain relief and promotes relaxation in patients' bodies (Sobczak and Kantyka 2014).

Indications and contraindications of Hirudotherapy

Canines, felines, equines, and ruminant animals such as cattle are typical patients exposed to leech treatment in veterinary medicine (Sobczak and Kantyka 2014). The indications for leech treatment in animals are analogous to those for human patients, who are frequently treated for inflammatory diseases, dysplasias, and vertebral sicknesses (Abdisa, 2018). The anti-venous congestive characteristics of leeches owing to antithrombotic, thrombolytic, anti-inflammatory, hypotensive, and bacteriostatic activity have been extensively established in reconstructive operations, with oedema being one of the major reasons. There have been instances of utilizing maggot and leech therapy in conjunction to treat partial digital amputation damage in dogs, with maggots assisting in the debridement of necrotic tissue and leeches assisting in the management of venous congestion (Vigani et al., 2011). Recently, a one In vivo study in diabetic rats demonstrated the antihyperglycemic action of leech saliva extract that has a synergistic effect with insulin; hence, it can aid in lowering insulin dosage in diabetic animals (dogs and cats) and humans (Abdualkader et al., 2013). Studies have also shown the effect of medicinal leeches in the preliminary treatment of polycythemia vera in a cat where phlebotomy was not possible due to hyperviscosity of the blood. Other diseases treated in animals by leeching include mastitis, mud fever, tendinitis, tenosynovitis, ataxias, and ear eczema (Sobczak and Kantyka 2014). Hirudotherapy can be used in conjunction with antibiotics and anti-inflammatory medications to improve outcomes. The literature on hirudotherapy is scarce, particularly in veterinary medicine. As a result, further research is required to determine the therapeutic potential of leech treatment in the future. Bleeding diseases such as haemophilia, pregnancy, breastfeeding, and anaemia, acute infections, immune-suppressive illnesses, and malignant skin abnormalities are all apparent contraindications to leech therapy that must be addressed before using leeches (Sobczak and Kantyka 2014). Using the same leech on another patient must be avoided since there is a danger of disease transmission to other animals.

Conclusion

Describing the myriad medical benefits of leeches, it is critical for medical and veterinary experts to comfortably keep medicinal leeches under laboratory settings for treatment and research. Knowing the medical perspectives of leech treatment in animals, veterinarians must provide appropriate client education on leeching as well as its benefits and drawbacks.

Furthermore, based on the facts stated above, future scientific investigations in human and veterinary leech therapy are needed, which will greatly benefit our human and animal patients.

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Number of Ovulation Observed in Black Bengal Goats by Ultrasound Imaging and its Subsequent Confirmation through Exploratory Laparotomy

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Abstract

By ultrasound imaging technique a single number of CL was counted for each animal under each treatment group on the 11th day of oestrous cycle, with an exception of three CL in one animal of the first treatment group. Out of total 17 CL counted, 11 were found on the left ovary. The counting was confirmed through laparotomy.

Introduction

Over thousand years, goats have been utilized for their milk, meat, and hair and skin production all over the world. In India, goats are generally reared by small and marginal farmers and land less labourers with minimum or without any investment. Black Bengal goats are found in West Bengal, north-eastern part of India and Bangladesh. A new field ultrasonography has been opened, and surely successful outcome will be forthcoming in the next years to improve reproductive management of the goat, the fastest growing ruminant population in the world (Rubianes and Menchaca, 2003). The understanding of the dynamics and regulation of follicle development in the goat has increased in recent years due to the use of ultrasonography. Previous findings observed in other ruminants extend to this species such as: the wavelike pattern of follicular growth with waves emerging every 5-7 days; the presence of follicular dominance, particularly during wave 1 and the ovulatory wave; and the role of progesterone concentrations on follicular wave turnover (Rubianes and Menchaca, 2003).

Review of Literature

Pierson and Ginther (1987) studied ultrasonography in heifers and concluded that ultrasonography was a reliable method of identifying and measured the number of follicles from 2 to ≥ 4 mm in diameter. Boyd and Omran (1991) reviewed the diagnostic ultrasonography of the bovine reproductive tract to study reproductive physiology. They documented that high frequency transducer with 7.5 MHz proved better in investigation of the reproductive tract. This technique was non-invasive, safe for breeding potential of the animal, and viable of the conceptus. It was investigated by Schrick et.al. (1993) that transrectal ultrasound examination performed in ewes in dorsal recumbancy, using 7.5 MHz transducer, was an accurate method for evaluation of number of CL on the ovaries. Dickie *et al.* (1999) studied that the limitations of ultrasonography for detecting the number of CL could be overcome by analyzing the ovulation rate at the 10th day of estrous when the CL were well developed. They also got the result that when ewes were scanned in the standing position using a 5 MHz transducer, the result was disappointing with 38 percent accuracy rate. Gracia *et al.* (1999) reviewed ovarian follicular dynamic in cattle and ultrasonographic observation of follicular wave, and some implementation to improve the fertility. Veronesi *et al.* (2002) studied that the CL diameter could be estimated by performing several measurements of structure with prolonged ultrasound examinations. The accuracy of the technique could be improved by evaluating the functional status of the CL. The patterns of various ovarian hormones were investigated by Rubianes and Menchaca (2003) using ultrasonography, along with other manipulation of ovarian follicular growth in goat. Medan *et al.* (2003) conducted experiment on Shiba goats, and used B mode ultrasonography to monitor the ovaries. It was studied by Vinales *et al.* (2004) in ewes that, ultrasound scanning provided a highly accurate method for determination of the number of CL and follicle ≥ 4 mm diameter, but its predictive value and sensitivity were lower for smaller diameter follicles. They also investigated that the result of ultrasonography in a standing position with 7.5 MHz probe might give better accuracy.

Methodology

The experiment was conducted in Completely Randomized Design (CRD) with three treatments, viz. i) Nutritional Effect and buck effect (TG-1), ii) Nutritional effect (TG-2) and iii) Control (TG-3). There were five female Black Bengal kids of three months of age in each group. The first group of animals was allowed to graze *ad lib* and the only were kept with a buck while the other two groups of animals were stall fed with the similar type of grass *ad lib*. The animals of TG-1 and TG-2 were also provided with concentrate feed supplement (21% CP) @ 10g per kg body weight per day in two divided doses. The animals under control group were neither provided the concentrate feed mixture nor kept with the buck. All the animals were placed in comfortable sheds and were under standard management practices. The data were analysed statistically by the analysis of variance (ANOVA) method, described by Cochroan and Cox (1967) and Panse and Sukhatme (1967). Error mean square by Fisher and Snedecor's F-test method was followed to test significance of different sources of variation. The standard error (S_e) and test of significance have been provided in the tables of results to compare the mean values. Ultrasonography was done by - Digital Ultrasound Imaging System (CTS-900V) made by Shantou Institute of Ultrasonic Instruments (SIUI), China. The procedure for laparotomy was followed as per the standard protocol by O'Connor, J. J. (edt), Dollar's Veterinary Surgery, 4th edition.

Results and Discussion

Observation of number of ovulation by Ultrasound imaging

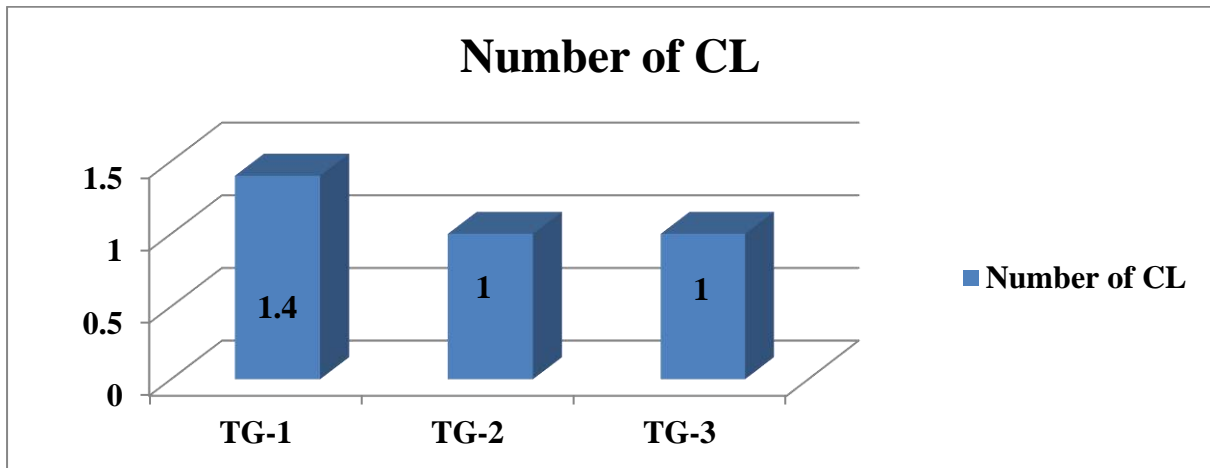
Trans rectal ultrasound imaging is an important technique for detection of ovulation in small ruminants, which provides a means for repeated, direct non-invasive monitoring of ovarian structures. It is also useful to detect pregnancy as early as 20-25 days post-mating. It can also help in monitoring of embryonic development, transfer implantation, feto-maternal relationship as well as foetal growth and placentation. Real-time imaging systems can help in diagnosis of reproductive disorders. It is powerful tool for studying ovarian physiology (Ginther *et.al.*, 1989; Vinales *et al.*, 2004).

Trans rectal Ultrasound imaging was performed to observe the number of *corpora lutea* (CL) on the ovaries of the animals of three treatment groups on the eleventh day of estrous cycle, when the CL were most prominent (Dickie *et al.* 1999; Veronesi *et al.* 2002; Vinales *et al.* 2004). It was evident from the present study that, the average number of CL on the ovaries of the animals under first, second and third treatment groups were 1.40, 1.0 and 1.0, respectively (Table 1 and Fig. 1). Number of CL found on left ovary of the first group of animals was 1 from each of the four animals and 2 from the fifth one, whereas, one CL was found from right ovary only from the fifth animal. One CL from each of the three animals on the left ovary and each of the rest two animals on the right ovary was observed through ultrasonography of ovaries of the animals under the second group. For animals of the third treatment group, one CL from each of two animals on left ovary and one CL from each of rest three animals on right ovary were detected. Out of total seventeen CL counted, eleven were found from left ovary and rest six from right ovary (Table 1 and Fig. 2). The present data was supported by the observation of CL through ultrasonography and laparotomy of the Black Bengal goat under NFBSFARA project (Annual Report NFBSFARA-2011-2012) where more than 50 percent ovulation was from left ovary.

The observations of the present study corroborated with the findings of the previous workers regarding the procedure and observation through trans rectal ultrasound imaging technique. Trans rectal ultrasound examination was performed in ewes in dorsal recumbancy or in standing position, using 7.5 MHz transducer to know the number of CL on the ovaries which was an accurate method of evaluation of CL (Schrick *et.al.* 1993; Vinales *et al.* 2004). On the tenth day of oestrous cycle, CL were well developed and could be scanned by this procedure (Dickie *et al.* 1999).

Table 1: Number of *corpora lutea* on the ovary of female under different treatments observed through ultrasonography

Animal number in respective treatment group	Number of <i>corpora lutea</i> on the ovary of female observed through ultrasonography					
	TG-1*		TG-2*		TG-3*	
	Left ovary	Right ovary	Left ovary	Right ovary	Left ovary	Right ovary
1	1	0	1	0	0	1
2	1	0	0	1	0	1
3	1	0	0	1	1	0
4	1	0	1	0	1	0
5	2	1	1	0	0	1
Mean	1.40		1.0		1.0	



[X-axis represents different treatment groups and Y-axis represents number of CL]

Fig.: 1 Average number of *corpora lutea* on the ovary of female under different treatments observed through ultrasonography.

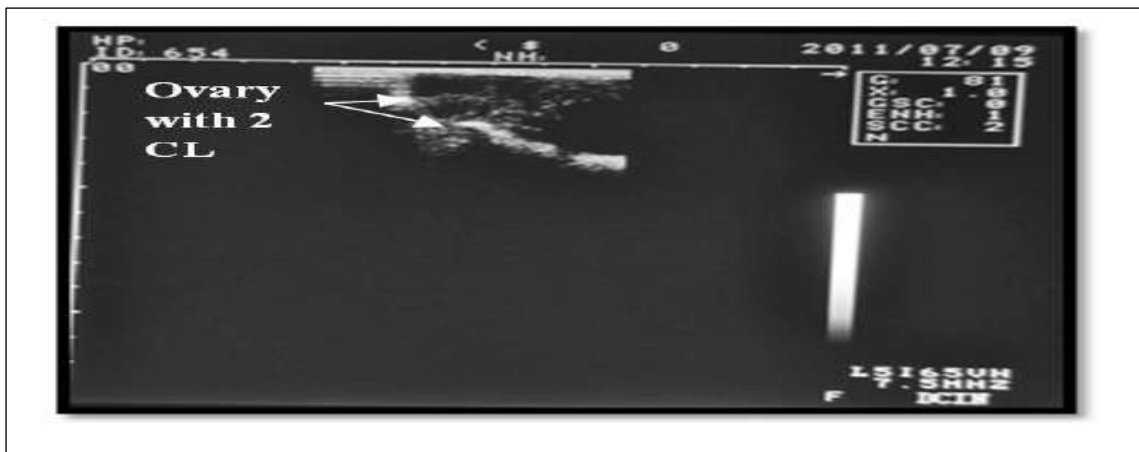
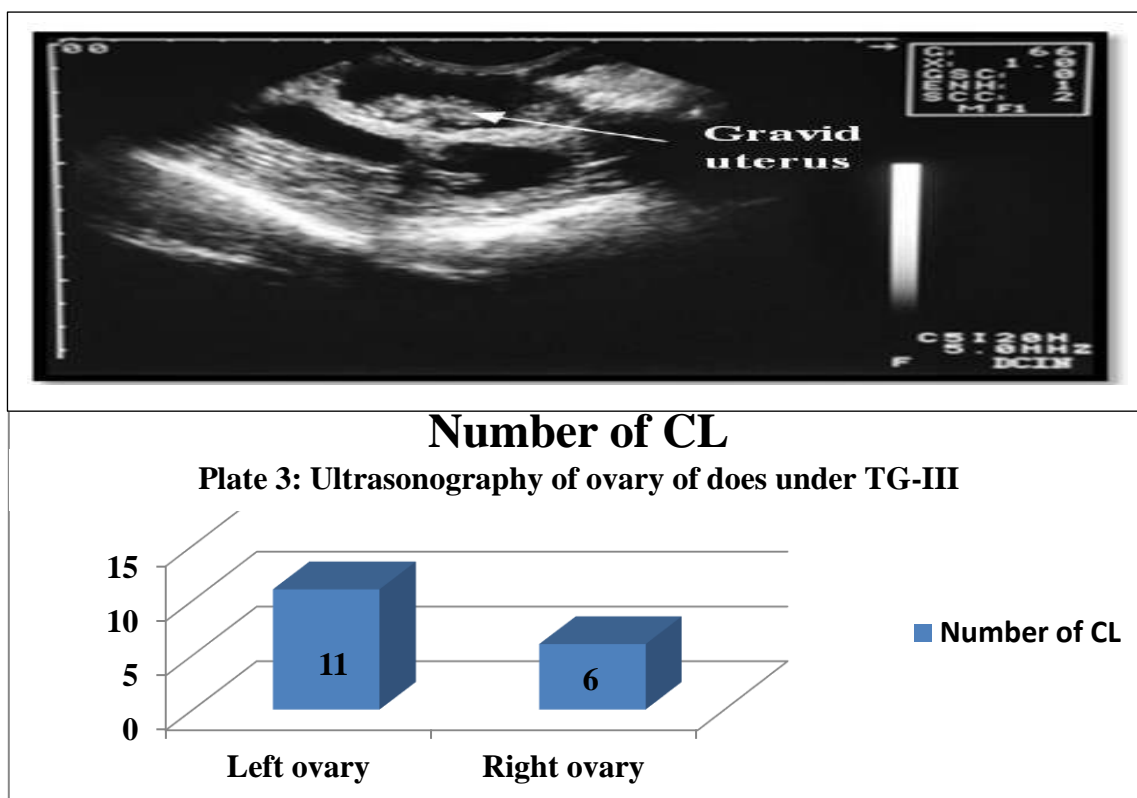


Plate 1: Ultrasonography of ovary of does under TG-I and gravid uterus (left lower plate)



Plate 2: Ultrasonography of ovary of does under TG-II and gravid uterus (left lower plate)



[X-axis represents ovaries and Y-axis represents number of CL]

Fig. 2: Total number of CL on left and right ovaries of all the experimental animals

Observation of number of ovulation through exploratory laparotomy

A laparotomy is a surgical procedure involving a large incision through the abdominal wall to gain access into the abdominal cavity. Laparotomy is of two types, namely, exploratory laparotomy and therapeutic laparotomy. In exploratory laparotomy or diagnostic laparotomy (abbreviated as ex-lap), the internal abdominal and lower abdominal structures are observed thoroughly (<http://en.wikipedia.org/wiki/Laparotomy>).

Exploratory laparotomy was performed to know the actual number of *corpora lutea* (CL), which represented the number of ovulation and eventually the number of kids will born, on the ovary of the animals under experimentation. This particular experiment was performed after ultrasound imaging on the eleventh day of oestrous after doing ultrasonography, when the CL were prominent, which were supported by earlier workers (Dickie *et al.* 1999; Veronesi *et al.* 2002; Vinales *et al.* 2004). From the present study, it was found that the number of CL, examined through laparotomy were same as found by ultrasonography (Table 1 and Table 2). As all the animals were in first parity, most of them had one CL on the ovary, with an exception in one animal of the first group of treatment. It had been examined that three CL were present of that particular animal, two on the left and one on the right ovary. As the animals of the first treatment group were given good plane of nutrition, the animal could successfully carry the pregnancy and terminated into successful kidding in spite of having three ovulations.



Plate 4: Genital tract along with both ovaries showing CL and follicles

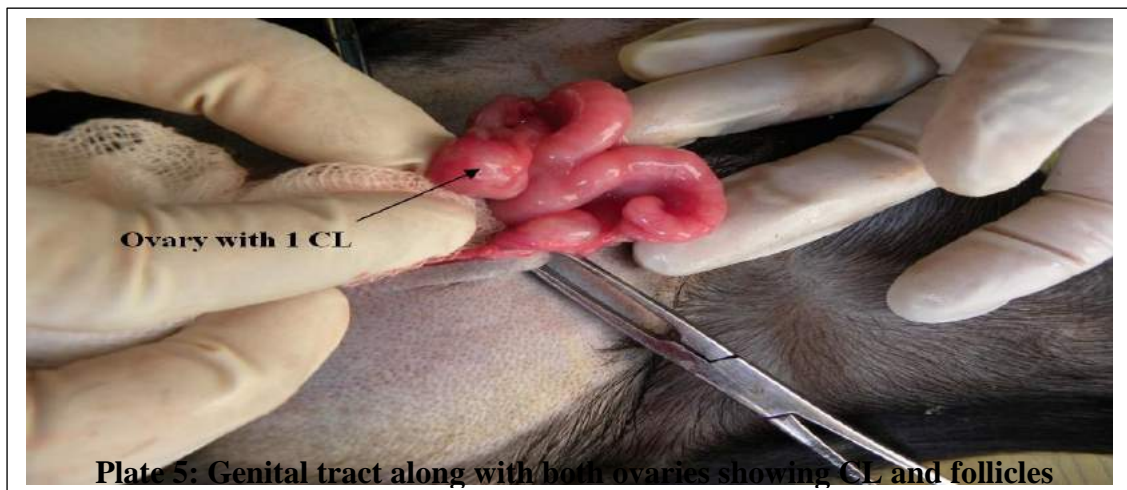


Plate 5: Genital tract along with both ovaries showing CL and follicles



Plate 6: Left Ovary with one CL and right ovary with several small follicles

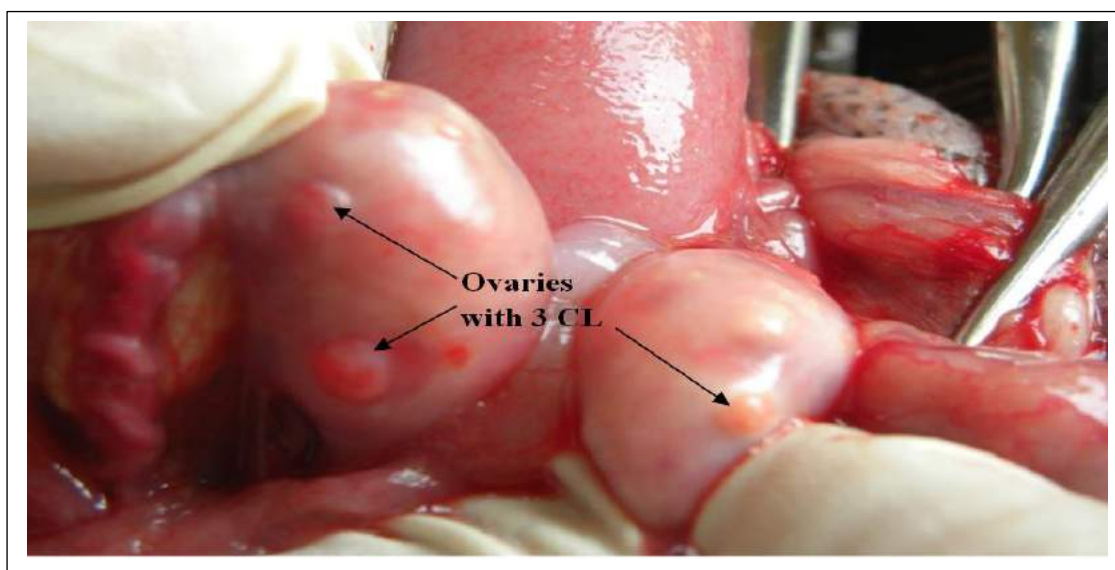


Plate 7: Left ovary with two CL and right ovary with one CL

Table 2: Number of *corpora lutea* on the ovary of female under different treatments examined through laparotomy.

Animal number in respective treatment group	Number of <i>corpora lutea</i> on the ovary of female examined through laparotomy					
	TG-1*		TG-2*		TG-3*	
	Left ovary	Right ovary	Left ovary	Right ovary	Left ovary	Right ovary
1	1	0	1	0	0	1
2	1	0	0	1	0	1
3	1	0	0	1	1	0
4	1	0	1	0	1	0
5	2	1	1	0	0	1
Mean	1.40		1.0		1.0	

Conclusion

By ultrasound imaging technique, a single number of CL was counted for each animal under each treatment group on the 11th day of oestrous, with an exception of three CL in one animal of the first treatment group. Out of total 17 CL counted, 11 were found on the left ovary. Exploratory laparotomy was conducted on the same day, and the numbers of CL were found to be same, as seen by ultrasound imaging.

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Punganur Cattle: A Native Breed of Andhra Pradesh

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The state “Andhra Pradesh” was found to experience hot and humid equatorial tropical climate with temperature humidity index (THI) crossing the critical point for many days in a year and the livestock population is expected to be under significant heat stress. In fact, the comfort/ strain inflicted by heat stress, for instance, must be evaluated considering both the severity of the stress as measured by THI and also the adaptability of the stock as measured by changes in physiological, haematological, hormonal and gene expression pattern of HSPs.

Stress represents the reaction of the body to stimuli that disturb homeostasis which end up with detrimental effects (David *et al.*, 1990). Animals are being exposed to different environmental conditions during a complete year. Among the stressors, heat stress has been of major concern in reducing animal's productivity in tropical, sub-tropical and arid areas (Silanikove *et al.*, 1997).



Picture of a Punganur cow

Heat stress induces numerous physiological responses where an appreciable portion of energy is deviated for thermoregulation compromising the production behind. Interestingly, it was found that

the heat stress responses were less pronounced in indigenous compared to exotic breeds owing to its evolutionary process and are better adapted to hot and humid tropic maintaining their production.

“Punganur” a native cattle breed of chittoor is one of the Worlds shortest humped cattle with long tail with switch touching the ground, white and light grey in color with a broad forehead and short stature. The average height is 60-100 centimeters and weight 115-200 kgs. Punganur cattle fall in the category of Indigenous cattle breed owing to its evolutionary process and no studies have been conducted so far to evaluate the effect of varying THI on biochemical, hormonal and gene expression pattern of HSPs in Punganur cattle at various physiological states. Such studies may help us to evaluate the adaptability of stock and thus promote the propagation of indigenous breeds which maintain their production in this climate change scenario.

Punganur cattle are well known for its dual purpose and is said to be on the verge of extinction. In the process of sustaining the deteriorating population of these animals necessary studies need to be conducted out to enhance the productivity and improve the reproductive performance.

The only work done until recent times was by Naik *et al.* (2013) established base line physiological and hematology values in Punganur cattle in different age groups (bulls, cows, young bulls, heifers, and male and female calves) and during different seasons (monsoon, winter and summer). The mean RBC and WBC counts differed significantly ($P<0.01$) among the age groups and were higher during summer than either during monsoon or winter seasons. The Hemoglobin content was not significantly different among the age groups but was significantly higher ($P<0.01$) during summer than during either monsoon or winter seasons. The mean PCV (%) in different age groups and also during monsoon, winter, and summer seasons were not significantly different. The mean ESR (mm/hr) in different age groups was not significantly different but was significantly ($P<0.01$) higher during summer than during winter. The DLC count (%) among different age groups of Punganur cattle was significantly different ($P<0.01$) for Neutrophils, Eosinophils, Basophils, Lymphocyte except for monocytes. Neutrophils and lymphocytes were significantly higher ($P<0.01$) during summer, Eosinophils and Basophils during monsoon while Monocytes count was significantly higher ($P<0.01$) during winter than during other seasons.

Conclusion

It is very important to conserve our native breeds of cattle which are on the wedge of extinction. Punganur is one such breed which requires immediate attention and it is of paramount importance to propagate the breed owing to its native characters like disease resistance and thermo tolerance. Necessary research need to be conducted in this area for exploring and validation of the same.

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Bio security practices: Essential for successful poultry farming

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Introduction

Poultry is one of the fastest growing segments of the agricultural sector in India and contributes to approximately 10% of all meat and eggs produced in the world each year. Unfortunately, they are highly susceptible to disease outbreaks that may cause irreversible economical loses to the poultry industry. Most of the poultry industries in the world have developed biosecurity measures to maintain the safety of poultry from biological hazards and be used for protection and disease control of the poultry. The first step in the biosecurity on the farm is to assess the farm and then, utilizing the data as a reference. Any general biosecurity program should include three major elements: isolation, traffic control, and sanitation. In addition to these procedures, it is important to follow all regulations recommended by the biosecurity program and implement them carefully in the farm. Biosecurity level should be more strengthened in breeder farms and during disease outbreak. Finally, farm workers should be educated on the importance of the biosecurity program and should be trained in the implementation of the program regulations.

The term "biosecurity," is derived from two words; "Bio" means "life" and "security" implying to protection. Hence, "biosecurity" refers to a type of program that is designed to protect life (Shane *et al.* 1995). Biosecurity as a set of practices designed to prevent disease causing

organisms from coming in contact with resident birds on the farm. These practices when followed correctly will reduce the potential for the introduction and spread of disease causing organisms onto and between sites (Cardona and Kuney, 2001; Woodger, 2005).

Objectives

- To prevent the introduction of infectious disease agents to poultry
- To prevent the spread of disease agents from an infected area to an uninfected area
- To minimize the incidence and spread of microorganisms of public health significance

Major route of disease and pathogen transmission

1. **Animals and other birds** - transfer of birds from production area to production area, dead bird disposal, other animals and wild birds, feral and domestic animals, including other livestock and pets, insects, rodents, domestic birds
2. **People** - farm personnel and family members living on site, contractors, maintenance personnel, neighbor, service person, visitors. Disease can be transmitted by hands, boots, clothing, dirty hair, equipment's
3. **Vehicles**
4. **Air**- transmission as an aerosol or dust
5. **Water supply**- Water supplies may become contaminated with faeces from contact with avian or other animal species
6. **Feed**- feed may be contaminated by the raw materials used, post-production and during transport, or by exposure to rodents and birds on the property. Bacteria and mold in poor quality or damaged feed may also be a concern.

Management factors in disease prevention

The following managerial factors help to reduce the spread of disease and stress to the birds-

a) Isolation

- It is not advisable to rear birds of different age groups in the same house. Wherever possible, it is advisable to practice the all-in-all-out system.
- Proper layout of houses, appropriate designing to prevent any entry of rodents, proper ventilation, and the designing of feeders and drinkers to avoid spillage, are basic essentials in disease prevention.

b) Litter management

- Wet litter is a potential source of disease transmission. Maintain proper litter conditions as suggested earlier.

c) Quality chicks

- Ensure that chicks are received from a hatchery where adequate preventive care is taken for breeder birds to guard against mycoplasmosis, salmonellosis and infectious bursal disease.
- Check for a history of vaccination against Marek's disease. Look for signs of dehydration.
- Ensure that the received chicks are healthy and are within the normal weight range.
- A good balanced feed prepared according to nutrient requirements at different ages will ensure proper health and good immune status in birds. Addition of coccidiostats and vitamin-mineral supplements are essential.

d) Water quality

Poultry farmers often fail to provide the birds with good quality water. Both the microbial and chemical quality of the water need to be tested before establishing a poultry farm in a given area. The desirable quality guidelines for drinking water on poultry farms are as follows: Total hardness : 60-180 ; pH : 6.8-7.5; Nitrate : 10 mg/liter; Nitrite : 0.4 mg/liter; Total bacterial count : 0/ml; Coliform count : 0/ml; Calcium chloride : 60 mg/liter; Sodium : 50 mg/liter; Sulphate : 125 mg/liter.

Chlorination is the best and cheapest method to get rid of micro-organisms. Five to eight grams of *bleaching powder* with about 35 percent available chlorine should be added to 1000 liters of drinking water to maintain a chlorine level of 1 to 2 ppm at delivery. A minimum contact time of one hour should be given before offering the water to birds. Products containing *Quarternary ammonium compounds* like quat, quatovet, encivet, sokrena etc. may be used as water sanitizers as per the manufacturers' specifications.

e) Dead bird disposal

Mortality is inevitable on every poultry farm, and it varies with the prevailing disease and sanitary conditions on the farm. When birds die, their carcasses remain as a source of infection for pen-mates and other birds on the farm (or other farms). Diseased and ill birds also discharge infectious material into the environment and act as reservoirs for disease-producing organisms. In general, the following points should be observed while disposing the carcasses:

- Remove the dead birds from the flock as soon as possible;

- Take the necessary precautions to prevent spillage of infectious material from the carcasses during transportation from the farm or post-mortem room to the disposal site;
- Take sound bio-security measures at the disposal sites to prevent disease transmission.

f) **Disposal of poultry farm manure**

After the pen is emptied, deep litter and caged layer droppings should be removed to a field far from the poultry shed, and spread to dry in the sun. It should be disposed off as soon as possible for manure or other purposes and not allowed to remain accumulating for a long period. Composting is better, since the heat produced will destroy the pathogens.

- **Disinfection-** *Disinfection* is the process or act of destroying pathogenic microorganisms. Phenol, cresol, chlorine compounds and iodophors can be used for disinfecting surfaces as well as the egg room, feeders, drinkers, buildings and footwear; *liquid formalin* at 5 percent level, or *formaldehyde gas by fumigation*, will also serve as an effective disinfectant. *Sun-drying* may be practiced for washed equipment; for cement surfaces-dry heat in the form of flame is recommended.

g) **Rodent control**

Keep rodents out from the initial stage of farming itself, since once the farm is infested, it is difficult to get rid of them.

- Remove piles of unused equipment and empty gunny bags as they serve as breeding places for rats, mice and squirrels.
- Remove spilled feed daily. Store feed in well-ventilated, rodent-proof rooms. Use *traps* in the initial stages and later *rodenticides*.

h) **Insect control**

Counter measures against insects are part of maintaining a sanitary environment, as insects play a significant role in transmitting disease-producing micro-organisms, tape worms, etc. Insect or fly control measures include:

- Avoiding stagnation of water in and around the farm premises.
- Provision of proper drainage facilities, attending immediately to leaky drinkers, water lines, etc. Use of insecticide sprays or dusting at required intervals,
- Keep the surroundings clean by covering the area with treated soil devoid of vegetation or by growing grass lawns.

Conclusion

Protecting poultry flocks from microorganism contamination is an extremely important component of commercial poultry production environment. The effectiveness of a biosecurity program can be optimized by regional participation. While any level of biosecurity is helpful, if all poultry producers in a given area utilize best management programs, the program as a whole will be more effective. Practicing sound biosecurity procedures every day as part of a best management program will help to reduce the possibility of contracting a disease and will reduce the spread of disease.

Basics of Wildlife Forensics

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“Humanity can no longer stand by in silence while our wildlife are being used, abused and exploited. It is time we all stand together, to be the voice of the voiceless before it's too late. Extinction means gone forever.” — Paul Oxtan

The topic encompasses two broad areas of science – “Anatomy” and “Forensic Science”. Literal meaning of ‘Anatomy’ is study of form and structure of animals. But the term *forensic science* involves *forensic* (or *forensis*, in Latin), which means a public discussion or debate. In a more modern context, however, *forensic* applies to courts or the judicial system. Combine that with *science*, and forensic science means applying scientific methods and processes to solving crimes (from Forensic Wikipedia: 15/08/2018).

Wildlife crime is a burning problem in India which is due to numerous reasons like deforestation, habitat destruction, human settlement etc leading to shrinkage of forest land facilitating easy access for poachers’ to go for wildlife poaching and most of the mammals are now endangered. Efforts are being made to preserve wild animals by establishing wildlife sanctuaries, national parks and to taper the incidences of poaching and trading of wildlife under WLP Act 1972.

Due to this reason, identification of animal species became important in the investigation of crimes in case of poaching and trading of animal parts. In other way wildlife

forensics is the application of scientific knowledge to legal cases involving wildlife. It involves use of scientific procedures to investigate wildlife-related crimes involving the wildlife trade, poaching, illegal hunting activities, and even oil spills. Role of Wildlife forensic specialists is to collect evidence from wildlife inspectors or wardens of respective areas, work together with them to investigate and prosecute crimes. They try to identify the species in question to which an animal, animal part, or product belongs. Here comes the knowledge and skill of Anatomists how best it could be applied in wildlife forensics and crime control.

The ultimate answer to this crime investigation for an anatomist to challenge is to go for species identification of the exhibit concerned. To undertake this major step, four parameters to be taken into consideration viz. Morphology and Morphometry, chemical test, DNA profile, and meat analysis.

When we say about morphology and morphometry in Wildlife forensics the forensic experts compare the shape and size of the objects, exhibits or trophies and try to analyse the data collected from the crime scene to summarize and submit to the Court of Law. Morphometric study actually retains the size information in the analysis, and shape data is the comparison of shape of objects collected from different sources and match them with one another, correlate with the morphometrics for a final report. Again geometric morphometrics is another approach to be considered in forensics to address various forensic problems of different areas.

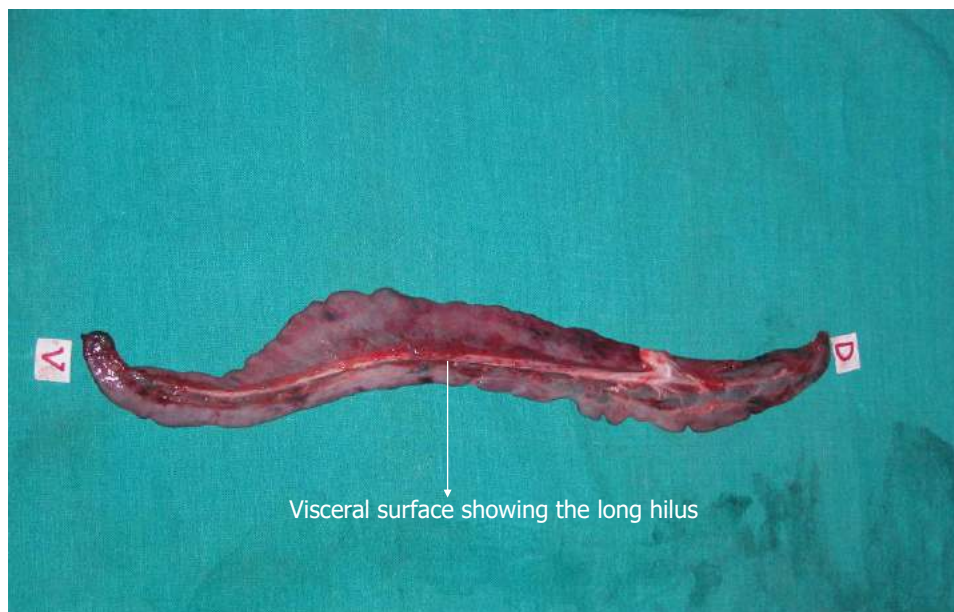


Fig. 1: Recording of detailed morphology and accurate morph metrics is an important tool in forensic examination

One of the vital role played by Forensic is chemical evidence for which chemical test is performed from samples collected from crime scene. Chemical evidence can be any chemicals collected from suspect or victimized people, various objects, or in solutions from the crime scene and or recovered from related areas. DNA profile is another area of study by way of which the Forensic experts can authenticate valuable information connected to species identification to solve the unsolved crimes.

Meat analysis is another challenging approach of forensic science to solve various vetero-legal or medico-legal cases. Because of high income from cross border illegal wildlife trade in international market it is often seen that due to unavailability of reliable diagnostic techniques at the appropriate time most of the illegal merchants get caught free in the court. Therefore, one should make use of sophisticated analytical techniques to address the identification of suspected wild species in question.

What are the investigations conducted in the crime scene by the forensic experts? They are:

1. Evidence collection:

Photographs, video recordings of the crime scene is very important. The area of crime should be barricaded and properly documented with photo graphs and videos. Human movement into the barricaded area should be restricted so that there is no loss or



Fig. 2: Samples should be properly collected, sealed, packed and labelled. In this photo hair samples were collected from dead Pygmy hog.

tempering of vital evidence. While handling wildlife crime biological samples should be collected as per the standard guidelines and protocols.

2. **Foot print analysis:** Forensic podiatry examination is an important tool for forensic experts to book the culprit. This is done by comparing a shoe collected from the crime scene with the confirmed shoe wore by the culprit by examining the pattern of shoe outsoles, inside of both suspected and confirmed shoes, identification of the shoe manufacturer, model and size, compile the findings and place before the court of Law.
3. **DNA Profile or DNA finger printing:** DNA profiling or DNA fingerprinting is a technique is an important tool in criminal investigations, wherein the DNA of the suspect is compared with the DNA evidence collected to ascertain whether the profiles tally to book the culprit (s).
4. **Brain mapping:** It is a test done to reveal guilty knowledge of the suspect where forensic experts use unique techniques to find out whether a suspect's brain can recognise incidence from the crime scene which an innocent suspect would not have any knowledge about the crime
5. **Lie detector:** Lie detector is a technique used by the psychologists and practitioners have study whether there is any change in the behaviour, speech, and peripheral physiological responses to recording brain activity.
6. **Narco- analysis:** It is a psychological investigation of a suspect in conscious or unconscious unwillingness to express memories or feelings by the use of a barbiturate drug.
7. **Suspect detection system:** The technology used in forensics to identify the unrevealed adverse intention of culprits before they committing a crime. The system can be used to quickly identify the criminal from among a general public.
8. **Forensic Ballistics:** Forensic ballistics is the examination of evidence of use of firearms that is used to commit the crime, the bullet fired from the gun in crime act which leaves behind the marks of the bullet and cartridge case.

What are the techniques applied in forensic research? Answer is about three techniques viz species identification, cause of death or injury, and techniques to book the accused. Therefore, to evaluate forensic evidence on wildlife two important issues should be considered: while collection of materials from the crime scene for investigation, one has to record whether whole animal, animal part, whole organ or part of it or even some products like cloth, jewellery or even processed meat is collected, and to ascertain the cause of death of the animal. But hair collection from the scene of crime is extremely important when other biological sample is

either putrefied or destroyed by using some unfair means by the culprits. Hair is an appendage of the skin which resists putrefaction; thus, is an evidential value when other evidences are not available or putrefied or destroyed.

Now a day's wild life crimes have become a threat in our nation and to check this is a great challenge to the wildlife scientist. How wildlife crime is committed? Answer is – definitely by way of wildlife trade. Then, what are the ways to wild animal trade? Answer shall be – by three ways: (1) live animal trading, (2) trading of animal parts and (3) animal products trading (smoked and dried meat, mongoose hair paint brush).

In Forensic Science- Wikipedia it was stated that in the past years documentation of forensic scenes became evolved and experts started using laser scans, drones and photographs for 3 dimensional point clouds of accidents or crimes. This resulted in accuracy to provide evidences in the court and also to preserve the images for future.

Forensic science can be broadly divided into the following subdivisions -

Art forensic: Concerned with art authentication method to detect forging, faking and copy of arts e.g. paintings.

Blood stain pattern analysis: to scientifically study the blood spattering patterns in crime scene.

Computational forensics: Concerned with development of algorithms and software for forensic examination.

Criminalistics: Use of knowledge of various sciences for answering biological evidence related questions and impression evidences e.g. finger prints, foot wear impression, tyre tracks, ballistics, firearms, etc as evidence in criminal investigation.

Digital forensics: Application of proved scientific techniques to recover data by forensic scientist.

Ear print analysis: Two dimensional representation pictures of external that touched a specific surface.

Forensic accounting: Use of interpretation of accounting forensic evidences.

Forensic aerial photography: Study of aerial photographic evidences.

Forensic anthropology: Use of anthropology in legal side to identify the recovered human skeleton remains.

Forensic archaeology: Use of both archaeological techniques and forensic science for law enforcement.

Forensic botany: study of plant life for accruing some crime information.

Forensic chemistry: For detection and identification of illicit drugs.

Forensic dactylocopy: Study of fingerprints.

Forensic DNA analysis: Studies the uniqueness of individual's DNA for solving forensic questions. In humans it is used for paternity/ maternity testing as well as crimes levied on individuals. However, in wildlife it is a different issue i.e. to identify the species in question and bring the culprit to the court of law.

Forensic odontology: Study of uniqueness of dentition or teeth.



Fig. 2: Recording of dentition with measurements is an important tool for forensic analysis

Forensic serology: Study of body fluids.

Forensic Toxicology: Study of effects of drugs and poisons.

Forensic video analysis: Scientific examination, comparison and evaluation of videos in legal matters.

Mobile device analysis: Examination and evaluation of evidences recovered from mobile phones.

Trace evidence analysis: Study of trace evidence of glass, paint, fibre and hair with the help of micro-spectrophotometry.

Wildlife forensic Science: Application of scientific knowledge from a wide range of disciplines to solve crimes of non-human biological evidence viz. poaching, animal abuse, trade of endangered species etc.

The forensic science is itself in a transitory period and therefore, the role of forensic science is challenging. Earlier, it played a supporting role and now it is the playmaker in much type of crime investigations providing quick and reliable information to crime investigations. As of today forensic science is no longer a profession of individual experience, it has turned into expertise of practitioners whose skilled knowledge works in solving different issues on wildlife crimes. New techniques and tools, updated information should be shared with the field

veterinarians and forest staffs and officials so that these can be used while collection, transportation and recording of crimes. Time to time sensitization programmes, workshops and trainings should be conducted for sharing of knowledge and information amongst various stake holders involved in wildlife crime control.



Fig. 4: Sensitization programme conducted for field Veterinarians on the topic “Wildlife Forensics for Field Veterinarians”.

Challenges before wildlife forensics:

1. Information: Most of the time accurate and correct information is not provided to the forensic experts to carry out the investigation.
2. Technological hindrances and unavailability of certified laboratories: Up to date equipments and technologies are not available for wildlife forensics.
3. Manpower: Due to lack of well trained officials the forensic experts face problem at the time of collection of information, evidence, materials from the scene of crime.
4. Sample preservation: Collection of appropriate samples in required quantity in proper preservatives is highly essential for proper crime investigation.
5. Non availability of species specific antibodies for serological analysis.
6. Non availability of species specific genetic marker for species identification for all species.
7. More field based innovations in terms of wildlife forensics is need of the hour. There has to be a mechanism whereby easy collection of samples, quick transfer and analysis is possible.

8. Red tapism often leads to difficulty in wildlife forensics. Often there is no proper coordination between various departments involved in crime control.
9. Awareness for the requirement and utility of wildlife forensics.
10. Lack of infrastructure.

These are the current challenges for multi disciplinary forensic expert team who has to come across many a times in reality to work for conservation of wildlife and bring the culprits to the court of law as ***“All objects in the universe are unique. No two things that happen by chance ever happen in exactly the same way. No two things are ever constructed or manufactured in exactly the same way. No two things wear in exactly the same way. No two things ever break in exactly the same way”*** quoted by famous forensic expert Joe Nickell.

Successful Medical Management of *Notoedres cati* Infestation with Ivermectin Therapy in a Cat

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Abstract

Notoedres cati is one of the most common mite in cat causing dermatitis. Severe crusted and pruritic lesions noticed in both ear pinna and forehead of one year old female domestic cat. The skin scrapping from the affected area examined under a light microscope revealed severe mite infestation. The condition was treated with ivermectin injection at weekly intervals. After medical therapy animal showed marked improvement and regrowth of healthy hair noticed after three weeks. Animal had an uneventful recovery.

Introduction

Notoedric mange also known as feline scabies. This disease is highly contagious skin infestation. It primarily affects felids but it can also cause infestation in rodents, lagomorphs and sometimes in dogs and foxes. This skin disease also has zoonotic potential (Senthil Kumar *et al.*, 2008). *Notoedres cati* opportunistically infest humans also (Griffin *et al.*, 1993). Common clinical signs of this disease condition include cutaneous lesions in the ear, face or neck and extreme pruritus (Kwochka, 1987). Ivermectin is widely used endoecticide in veterinary field and it possess wide range of safety, low dosage requirement

and persistent action (Senthil Kumar *et al.*, 2008). This case report reveal the successful medical management of Notoedric mange in cat with ivermectin therapy.

Case history and observations

A one year old female domestic cat weighing 1.8 kg body weight was presented to the University Veterinary Hospital, Mannuthy with severe pruritic and crusted lesions in dorsal aspect of both ear pinna and forehead. Animal showed severe itching and restlessness. External lesions were started since one week. Small wounds were noticed in the ear pinna due to self-inflicted trauma by the animal. On haematological examination revealed granulocytosis and eosinophilia. Serum biochemistry values were within the normal range.

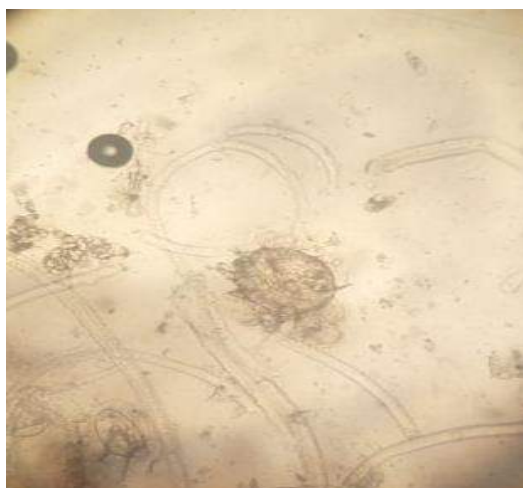


Fig. 1 *Notoedres cati* (10X)



Fig. 2 Crusty lesions in the ear pinna

Treatment and Discussion

Skin scrapings were collected from multiple sites of the lesion and processed in 10% KOH solution and examined under 10X and 40X of light microscope revealed live *Notoedres cati* mites with its eggs and various stages of life cycle. Animal were treated with ivermectin injection (1%) at the dose rate of 0.2 mg/kg subcutaneously at weekly intervals for one month. Concurrently treated with antibiotic ceftriaxone at the dose rate of 15 mg/kg IM for five days and multivitamin drops orally. Crusty lesions were disappear and healthy hairs came in the affected areas within three weeks. Treatment response was assessed weekly skin scraping examination. Skin scrapping was negative from second week onwards.

Skin disease in cats are most commonly caused by external parasites. *Notoedres cati* is the most common mite causing dermatitis in felines. *Notoedres cati* is responsible for the condition called notoedric mange in cats. The clinical signs of this mite infestation include intense pruritus, erythema, alopecia and crusting around the neck and head. The lesions having mouse like odour and it is highly contagious to other cats. In humans with contact of affected cats causing popular rash in the arms and trunk (Sivajothi *et al.*, 2015). This mite is belong to the family Sarcoptidae. According to Foley, 1991 this might not only attack cats but also affect foxes, dogs, rabbits and humans. The morphological appearance of mite include more circular in outline and the size varies between 150 μm to 220 μm . The mite have no projecting scales but in mid dorsally the striae were broken into a scale like pattern and the stout setae were absent and it possess dorsal anus (Walker, 1994).



Fig. 3 Recovery after 3 weeks

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Morphological and Morphometrical studies on the liver of Cobra (*Naja naja*)

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Abstract

Liver was collected from four adult female cobra snake of Tirunelveli district. Aim of the study is to find out the morphological and morphometric details of liver in snakes. Liver of cobra snake was very voluminous and wide and represented 3% of total body mass with an approximately elongated and divided into several lobes. Its colour varied from light to dark shades of brown. It lied in the entire middle portion of the coelomic cavity, enveloping the pancreas, duodenum and stomach. No distinct separate lobes were present instead the surface of the liver appeared lobulated. Single gall bladder was present about 10cm away from the caudal border of the liver. Caudal caval vein was seen at the centre of the ventral surface of the liver. Portal vein was embedded in the dorsal surface. The presence of these two veins make the liver appears to be lobulated. Blood supply to the liver was by the hepatic artery and portal vein. The length of the liver was 25.01 ± 0.23 cm. The breadth of the liver at cranial end, middle and caudal end were 1.1 ± 0.66 , 2.2 ± 0.52 and 0.6 ± 0.87 cm, respectively. The thickness of the liver was 0.8 ± 0.11 cm.

Key words: Morphology, morphometry, liver snake

Introduction

Snakes are elongated legless carnivores. It is reptile of suborder serpents. The liver is the largest internal organ in a snake, filling the space between the heart and stomach. Function of liver is to produce bile, a digestive enzyme. Henninger (1982) focussed on the microscopy of the liver of Terrapene. Eastwell and Richardson (2019) described the morphology of several testudines. Studies were made on the morphology and morphometrical description of the liver of snake.

Materials and methods

The liver was collected from two male and two female adult healthy fresh predator killed dicellate cobras. The snakes were clinically healthy with an average age of 4 years. The average weight was 3.5 kgs. Coelomic cavity was opened to expose the viscera and identified the organs of digestive system and anatomical relationships of liver with the surrounding organs were studied. The morphometrical parameters like length, breadth and thickness of the liver was also measured.

Results and Discussion

The liver was very voluminous and wide representing 3% of total body mass. It had an approximately elongated shape and colour varied from light to dark shades of brown (Fig. 1). According to Chiodini *et al.* (1982) the liver of cobra was long spindle shaped dark brown in color. It filled the entire middle portion of the coelomic cavity and enveloped pancreas, duodenum and stomach (Fig. 1). Liver was found in the second quarter of the viscera and gall bladder in the third quarter (Fig. 2).

The liver was divided into several separate lobes. Turtle liver had 3 lobes namely right, left and central lobes (Moura *et al.*, 2012). No distinct separate lobes were found in liver of the present study (Fig. 1). Dorsal and ventral surface of liver had sulcus. This gives the liver of snake to be lobulated appearance. Portal vein was located in the dorsal sulcus and caudal vena cava was located in the ventral sulcus (Fig. 3 & 4).

Single well developed gall bladder was found. It was located 10 cm away from the caudal tip of the liver. Multiple bile ducts passed from gall bladder through the pancreas into the duodenum. Gall bladder, pancreas, duodenum and spleen are closely associated (Fig. 2). In some snakes, pancreas and the spleen were fused and formed splenopancreas (Douglas, 2012). Blood supply was through hepatic artery and portal vein.

The average total length of the liver was 25.01 ± 0.23 cm. The average breadth of the liver at cranial, middle and caudal end were 1.1 ± 0.66 , 2.2 ± 0.52 & 0.6 ± 0.87 cm, respectively. The average thickness of the liver was 0.8 ± 0.11 cm. According to Chiodini *et al.* (1982) the total length of liver in male cobra was 311 cm and 323cm in female.

Figures:



Fig.1: Photograph showing the visceral organs of cobra. (L – Liver, E – Esophagus, S – Spleen, G – Gall bladder, P – Pancreas, D – Duodenum, St – Stomach, J – Jejunum and K – Kidney)

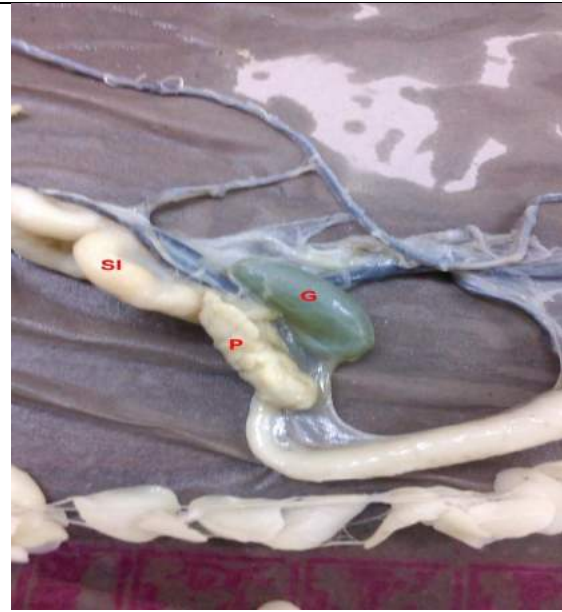


Fig. 2: Photograph showing relationship of the gall bladder (G) of cobra. P – Pancreas, SI – Small intestine



Fig. 3: Photograph showing the dorsal surface of liver with capsule and depression for hepatic vein.



Fig. 4: Photograph showing the ventral surface of the liver with capsule and depression for caudal caval vein (V).

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Thermal stress and its significance in buffaloes

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Thermal stress is a major concern for all livestock production systems owing to its negative impact on health and production. India loses 3.2 million tonnes of milk production at present due to thermal stress and global warming was further hypothesized to reduce milk production by 15 million tonnes by 2050. Intergovernmental panel on climate change (IPCC) anticipates a rise in Earth's surface temperature by 0.2 °C per decade and thus, it might lead to an overall spike of around 1.8 °C to 4.0 °C by 2100 (IPCC, 2007). The major factors that contribute to thermal stress are the relative humidity (RH) and ambient temperature (AT). The inability of the animal to lose heat to the surroundings is defined to be thermal stress; it results in accumulation of heat load due to inability to loss heat to the surroundings. Thermo-neutral zone (TNZ) is the range of ambient temperature where homeotherms survive without any expenditure of energy to maintain body homeostasis. Any deviation in the ambient temperature which goes beyond the upper critical temperature could terminate in heat stress (Sahu et al., 2019). Most of the livestock species experience stress of varying degrees but are able to cope with these environmental stressors through behavioral measures such as sweating, panting, drinking water, shivering or by regulating their metabolic rates.

As the ambient temperature around the animal rises, above the body temperature the animal gains heat via conduction/convection/radiation. High RH accompanied by high AT further compromise animal's ability to lose heat to the surroundings. However, animals being homeotherms operate

numerous behavioral (shade seeking, orientation of body posture away from sunlight, wallowing, reduced motor activity) and physiological alterations including increased respiratory rate to promote evaporative heat loss and increased heart rate to deviate blood flow from the centre to the periphery to allow heat loss via skin.

Buffaloes are the most affected species due to their anatomical features. Buffalo population in India comprises of 109 million which contributes to 50% of India's milk production. Buffalo population contributes to livelihood of small, medium and large scale farmers all over the country. Despite of their high milk production, buffalo population in India is being constantly challenged by thermal stress due to hot and humid equatorial tropical climate and are insufficiently thermo tolerant compared to cattle because of differences in heat regulating mechanisms such as black skin and lower density of sweat glands. Thermal stress results in reduced feed intake which is a thermoregulatory mechanism to reduce further heat accumulation, altered hemato-biochemical profile and deviation of energy for thermoregulation i.e. less blood and nutrient flow to udder which deviate energy for heat loss mechanisms. These disturbances finally impair production and reproduction parameters in buffaloes.

Hence it is very important to manage buffaloes during summer to maintain optimum productivity, health and well being of the animals.

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Methods for effective Wild boar management

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Abstract

Wild pigs/boars are mainly present all over the world and are considered as major threat to both environment and agriculture. Population of pig's increase at higher rate due to more litter size per Farrowing. Moreover, now as days these wild pigs are also responsible for some zoonotic disease and are also a direct threat to human. So continuous check on wild pig's population is need of hour. Current article deal with some managemental aspect which can be used to control wild boar population and thus reduce harm cause.

Introduction

Mainly boar is a term given to male swine and today's pig has ancestral relationship with wild boar and other species. Just like human, these wild boars have mono-gastric stomach and can survive on both animal and plant material. Currently wild boar belongs to:

Kingdom - Animalia
Phylum - Chordata
Class - Mammalia
Family - Suidae
Genus - *Sus*
Species - *S. scrofa*

There are many variations in wild boar colour, shape and size. Majority of wild boar has black body coat and has 44 teeth (Sweeney et al. 2003). These teeth grow in size and become very sharp due to friction between canines of lower and upper jaw. Sometime the canines can also

form tusk and grows up to 4 inches (Mayer and Brisbin 1988). In general, wild boars make changes to local micro-environment and ecosystem by various activities such as rooting, wallowing, foraging, and hunting. It can cause loss of \$1.5 billion to agriculture and the environment. Rooting by pigs means digging of soil, up-rooting plants, exposing bare soil, and enhancement in growth of unwanted and harmful weeds. They also lead to damage lawns and gardens. By wallowing activity pigs destroy various small water bodies and springs, and also they also cause physical damage to stock water troughs. Various activity of wild boar also changes the turbidity of water and in turn has devastating impact on variety of aquatic flora and fauna, most notably freshwater mussels and insects (Kaller and Kelso 2006, Kaller et al. 2007)

Wild boar mainly relishes on hard mast crops and thus creates competition with black bear and mule deer. Many time boars destroy cultivated crops of farmer and can lead to various health hazards to other livestock and human. Boars can also lead to spread of some pathogens such as *E.coli*, *Campylobacter*, *Salmonella*, *Cryptosporidium*, and *Giardia* (Kaller et al. 2007). These vertebrates also act as host or intermediate host to at least 37 parasites and many of them are zoonotic potential. So to make long story short we can easily say that wild boar has devastating effect on flora and fauna so their effective management is needed.

Various method of boar management-

Trapping- It is most common and easy method of swine control and can reduce swine population up to 80-90% (Choquet et al 1993). Many time after trapping pigs are euthanized. Various kinds of traps are there such as cage trap, drop gate trap, tunnel trap etc (Barrett and Birmingham 1994). To get maximum use of cages, various bait such as some food items like shelled corn, are also used which lure the animal to get in the cage for feeding. Building material and door of cage should be of good quality.



Fence trap

(<https://www.growingproduce.com/fruits/how-farmers-can-get-a-handle-on-wild-hogs/>)



Wood trap

(<https://www.wildpiginfo.msstate.edu/traps/types.php>)

Snares- Some place where cage trap is not feasible, different kind of snares can be used. Snares are cheaper in cost and have portability. Snares are generally made up of a loop of cable which can close easily but difficult to open. Many time snare are connected to immovable anchor (Mapston 1999). Mainly snares made for leg or neck are used.



Hog snare

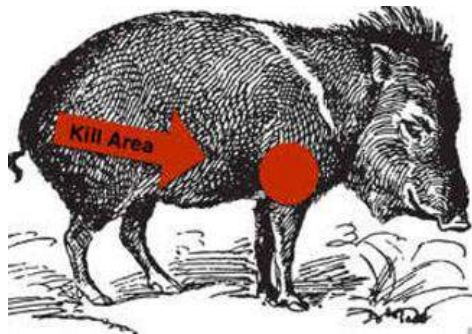
(<http://www.hoghuntinginfo.com/hog-snaring.php>)



Hog snare cable

(https://www.farmshow.com/a_article.php?aid=23935)

Shooting- It is the most common technique used worldwide. Shooting can be implemented in various ways such as sport hunting, aerial shooting, night shooting etc. For shooting management sometime special things are also needed such as in aerial shooting aircraft is needed. For night shooting sometime thermal imaging and night vision instrument are needed.



Best area to shoot a pig

(<https://www.hogwildok.com/blog/35-best-place-shoot-hog.html>)



Pig shot dead

(<https://www.wpr.org/dnr-hunters-look-out-feral-pigs>)

Judas pig- It is technique in which pig are tagged with radio technique and are released so that entire herd can be traced. Mainly adult females are generally used for this technique.

Dog hunting- In some places hunting of wild pig with well-trained dog is also done. Sometime dogs are used to kill the pig but sometime these canine are used only for just capturing the wild pig.



Pig hunted by dog

(https://www.tripadvisor.com/LocationPhotoDirectLink-g51602-d6731625-i232557793-Hog_Wild-Purcell_Oklahoma.html)



Dog hunting pig

(<https://www.gamegear.co.nz/blogs/our-adventures/more-than-just-a-pig-story>)

Intoxication with chemical– In some places chemical toxin are used to kill wild boar. It is powerful and cheap way of eliminating the boar population. By chemical method cost can be reduced to 11 and 80% as compared to shooting and trapping respectively (Coblentz and Baber 1987). In Australia warfarin poisoning is the way to have a check on pig population (Saunders et al 1990). Some other product used in other country are pig out, sodium nitrate and now a day's attempt are also been made to eliminate wild boar in more humane manner.

Fencing- Fencing a boundary is also a way to keep out wild boar population. Fencing is not 100% reliable but it can put some restriction in boar herd movement. Many design of fencing is available in market and a farmer can use as per his/her requirement. Electric fencing provides better results than normal fencing but it is costlier. Normally fencing should have 4-6" spacing (Liattauer 1993) and 36" high from the ground (Mapston 1999).

Contraception- It is also one of the effective way to control pig population. GnRH injection reduce both ovary and testicle weight, it also reduces the testosterone and progesterone level and therefore decrease the libido of wild boar and also reduce the conception are in female (Killian et al 2003).

Conclusion

Proper knowledge about effective management of wild hogs will provide benefit to rural people and in-turn enhance the agricultural productivity and income of farmers.

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Strategy to Prevention and Control of Pandemic Zoonoses

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Abstract

Zoonoses are a significant public health concern and cause considerable socioeconomic problems globally. Zoonoses lead to millions of deaths annually. Out of all microbial diseases, 61% are zoonotic with 13% species regarded as emerging or re-emerging. Among emerging infectious diseases, 75% are zoonotic with wildlife being one of the major sources of infection. Different factors such as climate change, urbanization, animal migration and trade, travel and tourism, anthropogenic factors, and natural factors responsible for emergence or re-emergence of zoonotic diseases. Multisectoral approach and key strategy for control of pandemic zoonoses are monitoring and surveillance of pandemic zoonotic diseases, implementation of One Health measures, capacity building programme, and policy at international level, financial support for developing countries by international organization, new diagnosis and scientific technique for zoonoses, re-governing animal origin food systems, biosecurity and awareness about pandemic zoonoses.

Keyword:-One Health, Pandemic, Prevention and control, Zoonoses.

Introduction

Zoonoses are diseases that can be transmitted from animals to human and vice versa. Almost all types of pathogens, including viruses, bacteria, parasites, fungi, spirochetes, mycoplasma and chlamydia, can cause zoonoses, and more than 800 pathogens have been shown to transfer between humans and vertebrates, with more than 73% of infectious diseases in humans originating from animals (Liu et al., 2014). More than 60% of the 400 emerging infectious diseases that have been identified since 1940 are zoonotic, and these pathogens are public health significance (Jones et al., 2008). The different factors that are responsible for emergence or re-emergence of zoonotic diseases are etiological changes in mans, environment and agricultural operations, increased movement or traveling of man, handling of

animal by-products and waste, overcrowding of animals, increased trade in animal products, globalization, drug resistant organisms, changing livestock farming practices, climate change, Increased contacts with a wildlife reservoir, accelerated degradation of the natural environment (deforestation, building of dams, land consolidation), global warming, disaster and changes in microbial pathogens due to genetic shift and drift. Climate change can impact physiological status of hosts, pathogens and vectors, distribution in life cycle of virus, and can also drive novel cross-species viral transmission (Poley and Thompson, 2009). Zoonoses may cause a serious health hazard to the international community. About 58–61% of the human diseases are communicable and up to 75% are zoonotic. Zoonosis involves the interaction of humans, animals, and environment, and therefore a multi-sectorial approach is required to ensure effective control measures (Aenishaenslin et al., 2013).

Strategy to prevention and control of pandemic zoonosis are as follows

Monitoring and Surveillance of pandemic zoonotic diseases

Develop efficacious method of monitoring and regulating practices associated with zoonotic disease, including food systems from farm to fork. Surveillance is crucial to prevent and control zoonotic diseases. It can be used to detect early infection, affected humans and animals, reservoirs, vectors and endemic areas including the “hotspots”. It aid in the adaptation of control strategies against emerging and re-emerging diseases to improve human health status, to manage disease properly, and to minimize morbidity and mortality of humans and animals. Pandemic zoonotic diseases like severe acute respiratory syndrome and highly pathogenic avian influenza can spread across the globe to affect global communities, coordinated surveillance approaches at local, regional, national, and international levels are essential to control these types of pandemic zoonoses. All potential sources of zoonoses including exotic animals and birds, pet and companion animals, aquatic animals, wildlife and rodents need to be subject to surveillance. There are different types of surveillance that need to be conducted (Van der Giessen et al., 2010). Successful and functional surveillance requires well equipped laboratory, adequate modern diagnostic facilities, skilled manpower and enough funding. The four surveillance types can be practiced for the control of zoonoses are pathogen surveillance to detect and identify pathogens. In serological surveillance to detect the presence of pathogens in the blood of humans or animals through monitoring immune responses. In syndrome surveillance to determine the propensity of diseases through data analysis based on symptoms of diseases. Risk surveillance is used to detect risk factors responsible for the transmission of disease.

One Health approach for control of pandemic zoonosis

Zoonoses are complex, responsibility for their prevention and control falls across multiple sectors. For the prevention and control of pandemic zoonotic diseases, international organizations and many researchers described the relationship among human, animals, and environments and adopted a concept known as “One Health Concept”. This One Health concept was adopted to properly deal with global health challenges. The one health concept encourages collaborations among wildlife biologists, veterinarians, physicians, agriculturists, ecologists, microbiologists, epidemiologists, and biomedical engineers to ensure favourable health for animals, humans, and environment. World Health Organization, Food and Agriculture Organization, World Organization for Animal Health, US Centres for Disease Control and Prevention, US Department of Agriculture, United Nations System Influenza Coordination, and European Commission recognize the prevention and control strategies involving the one health approach (Dahal and Kahn, 2014). One Health concept and research offers an approach to break down traditional sectoral barriers to achieve effective control of zoonoses. A promising progress in the wake of the avian influenza pandemic is the establishment of collective zoonoses working groups in many countries and other international collaborations.

Capacity building programme at national and international level

Need a Capacity building programme at national level for strengthen existing and build new capacities among health stakeholders in all countries to improve outcomes and to help them understand the human, animal and environment health dimensions concern to zoonoses and other emerging and re-emerging diseases.

National and International policy for pandemic zoonoses

For control of pandemic zoonoses requires strong policy frameworks rules, regulation and judicious legal mechanisms to accompany policy frameworks. It also demands well-functioning institutions that have adequate capacity, adequate financing and a clear plan for implementing interventions.

Financial support for developing countries for control of pandemic zoonoses

The developed countries and international funding donor agencies such as World Health Organization, Food and Agriculture Organization of the United Nations, World Organization for Animal Health, US Agency for International Development, US Department of Agriculture, European Union, Department for International Development Agency need to support the developing countries for effective zoonoses control.

Awareness of pandemic zoonoses

Raise public awareness and increase knowledge of zoonotic and Emerging /re-emerging disease risks and prevention at all levels of society to build widespread support for risk-reduction strategies. Awareness about health education. Mass media and electronic information system, social networks and other communication channels can play a significant role in increasing public awareness to control pandemic zoonoses.

Improved innovations and new scientific technologies for diagnosis, prevention and control of pandemic diseases

Without more fundamental knowledge of pathogen epidemiology and more rapid and inexpensive genome sequencing, every new serious emerging zoonotic disease will continue to take us by new challenges. However, additional investments in new technologies, particularly biotechnologies, bio information and communication technologies, could stimulate the innovation in disease surveillance, rapid diagnosis, response and control measures.

Transforming and re-governing animal origin food systems

For control of pandemic zoonosis as well as for preventing future outbreak of zoonotic disease also requires improvements in policy, regulation and monitoring of traditional animal origin food markets. Millions of people depend on unorganized food markets that occur in public spaces where small-scale retailers come together to sell fresh produce, fish and meat from domesticated animals and in some cases, from wild animals where sanitary and hygienic practices are poor control. Many recent zoonotic pandemics originated in wildlife. To control of pandemic zoonoses and reduce risks of future zoonotic diseases, meat from both wild and domesticated origin and the places in which the meat is sold should be subject to similarly strict sanitary standards (Pike *et al.*,2010).

Sustainable co-existence of agriculture and wildlife habitats

Support integrated management of landscapes and seascapes that enhance sustainable co-existence of agriculture and wildlife, including through investment in agro ecological methods of food production that alleviate waste and mitigate pollution while reducing risk of zoonotic disease transmission. Reduce destruction of natural habitat and fragmentation of wildlife habitat by strengthening the implementation of existing commitments on habitat conservation and restoration.

Biosecurity and other control measures

Identify key drivers of emerging diseases in animal husbandry sector, both in industrialized agriculture and small-scale holder production. Develop new practices that strengthen the health, opportunity and sustainability of diverse smallholder systems. Breaking the chain of zoonoses through practicing biosecurity in livestock system. Other important activities for the control of zoonoses include issuing laws and regulations related to the isolation and quarantine, establishment strong and effective disease reporting systems, farm biosecurity, mass vaccination of animals, test and slaughter or culling of animals. Decontamination of infected pathogenic materials is needed to reduce the chances of acquiring new infections in the populations. Many neighbouring countries are affected by zoonotic disease, for those proper coordinated approaches need to be adopted for zoonoses control. Ensure safe food production of animal origin and ensure safety of infectious laboratories to avoid the accidental spread of zoonotic infections and bioterrorism need to be ensured.

Conclusion

The implementation of multisectoral approach is required for the effective prevention and control of pandemic zoonosis. The strategy to combat these pandemic zoonoses needs One Health approach, a strong public health structure, effective risk communication, epidemic, pandemic preparedness and rapid response. Strong interrelatedness among animals, humans, and environment, research focusing on the one health approach need to be prioritized to identify critical intervention steps in the transmission of pathogens. Active surveillance targeting all components of the one health approach need to be implemented to early and accurately detect pandemic zoonoses. Integration of efforts of local and national governments, coordination of budgetary resources, awareness of zoonotic disease is necessary for prevention and control of pandemic zoonoses.

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Opportunities and Challenges in Meat and Poultry Sector in India

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Abstract

In India, the chicken business is one of the most important contributors to the rural and semi-urban economies. India is the world's third-largest egg producer and fourth-largest producer of chicken meat. The poultry industries organized sector accounts for 70% of the total output. In India, the broiler and layer industries are controlled by the southern states. Poultry production in India is a vertically integrated industry that rivals that of several Western countries. In the future, the feed industry must ensure that feedstuffs are of high quality, free of diseases, and environmentally friendly.

Introduction

India has the world's greatest livestock population. India produces 5.3 million metric tonnes of meat and 75 billion eggs each year. India produces the most buffalo meat and the second most goat meat in the world. Processing levels in poultry are currently at 6%, while meat processing levels are at 21%. The Indian government has taken attempts to modernize municipal abattoirs in order to supply consumers with safe and hygienic meat. Export-oriented businesses have made major investments in the construction of big abattoirs and meat processing plants using cutting-edge technologies. Meat output increased from 2.3 million tonnes at the end of the Tenth

Five-Year Plan (2006-07) to 5.5 million tonnes at the end of the Eleventh Five-Year Plan (2011-12). Meat output was 5.95 million tonnes at the start of the Twelfth Plan (2012-13), and it climbed to 7.7 million tonnes in 2017-18. The chicken industry meets the majority of protein and nutrient needs. India is now one of the world's largest producers of eggs and broiler meat. When we look at the big picture, we can see that agricultural production has been increasing at a pace of 1.5-2 percent per year, while egg and broiler production has been increasing at a rate of 8-10 percent per year. India produces the 3rd most eggs and the 6th most broilers in the world. In 2018, the Indian broiler and egg market was worth INR 1,750 billion, while in 2019, it was worth INR 2049 billion (DAHD, 2020).

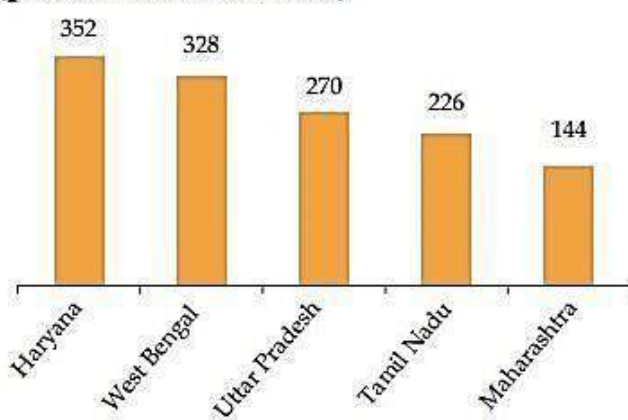
Advantage in India (Pashudhan Praharee, 2020)

- Fastest growing economy in the world
- Largest producer of several agri-commodities
- Second largest Consumer market
- Significant investments in world class ports, logistics & supply chain infrastructure
- Proactive Government policies
- Investor friendly incentives
- Highly skilled manpower pool

Meat production in India

The largest producer of meat in the country is Uttar Pradesh producing 23% of the total meat followed by West Bengal contributing 12% to the meat production. Andhra Pradesh is the third largest meat producer in the country which produces 7% of the total production.

Top 5 Poultry Meat producing states in India (production in 000'MT)



Source: Department of Animal Husbandry, Dairying and Fisheries (DAHD)

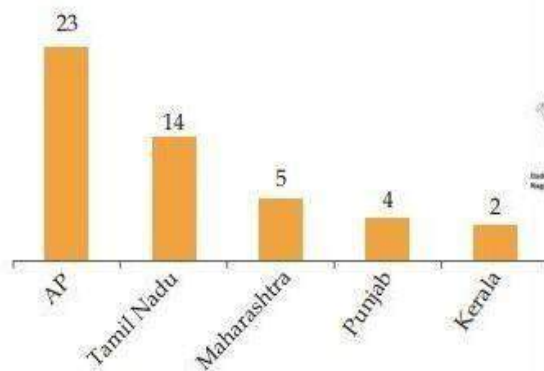
Figure 1 Poultry Meat Producing States in India



Egg production in India

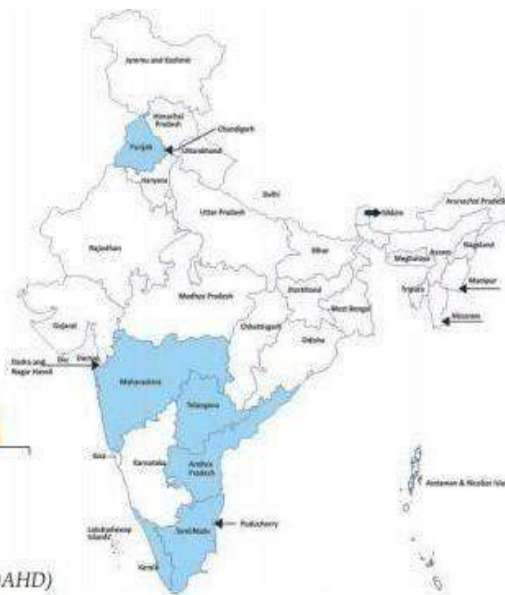
Andhra Pradesh, Tamil Nadu and Maharashtra are the top egg producers in the country.

Top 5 Egg Producers (in billion numbers)



Source: Department of Animal Husbandry, Dairying and Fisheries (DAHD)

Figure 2 Egg Producing states in India (DHAD, 2020)



Problems associated with the poultry industry

There are some threatening agents to the poultry sector,

1. More than 60% of broilers are produced in these 5 states of India (Andhra Pradesh with 20%, Karnataka, Maharashtra, Punjab, and West Bengal), while more than 60% of eggs are produced in these 5 states of India (Andhra Pradesh with 20%, Karnataka, Maharashtra, Punjab, and West Bengal) (Andhra Pradesh, Haryana, Maharashtra, Punjab, and Tamil Nadu).
2. International competition: opening up duty-free imports, removing trade obstacles, and so on.
3. Disease outbreaks: Fowl pox, Fowl cholera, avian influenza, etc.
4. Feed components and other logistics costs: Small-scale producers face issues such as high feed and transportation prices, costly vaccines, veterinary care services, and a lack of credit. It has been reported that some farmers have moved from layer to broiler production because to significant capital inputs, as broiler units may be harvested in six weeks (Hafez, 2010).
5. Antibiotic abuse in chicken farms is causing germs to develop multi-drug resistance (because of unsafe disposal of poultry litter).
6. Noticeable price fluctuations.

Challenges

1. Broiler pricing maneuverability refers to the management of broiler meat output during lean seasons, when consumer demand is lower. For example, Ganesh Chaturthi, Navratri festival, etc., or when consumer consumption increases, such as Ramzan (Sharma, 2021).
2. Over processed or frozen poultry meat is a result of a poultry producer's reliance on live bird sales.
4. Training of workers - The poultry industry has a scarcity of qualified workers.
5. Government food safety standards - Food safety is a major priority for both the government and the general public (Saravanan & Manjula, 2015).

Conclusion

The current nation has a market that is conducive to investment. The Indian chicken business is booming, and the country is on its way to becoming the world's second largest market. Every year, the rate of increase is staggering, averaging 12-15 percent. Poultry meat is widely accepted since it is not linked with any religious taboos and is less expensive than other nutritional sources. As a result, the chicken sector not only provides a low-cost supply of nutritional protein to consumers, but also job possibilities.

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Wildlife Conservation and Conservation Strategies

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Abstract

Wildlife conservation is an activity in which humans make conscious efforts to protect the wild species and their habitats. Wildlife plays an important role in maintaining the ecological balance. Conservation has several advantages for maintaining the healthy ecosystem, maintaining genetic resource, recreation and education. Habitat destruction and fragmentation, poaching, illegal trades, human wildlife conflict, pollutants are the major factors contributing to wildlife extinction. Safeguarding the critical habitats, sound planning and management for captive breeding, control of illegal trading and poaching are the big way for wildlife conservation.

Introduction

Wildlife conservation is the preservation and protection of animals, plants and their habitats. The conservation of wildlife is mainly focusing on maintaining the habitat, maintaining the breeding stock and prohibition of illegal trading and killing of any animal (Santra, 2018). Thus conservation of wildlife has not only concerned with biotic factors (plants, animals and microorganism) but also with abiotic factors. Hence, the conservation of wildlife resources is intimately related to the conservation of other natural resources.

Wildlife plays an important role in maintaining the ecological balance. Each organism on this earth has a unique place in food chain that contributes to ecosystem in its own special way. We can understand with the help of an example, killing of carnivores leads to an increase in number of herbivores which in turn affect the forest vegetation, thus due to lack of food in forest they come out from forest to agricultural land and destroy the crops. Once equilibrium and stability is disturbed it leads to many problems. But sadly today, many of the wild species are getting endangered. Habitat

destruction and fragmentation, poaching, illegal trades, human wildlife conflict, pollutants are the major factors contributing to wildlife extinction (Gupta, 2017). The most serious threat to wildlife comes from habitat destruction (Naz and Hussain, 2016). The extinction of wildlife species will certainly have a fatal impact on this environment. Therefore, it is very necessary for us humans to act in a way that ensures the conservation of wildlife and natural resources.

Necessity for conservation

1. *Balance of Ecological Nature*

The most important point being that the natural balance in an ecosystem is maintained. Reduction in numbers of one animal interrupts the eco system and the natural food chain, and leads to the threat of other species. This balance may be disturbed due to the introduction of new species, the sudden death of some species, natural hazards or man-made causes. For an example, when all the herbivorous animals in a forest killed, the tiger and lions enter human settlements and attack human beings and domesticated species. Like that the killing of snakes for their skin allows the rat population to increase enormously.

2. *Biological value*

Wildlife is considered to be an ecological asset and an indicator of environmental health. Biological value of wildlife is concerned with their contribution of productive ecosystems such as pollination, seed dispersal and planting, control of animal and plant population, recycling of nutrients and sanitation through scavenging (Santra, 2018).

3. *Commercial value*

Wildlife is a wealthy of the country and it is a good source of income (Singh, 2009). The wildlife has become an important source of revenue nowadays. Wildlife yields timber, firewood, hides, ivory, horns, fur, etc. and are harvested for commercial purposes. Live and dead animals can be stored in Zoos and Museums for exhibition

4. *Educational value*

Wild animals are used for various researches and scientific studies. For example, Rhesus monkeys are widely used in biomedical research.

5. *Recreation*

Wildlife forms a source of enjoyment and recreation to human beings. Many professionals like photographers and bird watchers are immensely benefited through visiting wildlife conservation places.

Conservation strategies

1. Preserve wildlife habitats

Habitat destruction is the major cause of declining wildlife population. Activities like logging, industrialization, urbanization, utilizing forest area for agriculture to suffice the human needs,

mining, are the human contributions to habitat destruction. Forest fire, climatic changes are some of the natural causes. By growing plants and saving trees, protecting remaining intact sections of natural habitat, introducing new ways to increase agricultural output without increasing land consumption and creating corridors are some of the solutions to protect our wildlife. Habitat corridors allow movement between isolated populations so it increases genetic diversity and also minimize the human wildlife interactions (Singh, 2009) Growing some flowering plants in the forest will attract insects which can also help in pollination. So the environment becomes greenery.

2. Stopping illegal trades

Apart from habitat loss, poaching is the next major threat to wild animals. This illegal act is being done for some ridiculous motives especially for the rare animal products such as ivory, fur, organs, skin and bones. On the other hand some animals have been hunted for food, religious purpose and some myth about medicinal values. But the actual truth is that the animal organs don't have any proven medicinal values. (Foster *et al.*, 2019). To prevent these activities, harmless and undetectable trackers need to be used to track the activities of animals. Outlawing the buying and selling of wildlife animal parts in black markets can significantly reduce poaching. International trade in wild plants and animals has to be regulated to appropriate legislative and administrative measures.

3. Reducing pollutants

Waste around the world increases day by day largely. According to survey, 5.25 trillion pieces of plastic debris present only in oceans. It creates a major threat to marine lives. Other than plastic waste oilspills, untreated sewage, industrial chemicals produce some toxic pollutants like carbondioxide, nitrogen dioxide, sulfur dioxide, ozone which will affect the humans as well as animals. At an animal level, it disturb the endocrine system, sex ratio changes and decreased reproductive parameters but also include teratogenic, genotoxic effects, immunosuppression and other immune related diseases (Erren *et al.*, 2009). By reducing the usage of plastics, recycling and reusing the wastes, ocean cleanup, using microbes and some plants to break down the chemical pollutants and treating industrial waste water to reduce pollutants are the steps to be taken to decrease the environmental pollution. The important thing is that all the wastes are produced by humans only so the care should be taken by us only to clean the environment.

4. Awareness

Creating awareness among the people who are involved in poaching, poisoning the animals to protect their livestock and superstitious activities. Youngsters should learn and be trained to impart knowledge about the value of wildlife and its management. Educating the young minds and involving them in wildlife conservation activities will create a great impact. Including wildlife education as a part in school curriculum will show some seeds of wildlife importance in the minds of future citizens. Encourage the community participation in management and conservation of wildlife will surely improve the status of wild species.

5. Adoption

Adoption is one of the methods to conserve the captive wild species. Many countries including India allowed adopting wild animals, birds which was kept in the zoo. One can contribute manifold in wildlife conservation by adopting animals through providing feed for them monthly/annually. Paying methods and payments differ from species and zoos. Animal adoption scheme will certainly help in running the conservation projects which will involve captive breeding programme especially for endangered species in various zoos. The adaption of animals by a person of the society will not only show his/her love towards wildlife but will also inspire others to follow. This small step will help in a big way for wildlife conservation.

6. Captive breeding

Captive breeding is the practice of breeding animals in a human controlled environment as a tool for conservation. It has been a popular management option for endangered species in recent years as animal population can increase more rapidly than in the wild. Captive breeding programs are generally not initiated until population numbers in the wild have fallen below sustainable levels (Philippart, 1995). Captive breeding need much more care to protect the population against genetic disease. However it is important to be aware that captive breeding alone cannot be relied upon to ensure the long term survival of endangered species.

Conclusion

The wildlife has many important roles in the improvement of human society. Their contributions to the maintenance of healthy ecosystem, maintaining of gene pool and recreation have been well documented. To help protect wildlife, it's important to understand how species interact within their ecosystems, and how they're affected by environmental and human influences. We have a great responsibility to save the precious species and the planet. If the present rate of degradation and loss of habitat continues, we will be left only with their photographs. Apart from government measures, active participation of citizens of our country is the most important thing in conservation of wildlife. By conserving wildlife, we're ensuring that future generations can enjoy our natural world and the incredible species that live within it.

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Role of Mesenchymal stem cells in the treatment of COVID-19 infected patients

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Abstract

The corona virus, which infected the people all over the world and till that there is no proper treatment available. Several researches have been going on worldwide to find out the proper treatment of this COVID-19 disease. The use of mesenchymal stem cells (MSCs) is the promising way for the treatment of this disease. Mesenchymal stem cells are multipotent stem cells which have the capability to differentiate into a variety of cell types, including bone cells, cartilage cells, muscle cell etc. and have immunomodulatory properties, which makes them an effective tool for treatment of many chronic diseases.

Introduction

The first case of COVID-19 was detected in Wuhan, China, in December 2019 after within few days the number of COVID-19 patients have rapidly increased not only in China, but also worldwide, including Germany, Italy, and USA, India and other counties and become epidemic. COVID-19 is a positive-stranded RNA virus having spike glycoproteins on their surface which cause respiratory disease. Currently there is no drug or vaccine is available to treat this disease worldwide. So in this report I suggest a new approach to improve patient's health to COVID-19 using mesenchymal stem cells.

Mesenchymal Stem Cells

Mesenchymal stem cells are multipotent stem cells which have the capability to differentiate into a variety of cell types, including bone cells, cartilage cells, muscle cell) and fat cells. MSCs have immunomodulatory properties including cytokines, immune-receptors and secretion of anti-inflammatory molecules which makes MSCs an effective tool for treatment of many chronic diseases. They are present not only in fetal tissues but also in many adult tissues.

Several reports suggested that on infection the COVID-19 virus specifically recognizes the angiotensin I converting enzyme 2 receptor (ACE2) by its spike protein in host cell and this

ACE2 receptor allow the entry of this virus into the host cell especially the alveolar type II cells and capillary endothelium cells. Some scientist use immunological therapy to treat the infected patients, However immunomodulatory effects were not so strong enough because of only one or two immune factors were used.

Scientists at School of Life Sciences, Shanghai University, and Shanghai, China used MSCs therapy on COVID-19 infected patients during Jan 23, 2020 to Jan 31, 2020. After few days (10 days) they found that all symptoms disappeared in all seven patients and no side effects were reported. After treatment, they found that peripheral lymphocytes were increased, the C-reactive protein decreased, and the over activated cytokine-secreting immune cells CXCR3+ CD4+ T cells, CXCR3+ CD8+ T cells and CXCR3+ NK cells disappeared in 3 to 6 days. In addition to this level of TNF- α was significantly reduced and IL-10 increased in MSC treated group compared to the control group. So this study clearly indicated that treatment of COVID-19 infected patients with Mesenchymal stem cells could be the promising are in near future.

Conclusion

Mesenchymal stem cells are multipotent stem cells and have immunomodulatory effects and secretes different types of anti-inflammatory molecules, which enhanced the immune system of the body and help in preventing of the chronic diseases. So Mesenchymal stem cells therapy is promising area for the treatment of COVID-.19 patients

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Role of semen sexing technology for farmer's economy

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Abstract

Sexual selection of desired sex plays important economic role in livestock industries. In India Farmers prefer female calves of cows or buffalos than male calves as they can produce milk which is more economically beneficial for farmers. X and Y chromosome determine the sex of the animal. . If X sperm fertilize the bovine egg results in the birth of female calves whereas Y sperms result in male calves. Semen sorting based on that defenses between X and Y sperm. The X sperm contains about 3.8% more DNA than the Y sperm in cattle and this difference in DNA content is used to sort the X- from the Y- bearing sperm. Flow cytometry based sorting is most efficient method because the purity of sexed semen is more than 90%. In flow cytometry (Fig.1) an a special DNA-binding dye Hoechst 33342 and electric filed is used and on the basis of charge X or Y sperms are separated and on the basis of requirement X or Y sperm in injected through AI gun in female reproductive tract.

Introduction

Farmers prefer female calves of cows or buffalos than male calves as they can produce milk which is more economically beneficial for farmers. According to the 20th livestock census 2019, there are total milch animals (in-milk and dry) in cows and buffaloes are 125.34 Million. Compared to the 2012 census, the milch animals (cow and buffalo) have an increase of 6.0%. Apart from this the demand of milk also increasing day by day and to meet the demand, it is very important to increase the milk production by producing the more female cows and buffalos. For this Sexual selection of desired sex plays important economic role in livestock industries.

There are two types of sperms in bovine i.e X and Y. If X sperm fertilize the bovine egg results in the birth of female calves whereas Y sperms result in male calves and the probability of occurring male or female calf is 50%. Selection of a desired sex calf is not possible with natural

breeding because in natural breeding there are 50%- 50 % change of occurring male or female calf, so for this a special technique known as Artificial insemination (AI) is required. By this technique preserved sexed semen is introduced artificially into the reproductive tract of the female and sex of the birth calf is depend on the type of semen (either it has X or Y sperms separately) and separation of X and Y sperms is done by Semen sorting technique and semen is known as Sexed Semen.

How Sexed Semen is produced?

The basic principle for semen sorting based on that defenses between X and Y sperm. The X sperm contains about 3.8% more DNA than the Y sperm in cattle and this difference in DNA content is used to sort the X- from the Y- bearing sperm. There are several methods for semen sexing but flow cytometry based sorting is most efficient method because the purity of sexed semen is more than 90%. In flow cytometry (Fig. 1) an a special DNA-binding dye Hoechst 33342 and electric filed is used which gives the different charge (+ or -) on the surface of sperm and separates the X or Y sperms on the basis of charge and then stored in liquid nitrogen at -196°C . Finally by using Artificial insemination method desired semen contain either X or Y sperm is transferred into the reproductive tract of the female cow or buffalo.

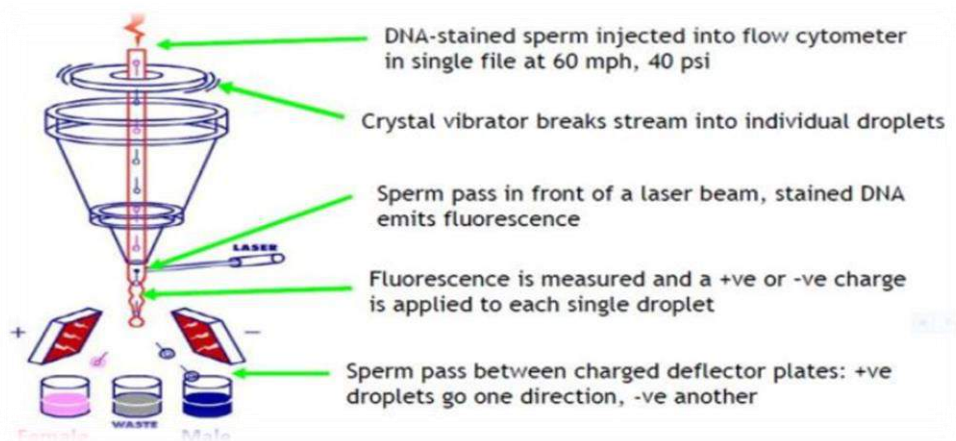


Fig. 1 Flow diagram of semen shorting by flow cytometry

Benefits of semen sexing:

- Pre-selection of sex can reduce the management
- Calves of desired sex can be produced.
- genetically superior female calf can be produced
- reduce the risk of dystoci
- no risk of introducing diseases

Limitations of sperm sexing:

- More than half of sperm sample are go as waste; only 30% to 40% semen is of desired sex.
- The equipment is highly expensive and special trained persons are require
- Currently there is no company producing sexed semen in India so it is imported from other country and due to this the cost is about 1500-4000 per dose.

Conclusion

The sexed semen is beneficial for the farmers as they requires only female calves which can reduce the maintaining cost on the male calf and by using this technology superior high milk producing calf can be produced which help in the doubling of farmer income.

A Glimpse on Zoonotic Tuberculosis and Its Public Health Significance in India

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Abstract

Bovine tuberculosis is a chronic bacterial zoonotic disease in animals, especially in cattle and it also affects the human. The disease causes loss in the productivity of animals thus possessing great economic losses. The true burden of the disease remains unknown due to lack of routine surveillance data from most of the developing countries like India where, the epidemiology of bovine TB continues to remain largely hidden towards attaining the End TB goal. Preventing and controlling bovine TB at the animal interface is crucial to avoid transmission to human, increase food safety and guard the livelihood of the people. Implementing One Health approach in India by the government, which acknowledge the interdependence of the health of people and animals will help the nation in the fight against TB. Our writing highlights the intercommunication between the components of the epidemiological triad and discusses the control and prevention strategies to reduce the spread of infection in bovines and also to humans.

Introduction

Tuberculosis (TB) is potentially a serious infectious and communicable disease that mainly affects the lungs and is a major cause of ill health. It is one of the top 10 causes of death worldwide and the leading cause of death from a single infectious agent (ranking above HIV/AIDS). Most of the human tuberculosis cases are generally caused by the bacterial species, *Mycobacterium tuberculosis*. Zoonotic TB is a less common form of TB in people predominantly caused by *M. bovis*, a closely related species, which belongs to the *M. tuberculosis* complex. The disease can also affect in other parts of the body called extra pulmonary TB.

Bovine tuberculosis is a chronic bacterial disease of animals caused by members of the *Mycobacterium tuberculosis* complex (MTBC), primarily by *M. bovis* and other members of the MTBC such as *M. africanum*, *M. canettii*, *M. microti* and *M. tuberculosis*, *M. caprae*, *M. mungi*, and *M. orygis* and contains known pathogens of humans, domesticated and wild species of mammals. The members of the *Mycobacterium tuberculosis* complex (MTBC) constitutes an exceptional genetically homogeneous group which are characterized by 99.9% similarity at the nucleotide level and identical 16S rRNA sequences.

Bovine tuberculosis is a major zoonotic disease, cattle serves as the main source of infection for humans. It also affects other domesticated and pet animals such as sheep, goats, equines, pigs, dogs, cats and wildlife species such as wild boars, deer and primates causing a general state of illness like loss of appetite, fluctuating fever, diarrhea, dyspnea, swelling of lymph nodes etc. Intermittent hacking cough, pneumonia, weight loss and eventual death are the major signs. The clinical symptoms may be sub acute or chronic, with a variable rate of progression. The bacteria can also lie dormant in the host without causing disease for a long periods.

Although the infection in cattle herds has been controlled in most of the developed countries but bovine tuberculosis remains a serious problem for animal and human health in many developing countries. Cattle are considered to be the major reservoir *M. bovis*, and are the main source of infection for humans. Nevertheless, the disease has been reported in many other domesticated and non-domesticated animals.

Epidemiology of Zoonotic Tuberculosis and its associated Public Health Risk

Bovine tuberculosis is worldwide in distribution, but the highest prevalence of bovine tuberculosis is found in Africa and parts of Asia. Still some countries have never detected TB, and many developed countries have reduced or eliminated bovine TB. However, significant pockets of infection remain in wildlife. The disease is contagious and can be transmitted directly by contact with infected domestic and wild animals or indirectly by ingestion of contaminated material.



Fig-1: Showing routes of transmission by direct handling of infected carcass without mask, gloves and protective body covering.

Globalization and modernization have led to an increase in complexity of TB disease burden across the globe. Bovine tuberculosis infection is generally correlated with individual

cattle's age, sex, body condition and with husbandry practices such as herd size, production system and proximity to wildlife including bovine tuberculosis maintenance hosts. Therefore, it is mandatory to understand the ecology and transmission of the disease. The interdependence between human, animal and environment plays a significant role in transmission of the disease. In India, farmers, dairy workers and livestock keepers are in close contact with the animals in their routine life while feeding and milking. They also live in close proximity area, which may lead to transmission of *M.bovis* from cattle to humans and vice versa.



Fig-2: Showing the poor husbandry practices of some tuberculosis positive farms.

The usual route of infection within cattle herds is by inhalation of infected aerosol, which are expelled from the lungs (by coughing). Calves can be infected by ingesting colostrum or milk from infected cows. Humans can become infected by ingesting raw milk from infected cows, or through contact with infected tissues at abattoirs or butcheries. However zoonotic tuberculosis infection remains an occupational hazard for farmers, abattoir workers, and butchers. In contrast, *M. tuberculosis* infection was initially restricted to humans and the reports of its transmission in other animals, especially cattle, was regarded as accidental infection due to the close proximity to infected humans, often in regions where human tuberculosis is common. Various epidemiological studies have been carried out to determine genetic relatedness between the strains isolated from cattle and human. A new scenario of tuberculosis caused by *M. orygis*, a new subspecies of the MTBC in animals and human, have been reported around the world. The origin has been traced to South-East Asia including India, Pakistan, Bangladesh and Nepal. The course of disease is slow

and takes months or years to reach the fatal stage. Consequently, an infected animal can shed the bacteria within the herd before the appearance of clinical signs. Therefore, movement of undetected infected domestic animals is a major way of spreading the disease.

Current trends of Tuberculosis and Zoonotic Tuberculosis

TB is a disease which is basically seen among those people who often are faced by poverty, economic distress, marginalization and discrimination. According to the WHO report, 2020; globally, an estimated of 10.0 million people were infected with TB in 2019. Geographically, most of the cases reported from South-East Asia (44%), Africa (25%) and the Western Pacific (18%) and with a smaller percentages from Eastern Mediterranean (8.2%), the Americas (2.9%) and Europe (2.5%). Among these regions, three countries which acquitted the largest global burden of tuberculosis are India (27%), China (14%) and the Russian Federation (8%) respectively. Although globally, the TB incidence and death rate that have been declining very slowly in recent years which is not enough or it is less than halfway to reach the 2020 milestone of the WHO's End TB Strategy.

An estimated of 1,40,000 (range, 69,800–2,35,000) new cases of zoonotic TB occurred globally in 2019 and approximately 11,400 (8.1%) died. This estimate is derived from data on *Mycobacterium bovis*, the most common cause of zoonotic TB globally. The COVID-19 pandemic threatens to reverse recent progress in reducing the global burden of TB disease. Trends and a comparison of progress with the 2020 milestone of the End TB Strategy, most WHO regions and many high TB burden countries are not on track to reach the 2020 milestone.

Why Bovine TB is a matter of concern in India?

Livestock plays an important role in Indian economy and also provides employment to about 8.8% of the population in India. Livestock sector contributes 4.11% GDP and 25.6% of total Agriculture GDP. India is home for over 303 million cattle population (Statista Research Department, Oct 22, 2020) and has been leading to highest milk production (198.4 million tons) in the world with 22 percent of global production (Economic Survey, in 2019-20). So, the Livestock production and agriculture are practically interconnected and being dependent on each other in India. A study conducted in the country by FSSAI revealed that only 62.3% milk sold meets the standard, which is far below the global average. Even though India is the largest milk producer in the world, milk sold in the country is not the standard set up by Food Safety and

Standards Authority of India (FSSAI). The fact that most of the people consume the unpasteurized milk in India, may lead to increase in transmission and continued spreading of bovine tuberculosis.

A total of 21.8 million (7.19%) cattle are estimated to be infected with bovine tuberculosis in India. Bovine tuberculosis remains endemic in developing countries including India where cattle and human coexist creating a huge economic impact on agricultural industries and animal productivity threatening the livelihoods of farmers and ranchers. In some developing countries it is estimated to account for up to 10% of human tuberculosis cases occurred due to *M. bovis* which is naturally resistant to one of the antimicrobials that is commonly used to treat human tuberculosis, pyrazinamide. The fact that cattle are the carrier of the pathogenic mycobacteria which will be an extraordinary challenge to the End TB strategy especially in high TB-burden countries like India.

Prevention and Control of Zoonotic Tuberculosis

In developing countries like India where most of the dairy farms do not follow the minimal husbandry practices. However, the clinical sign and symptoms of bovine tuberculosis are not specifically distinctive and, therefore, it is very difficult to give confirmatory diagnosis in field condition for veterinarians. Now a days, different types of diagnostic tests have been developed such as single intradermal comparative tuberculin test (SICCT), which is the standard method of bovine tuberculosis diagnosis in live domestic animals, Gamma interferon release assay, which is most widely used blood-based in vitro test and the definitive diagnosis is confirmed by bacterial culture and molecular techniques.

There is utmost requirement of a strong multi-disciplinary interrelationship between medical and veterinary professionals to estimate and determine the scale of problem involved in disease transmission at human-animal-interface. So to reach the WHO's END TB Strategy, the World Organization of Animal Health (OIE), the World Health Organization (WHO), the Food and Agriculture Organization of the UN (FAO) and the International Union Against Tuberculosis and Lung Disease (The Union) jointly launched the first-ever roadmap to tackle zoonotic TB in October 2017. It is based on a One Health approach recognizing the interdependence of human and animal health sectors for addressing the major health and economic impacts of this disease.

The following steps can be taken to tackle the zoonotic tuberculosis-

1. Implementing the Test and slaughter policy in the farms which is followed in some developed countries, but in India it is not allowed due to some cultural and religious issues. So alternative method like test and segregation can be applied to control bovine tuberculosis.
2. Routine tracing of tuberculosis animals and slaughter house surveillance. Suspected animals should be kept separately from other healthy animals in the dairy farm until further confirmatory diagnosis.
3. Food safety practices like consumption of pasteurized milk and properly cooked meat will also reduce transmission at the animal-human interface. Pasteurization of milk should be made compulsory as this will be the most effective method in reducing the public health impact of bovine TB.
4. Bringing awareness and training programmes among the people about the zoonotic tuberculosis by public health professionals and veterinarians.
5. Timely sharing of data along with great coordination and correspondence between human health and animal health sectors will help in counteractive action and control.
6. Sanitary inspection of carcasses at abattoirs, animals at farm level and on husbandry practices should be done routinely.
7. Ante-mortem and post-mortem inspection of animals should be carried out by respective professional before entering the food chain.
8. As the human and animals are interconnected, so there should be control of the reservoir host both in animals and humans.
9. Proper surveillance programs in both human and animal should be carried out at the national and regional levels.
10. Vaccination and treatment: Generally vaccination and treatment are not recommended in cattle because of it is cost effective and not reliable. But it could be considered in wildlife reservoirs in an effort to prevent the spread of the disease to cattle.

Conclusion

Bovine tuberculosis still is an endemic neglected zoonotic disease in India. The public health importance of bovine tuberculosis in India has so far been scarcely investigated that is why it is being identified as a high burden country for Tuberculosis. So application of One Health approach implemented by government at national and regional level is the only way to control these types of zoonotic diseases where multi-sectoral departments can work together for creating policies and

legislation, planning and executing control measures, exploring epidemic breakouts and leading investigations.

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Available on request

Lactoferrin a Potential Therapeutic Candidate

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Abstract

Milk is an essential nutritional supply for all animals, including humans. It contains a variety of proteins, both major and small, including lactoferrin (Lf), a member of the transferrin family. Aside from milk, it may be found in most biological fluids. It is considered to be an important host defense molecule during neonatal development. It is anticancer, anti-inflammatory, and immunomodulatory, as well as antibacterial, and antiviral against a wide spectrum of infections. These diverse activities are enabled by modes of action that involve not just Lf's ability to bind iron, but also its interactions with molecular and cellular components of both hosts and pathogens. Demonstrating an array of therapeutic applications, it is having a promising clinical application both in human and veterinary diseases from ocular infections to osteoarticular, gastrointestinal and dermatological diseases. Hence lactoferrin can be considered a product of therapeutic and nutraceutical interests due to its well documented functional versatility.

Keywords: Lactoferrin, Therapeutics, Medicine

Introduction

The evolution of multidrug resistant microorganisms is a serious impediment to the treatment of infectious illnesses affecting humans and animals. Antibiotics, which have been the preferred choice for treating infections for over a century, are being replaced by newer antimicrobial agents such as metallic nanoparticles and antimicrobial peptides. Milk, which is commonly referred to be a complete food, has been shown to be a rich source of a variety of bioactive peptides. Milk is rich in nutrients such as proteins, endogenous peptides, lipids, carbs, vitamins, and minerals. Whey protein present in milk is made up of components such as -lactoglobulin, lactalbumin, immunoglobulins, lactoferrin, transferrin, and many other

small proteins and enzymes. In the search for novel therapeutic and immunomodulatory agents for future, lactoferrin (Lf), the multifunctional whey protein projects a wide range of possibilities. Lactoferrin, also known as lactotransferrin, stands next to caseins in terms of abundance in milk. It is a non-hemic iron binding protein, which was isolated for the first time by Sorensen and Sorensen in 1939 from cow milk. A glycoprotein by nature, lactoferrin having a molecular mass of about 80kDa, exists as a single polypeptide chain having nearly 690 amino acid residues in it (Groves, 1960 Montreuil *et al.*, 1960; Derechin and Johanson, 1962). Even though it is present in milk throughout the lactation, its concentration is found more in colostrum. Human colostrum is the richest source of lactoferrin followed by its bovine counterpart. Besides milk, the presence of lactoferrin is seen in most of the body secretions as well as secondary granules of neutrophils. Lactoferrin has shot to the utmost prominence because of the wide range of biological roles, including: antimicrobial activity against bacteria, viruses and parasites; antimicrobial and antioxidant properties which contribute to the tissue regeneration; immunomodulation through the bridging of innate and adaptive immune response and anticancer activity (Brock, 1980; Brock, 2002). This article will provide medical and veterinary professionals with a better understanding of using novel therapeutic candidates like lactoferrin, which will motivate more scientific investigation into this biological agent.

Biological functions of Lactoferrin

Role in Iron Metabolism

The transferrin family of non-heme iron binding glycoproteins is thought to play a key role in iron metabolism, with roles in iron transport, cellular iron delivery, and the regulation of free iron levels in external secretions. Lactoferrin is a member of this family that can be found in a variety of external fluids, including milk and mucosal secretions, as well as being a major component of neutrophil secondary granules. Lactoferrin is a multifunctional natural iron-binding protein with ability to scavenge free iron in biological fluids and ingrown regions in order to avoid free, easy-to-use, damage and reduce the involvement of metals to assault microbial and neoplastic cells. Lactoferrin appears to have a broader functional role, mediated by both iron dependent and iron independent pathways, despite its structural similarity to transferrin. The beneficial effect of Lf on iron acquisition in the gut is through a process that involves a receptor-mediated absorption of iron-bound Lf through intestinal epithelial cells. The influence of Lf on iron metabolism indirectly involves physiological functions such as DNA synthesis, RNA, proteins and immunoglobulins (Van-Snick *et al.*, 1974; Van-Vugt *et al.*, 1975).

Therapeutic properties

Antibacterial Activity

The affinity of Lf towards iron and its iron scavenging ability resulted in deprivation of iron availability for bacteria and hence formed the basis of antimicrobial property. When iron binding ability of Lf prevented growth of iron dependent bacteria like *Escherichia coli*, it enhanced the growth of beneficial bacteria like *Lactobacterium* spp. and *Bifidobacterium* spp., with lower iron demands. The efficacy of Lf against Gram positive and gram-negative bacteria is illustrated in the Fig 1. (Bruni *et al.*, 2016).

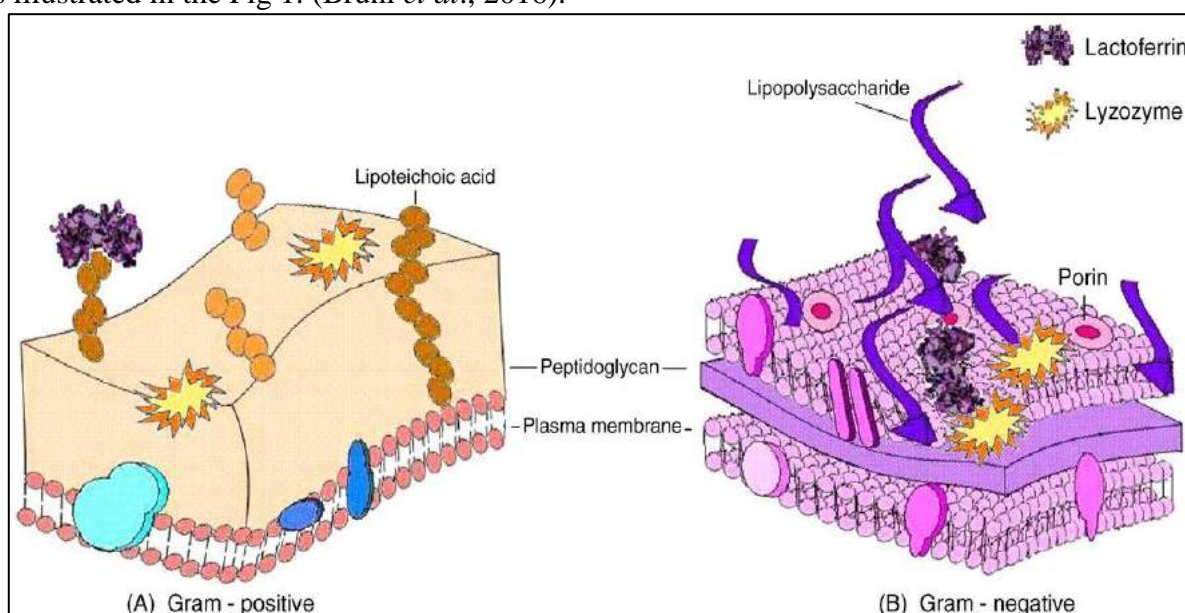


Fig 1: Mechanism of antibacterial action of lactoferrin

(A) Gram-positive bacteria: Lf is bound to negatively charged molecules of the cell membrane such as lipoteichoic acid, neutralizing wall charge and allowing the action of other antibacterial compounds such as lysozyme.

(B) Gram-negative bacteria: Lf can bind to lipid A of lipopolysaccharide, causing liberation of this lipid with consequent damage to the cell membrane.

Antiviral Activity

Lactoferrin has shown its effect by protecting the host via inhibition of a broad range of DNA and RNA viruses from binding to target cells and thus inhibited consequent intracellular replication, apart from modulating the systemic immune responses. Antiviral activity of Lactoferrin against human immunodeficiency virus, herpes simplex virus (types I and II) and cytomegalo virus has been reported by (Viani *et al.*, 1999). Mechanism of antiviral action of lactoferrin is illustrated in the Fig 2. Additionally, it has also been studied that lactoferrin causes stimulation of natural killer (NK) cells along with activation of T helper lymphocytes to release cytokines (Wakabayashi *et al.*, 2014) thereby acting as antiviral agent. Recently lactoferrin was

utilized as a therapeutic candidate for the treatment of Covid-19 based on its antiviral, anti-inflammatory and immune regulating properties (Wang *et al.*, 2020)

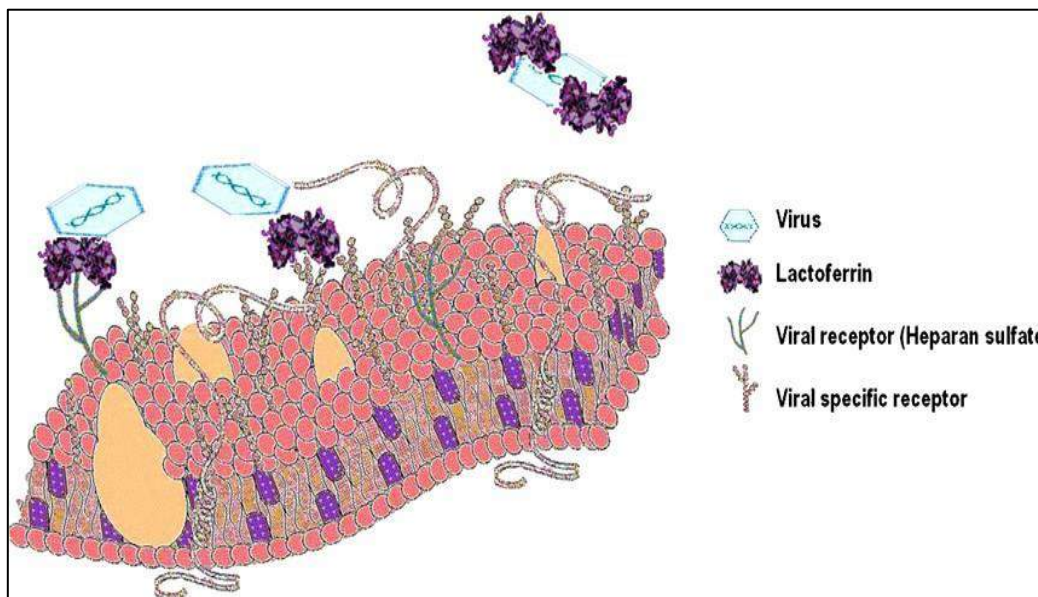


Fig 2: Mechanism of antiviral action of lactoferrin

Lf links to portion of viral receptor especially heparin sulphate that prevents the virus internalization into host cells.

Antifungal and antiparasitic Activity

The antifungal activity of lactoferrin has been recorded against *Candida* spp. which is attributed to its iron sequestering ability from the micro-environment. It has also been seen that lactoferrin possess potential antifungal action against body tineas such as *Trichophyton mentagrophytes* (Legrand *et al.*, 2004). Competition between parasite and lactoferrin with regard to sequestering environmental iron is the basis of the antiparasitic action of lactoferrin. Parasites like *Trichomonas foetus* and *Trichomonas vaginalis* possessed the ability to utilize lactoferrin as an iron (Fe^{3+}) donor. In order to cause alterations in host-parasite interactions, lactoferrin damages the parasite integrity. It has been reported that Lf and Lfcin increases T CD4+ lymphocytes for their antiparasite effect against parasites such as *Toxoplasma Gondii* (Legrand *et al.*, 2004).

Other biological activities

Antineoplastic activity of lactoferrin has been reported in cases of human mammary gland carcinoma (Legrand *et al.*, 2004), and also upon oral administration it was able to repress squamous cell carcinoma in neck and T- cell dependent tumor in head that is because of stimulation of NK cells as well as CD4+ and CD8+ cells. Activation of caspases producing

apoptosis induction, cell immunomodulation, cell cycle arrest, cell membrane modification, cell cycle arrest, anti-angiogenic action and metastasis inhibition are various action mechanisms responsible for anti-cancer activity of lactoferrin (Wolf *et al.*, 2003; Zhang *et al.*, 2004). The immunomodulatory activity of Lf has also been reported that is mediated by its DNA binding ability to activate various signaling pathways. The surface of various cells of immune system had negative charge that facilitates the binding of positively charged Lf molecule and triggering cell signaling pathways to cause cell proliferation, maturation and differentiation. Lactoferrin also enhances antigen presenting activity of B-cells and allows their interaction with the T cells to potentiate antibody mediated humoral immune response. Lactoferrin also modulates dendritic cell function to cause T- cell activation. Additionally, Lactoferrin modulates the immune response of gut associated lymphoid tissue (GALT) by triggering the intracellular signaling pathways to cause the activation, proliferation and differentiation of small intestine epithelial cells in a dose-dependent manner (Haversen *et al.*, 2002). In addition to this, anti-inflammatory activity of lactoferrin on its administration has been recorded by decreasing the expression of pro-inflammatory cytokines like TNF- α and monocyte chemotactic protein-1 (MCP-1) as well as markers for free radical induced oxidative damage (Legrand *et al.*, 2004).

Potential therapeutic applications and future perspectives of lactoferrin

Lactoferrin, in addition to its intrinsic iron-binding capacity, can be used as an organ-targeting ligand for drug administration and has a variety of positive benefits. Lf can be utilized as an adjuvant therapy for metabolic illnesses because of its antimicrobial, anticancer, antiviral, and immunomodulating properties. By regulating intestinal flora and avoiding diarrhoea, Lf may have a beneficial influence on illnesses of the gastrointestinal system. Lactoferrin is important in the restoration of glucose transport, especially under inflammatory conditions, due to its ability to absorb glucose from the intestine (Vogel, 2012). Some clinical investigations have shown that Lf lowers *Giardia lamblia* and reduces vomiting and diarrhoea. In children infected with rotavirus, clinical investigations have recently proved that Lf lowers *Giardia lamblia* colonization and reduces vomiting and diarrhoea. In immunocompromised patients, Lf has been shown to restore the humoral immune response. Because Lf suppresses early cervical maturation and lowers the generation of proinflammatory factors, it may aid in the prevention of preterm delivery in humans. Furthermore, Lf aids in the development of the brain and intellect. During pregnancy and neonatal stress, bovine Lf has been demonstrated to protect the brain from neuronal death and reduce inflammation (Wang *et al.*, 2017). A sufficient supply of bovine and

recombinant human Lf is currently available for the production of health-promoting nutraceuticals. Food preservation, fish aquaculture, infant milk formula, and dental hygiene are among the applications of Lf that are being exploited recently. Future applications of Lf could include clinical treatment of disorders affecting specific tissues and organs. Lf is also utilized in the pharmaceutical and food industries, as well as in the manufacture of feed additives. With the rapid advancement of transgenic technology, Lf may currently be created in cows, goats, and rice. Bovine Lf has been used as a health-promoting addition in commercial food items in Japan for decades, but the European Food Safety Authority recently certified it as an ingredient in food products. The processes behind Lf synthesis, regulation, and action need to be better understood as the consumption of milk grows, and this will aid comprehension of the critical roles of Lf in metabolism.

Conclusion

Lactoferrin has recently become the subject of significant investigation. Lactoferrin appears to have significant potential in practical medicine due to its unique antibacterial, antiviral, antiparasitic, immunomodulatory, and even antineoplastic characteristics. Nonetheless, much more study and numerous trials are required to have a better knowledge of its activity and interactions that will permit the full and safe use of it.

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Organic Aquaculture

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Introduction

Organic aquaculture is a holistic method for farming marine species in line with organic principles. The International Federation of organic Agriculture movement (IFOAM) defines organic aquaculture as a “system which promotes environmentally, socially and economically sound production of food and fibres and which dramatically reduces inputs by refraining from the use of chemo-synthetic fertilizers, pesticides and pharmaceutical. It refers to rearing aquatic organisms in a chemical free environment, which is totally based on ‘Organic Inputs’ and is not associated with any kind of health and ecological hazards. Organic aquaculture is the farming of aquatic animals like shrimps, fishes, bivalves etc. without using antibiotics, chemicals and fertilizers by preserving the ecosystem and biodiversity. Organic aquaculture also aims to provide fish and other products that are ecologically, economically and socially sound.

Organic aquaculture is based on the organic Agriculture farming technology, and these root causes continue to shape the organic aquaculture sector in many ways. Organic farmers in Australia and Germany first started to develop extensive “organic” carp production system in the early nineties. The first national general standards for organic aquaculture were established by France and the United Kingdom in 2000. The first global organic aquaculture criteria were established by the International Federation of Organic Agriculture Movements (IFOAM) in 2000. All over the world there are 240 number of certified organic aquaculture operations in 29 different countries in 2009, most of the operations are located in Europe.

Requirements for ‘Organic Aquaculture’

Utilization of chemical free manures- It is only possible if livestock is reared on pesticide/chemical free fodder without any antibiotics/medicine administration.

- Inorganic fertilizers - Not recommended
- Chemical free organic Seed – More than 95% of carp fish seed is being produced in hatcheries through induced breeding technology, where synthetic hormones are used to induce the fish to breed under captive conditions. It is not possible to meet the demand of seed by collecting seed from natural resources, which is associated with many other disadvantages. Hence, Induced breeding is required for producing quality seed of cultivable species in required quantities.
- Utilization of Organic supplementary feeds- supplementary feeds in aquaculture are generally formulated by using plant and animal based feed ingredients, e.g.

Use of plant based ingredients

- Rice bran, wheat bran, rice polish
- Mustard meal, soybean meal, groundnut meal
- Maize gluten, brewery waste, molasses

Use of animal based ingredients

- Fish meal
- Meat meal
- Bone meal

Availability of chemical free feed ingredients is again a matter of great concern. It is only possible if the agricultural crops are reared without pesticides and fertilizers. Fishmeal and bone/meat meal has to be manufactured from chemical and antibiotic free stock. Fish meal manufactured from stock captured from polluted water resources also does not fall in the concept of 'Organic Aquaculture'.

Principles

- Monitoring of environmental impact
- Natural breeding procedures without use of hormones and antibiotics.
- No use of inorganic fertilizers
- Feed and fertilizers from certified organic aquaculture and fisheries.
- No use of GMO's (Genetically modified organisms)
- Preference for natural medicines

Required labelling

Organic products could be labelled as

- “100 percent organic”, “organic”, or “made with organic ingredients”.
- Products labelled “100 percent organic” must contain (excluding water and salt) only organically produced ingredients.
- Products labelled “organic” must contain at least 95% organically produced ingredients.

- Products labelled “made with organic ingredients” must contain at least 70% organically produced ingredients.
- Products with less than 70% organically produced ingredients cannot use the term organic everywhere on the principle panel but can identify specific ingredients in the information panel.

Certification

- Certification is a procedure for verifying that a product or process conform to certain standards
- The certification can be made by first party, second party and third party.
- The certification process consists of three steps:
 - The standard setting
 - The accreditation
 - The certification
- Accreditation is a procedure whereby an authorised body gives formal recognition that an organisation is authorised to carry out the certification.

Why go to organic production

- Safe the soil, water and nature
- Value added for export products
- Avoid to sink our lands
- Improve health for both producers and consumers
- Help property reduction in remote areas.

Conclusion

Many organic aquaculture issues still need to be resolved, steps should be taken to encourage and enhance the biological cycles with respect to nutrients management and to retain the integrity of the organic products from farmer to consumer and conversion requirement for moving conventional aquaculture system into organic system. With continue emphasis on worldwide aquaculture will emerge as the most environmental friendly and efficient form of agriculture and as a partner in sustainable development.

Role of Enzymes in Livestock and Poultry Feed: Review

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Abstract

The animal's digestive system is not fully efficient. Swine and poultry cannot digest approximately one fourth of the diet they are fed because the feed ingredients contain undegradable harmful factors that hinder the digestive process and/or the animal is devoid of the necessary enzymes needed to degrade certain complexes in the feed. Supplementation of feed with enzymes enhances its nutritive value, thereby increasing the effectiveness of digestion. Animal feed enzymes help break down indiscriminating factors (e.g., fiber, phytate) that are naturally occurring in various feed ingredients. Exogenous enzymes are mainly added to enhance the accessibility of nutrients from feed ingredients. The animal feed industry uses enzymes that degrade crude fiber, starch, proteins, and phytates, and being proteins, they are eventually digested or excreted by the animal, having no residual effect on products like meat or egg.

Introduction

Enzymes that are produced within the animal are referred to as endogenous and enzymes that are added to animal feed are referred to as exogenous. Exogenous enzymes created for use as animal feed additives are produced by microbial fermentation of substances found in plants, animals or microorganisms. As a result, exogenous enzymes have low toxicity, making them safe for animals and people and less harmful to the environment than chemically derived feed additives.

While thousands of enzymes have been identified and several hundred are available commercially, only a fraction of these are produced on an industrial scale. Since the early 1980's, scientists have been using biotechnology to increase the production efficiency, quantity and quality of enzymes.

The intensive and rapid growth on world population are caused on increased demands of animal origin. Meat and meat products consumption (including beef, pork, goat, mutton and

poultry) has increased gradually, by almost 60% between 1990 and 2009; from 175,665 thousand to 278,863 thousand tons, driven in part by a growing world population (Henchion *et al.*, 2014). Feed cost is the largest cost (60-70%) in livestock and poultry production systems. To minimize this cost, many producers supplement feed with certain enzyme additives, which enable them to produce more meat per animal or to produce the same amount of meat in cheaper and faster way (Vohra and Satyanarayana, 2002).

The use of dietary exogenous enzymes on livestock production could be a promising strategy for improve animal digestibility and feed efficiency. The purpose of this review article summarizes role of exogenous enzymes and their use on different livestock species and poultry.

Reason for using enzymes in animal feed:

The ultimate aim of adding enzymes is to improve bird performance and profitability through enhanced digestion of dietary components (protein, amino acids, starch, lipids, and energy) in ingredients (Sheppy, 2001).

- To break down anti-nutritional factors that is present in many feed ingredients. These substances, many of which are not susceptible to digestion by the animal's endogenous enzymes, can interfere with normal digestion, causing poor performance and digestive upsets.
- To increase the availability of starches, proteins and minerals that are either enclosed within fibre-rich cell walls and therefore not as accessible to the animal's own digestive enzymes, or bound up in a chemical form that the animal is unable to digest (eg: phosphorus as phytic acid).
- To break down specific chemical bonds in raw materials that are not usually broken down by the animal's own enzymes, thus releasing more nutrients.
- To supplement the enzymes produced by young animals, because of the immaturity of their own digestive system, endogenous enzyme production may be inadequate. This will be especially true for newly hatched chicks with immature digestive systems.
- Shift of digestion to more efficient digestion sites.
- Reductions in endogenous secretions and protein losses from the gut resulting in reduced maintenance requirements (Cowieson and Ravindran, 2007; Cowieson *et al.*, 2009).
- Reduction in the weight of the intestinal tract and changes in the intestinal morphology (Jaroni *et al.*, 1999; Wu *et al.*, 2004).
- Changes in the microflora profile in the small intestine. As enzymes influence the amounts and form of substrate present within the gut, their use has a direct effect on the bacteria that make up the microfloral populations (Bedford and Cowieson, 2012; Apajalahti *et al.*, 2004; Vahjen *et al.*, 1998).

Types of enzymes used in livestock and poultry feed:

Four types of enzymes currently used in livestock and poultry feed. They are enzymes to break down fibre, protein, starch and phytic acid.

Fibre-degrading enzymes

Monogastrics (pigs and poultry) do not produce the enzymes to digest fibre. In feed containing ingredients such as wheat, barley, rye or triticale (viscous cereals), a large proportion of this fibre is soluble and insoluble arabinoxylan and β -glucan (White et al., 1983; Bedford and Classen, 1992). The soluble fibre can increase the viscosity of the contents of the small intestine, impeding the digestion of nutrients and thereby reducing the growth of the animal. It has also been linked with the incidence of digestive disorders such as non-specific colitis in swine, sticky litter and hock burns in poultry.

The fibre content of wheat and barley can vary considerably according to variety, growing location, climatic conditions etc. Hence there can be considerable variation in the nutritional value of these ingredients and feed containing them. In breaking down the fibre, enzymes (eg: xylanase targeting arabinoxylans, β -glucanase targeting β -glucans) can reduce this variability in nutritional value, giving rise to improvements in the performance of the feed and the consistency of the response. An additional benefit is the reduced incidence of certain digestive disorders.

Protein-degrading enzymes

Various protein rich raw materials contribute to the protein content in the feed and ultimately the amino acids that fuel lean meat deposition. There is considerable variation in the quality and availability of protein from the different raw materials found in monogastric diets. The primary vegetable protein sources such as soybean meal, certain anti-nutritional factors (ANFs) such as lectins and trypsin inhibitors can lead to damage to the absorptive surface of the gut, impairing nutrient digestion. In addition, the underdeveloped digestive system of young animals may not be able to make optimal use of the large storage proteins found in the soybean meal (glycinin and β -conglycinin).

The addition of a protease can help to neutralize the negative effects of the proteinaceous ANFs in addition to breaking down the large storage protein molecules into smaller, absorbable fractions.

Starch-degrading enzymes

Maize is viewed as the 'gold standard' of raw materials. But recent research suggests that at the ileal level, starch digestibility rarely exceeds 85% in broilers between 4 and 21 days of age (Noy and Skylan, 1994). The addition of an amylase to animal feed can help to expose the starch more

rapidly to digestion in the small intestine, and improved growth rates from enhanced nutrient uptake.

At weaning, piglets often suffer a growth check because of changes in their nutrition, environment and immune status. The addition of an amylase, usually in conjunction with other enzymes, to augment the animal's endogenous enzyme production has been shown to improve nutrient digestibility and absorption, hence growth rate (Close, 1995).

Phytic acid-degrading enzymes

Phosphorus is required for bone mineralization, immunity, fertility and growth and is an essential mineral for all animals. Swine and poultry digest only about 30 to 40% of the phosphorus found in feedstuffs of vegetable origin, with the remainder being tied up in a form inaccessible to animal – phytic acid. Hence, additional phosphorus must be added to the feed to meet the animal's requirements. More than half of the phosphorus consumed from such feedstuffs is excreted in the faeces, which can result in major environmental pollution. By adding a phytase to the feed, the phytic acid is broken down, liberating more of the phosphorus for use by the animal.

The two main benefits of phytase supplementation are, firstly, the reduction in feed costs from the reduced additional supplementation of phosphorus to the feed and secondly, the environmental from reduced excretion of waste products and the threat of pollution.

Conclusion

The constant population increase and the continued need for feed of animal have originated rise in demand for livestock products and continuous research on the use of additives for growth. The addition of exogenous enzymes is a useful tool to improve feed utilization, animal performance, immunity through improve gut health and economic efficiency and the mode of action may include reduce gut viscosity of viscous grain, digestibility of nutrients, utilization of nutrients and overcome the anti-nutritional substances. Nowadays, consumer and producers increase knowledge concern to the use of secure growth promoters and antibiotics in livestock production attend these reasons; many researchers have been investigated the use of exogenous enzymes in ruminant and non-ruminant animal production.

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