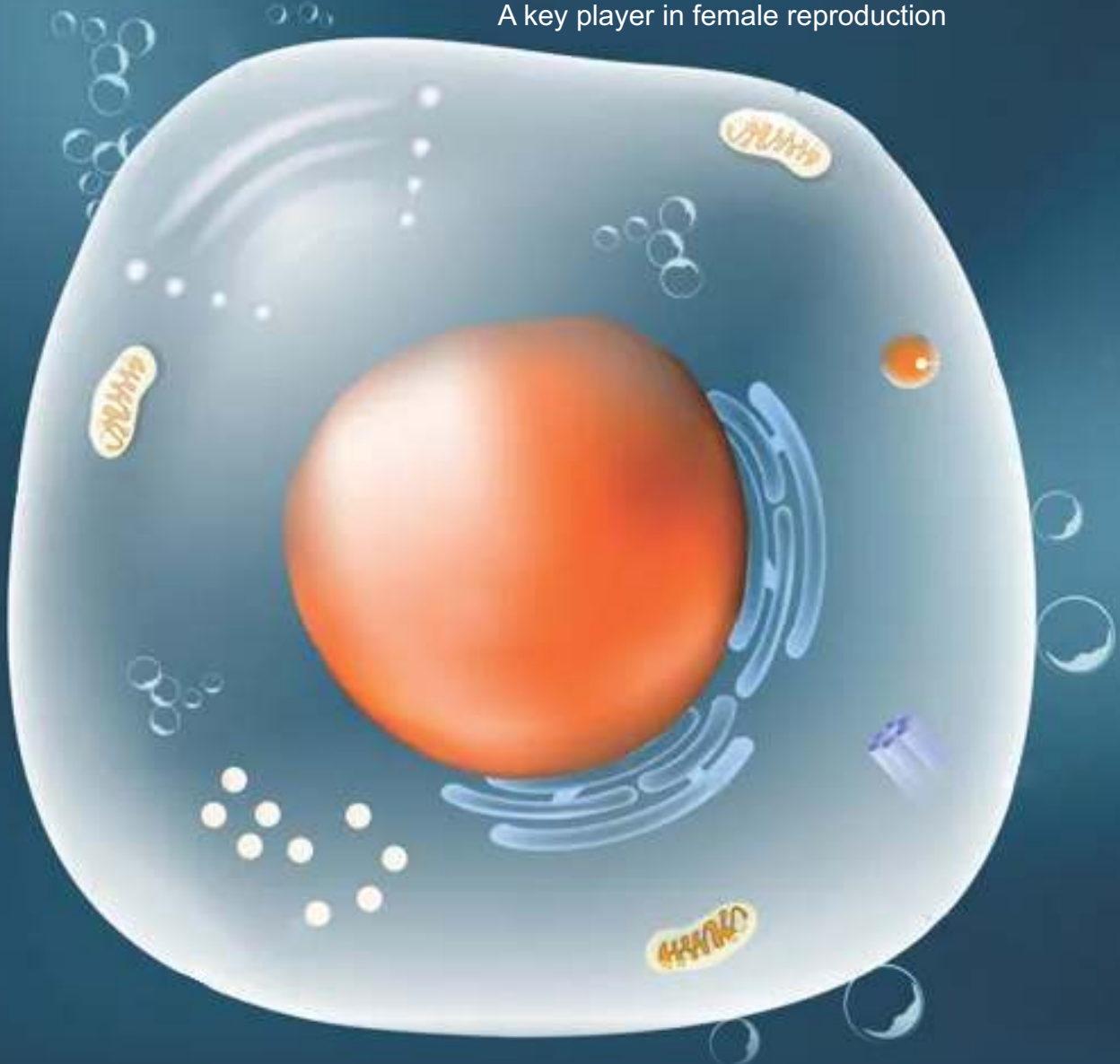


# RAKSHA TECHNICAL REVIEW

Publication of Indian Immunologicals Limited

February 2020  
Free Private Circulation

**Gonadotropin Inhibitory Hormone (GnIH)**  
A key player in female reproduction



**Scrub Typhus**  
A Reemerging Zoonotic threat in India

**Congenital Disorders**  
in Dogs



INDIAN IMMUNOLOGICALS LIMITED

# Reader's Desk



Excellent booklet which keeps the field veterinarians update with rest of the world of research, field experiences. Good initiative by IIL.

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## From the Editor's desk

Fertility is the natural capability to produce offspring. Animal reproduction remains a challenge not only in developing countries, but also in developed nations. Low fertility results in low production rate, increase in financial input, and reduced efficiency of livestock operations. GnIH, Gonadotropin Inhibitory Hormone regulates release of gonadotropins. The function of GnIH, has been intricately described by the author in the article *Gonadotropin Inhibitory Hormone (GnIH): A key player in female reproduction?*

The article *New Insights into the Diagnostic and Treatment Approaches for Subclinical Endometritis in Cattle* is interesting as the cases are common among high yielding dairy cattle. The article, *Surgical Affections of Udder and Teats in Large Ruminants*, describes various types of surgical methods to treat the animals.

*Zoonotic Tuberculosis* is an infectious and contagious disease of zoonotic importance. The article focuses on the different methods of diagnosis and treatment protocols. *Tyzzer's disease: A review*, gives details on disease prevalence, incidence, and its prevention and control measures in laboratory animals. *Laminitis in Horses- A Case Study*, studies the prevalence, diagnosis, treatment as well the successful recovery of the animal.

Under Grazers and Browsers section, *Kendrapara Sheep Breed's* origin, characteristics, performance etc., are briefed.

In this issue, we are covering Kankrej Cattle: The pride of Gujarat, describing its strength and utility as a dual purpose breed under Indigenous breed section.

In Companion Animal Section, the article *Congenital Disorders in Dogs* refers to the breeds that are at risk and its management and *Seizures in Dogs and Cats*, refers to its treatment as well as management are very attention-grabbing for canine fraternity.

In General Articles, *A Review of Bat Borne Zoonoses and Its Significance in Public Health; Biosecurity at Egg Processing Plant – Initiatives, Measures and Procedures; How Milk and Urine of Indigenous Cow Breeds are Useful and Failure of Transfer of Colostral Immunoglobulins* give a thoughtful insight on several issues on public health aspects.

The article *Scrub typhus - A Reemerging Zoonotic Threat in India* reviews various symptoms and signs in the affected host as well the preventive measures to control. *Tips for Summer in Pet Management*, illustrates the way how to take care of pets from heat.

This edition of RTR is covering a wider range of interesting articles. Based on your feedback, we wish to expand the horizon of RTR beyond the existing range. Please forward your feedback to [rtr@indimmune.com](mailto:rtr@indimmune.com).

Regards,

**Dr Prasanna A Deshpande**



## Managing Director's Message

Dear Patrons,

Greetings!

I'm glad to share with you all that the winning streak of IIL is now reaching new heights in FY2019-20. IIL is expected to grow by more than 50% growth compared to FY2018-19.

Novel Corona virus related epidemic is taking a toll!! World-wide nations are sounding alert and preparedness to counter the spread of COVID-19 related diseases. Several national and international scientific bodies are pooling their resources to find out the exact origin, cause and treatment methods for such highly contagious zoonotic diseases.

An important element in the on-going episode of COVID-19 is the indifference of human-kind to the sustenance of ecological balance. In our view, indiscriminate use of natural resources and infiltration into the habitats of zoonotic life could be a reason for spreading such diseases of catastrophic nature.

IIL wishes to foster the "One Health Initiative" and look forward to your continued support in taking this cause ahead. Wish your patronage helps RTR and IIL in "Shaping Global Healthcare by spearheading the One Health Initiative"

Warm Regards

**Dr K Anand Kumar**

## Gonadotropin Inhibitory Hormone (GnIH) : A key player in female reproduction?

Nilendu Paul, Pradeep Nag B S and Ankur Sharma

ICAR-NDRI, Karnal, Haryana and Theriogenology Laboratory, SRS of ICAR- NDRI, Adugodi, Bengaluru, Karnataka.

### Introduction

Ever since the discovery of gonadotropin releasing hormone (GnRH) in mammals during early 1970's, it was believed to be the only neuropeptide hormone regulating the release of gonadotropins from the anterior pituitary gland. But the year 2000 witnessed a major breakthrough in the field of neuro-endocrinology when a hypothalamic neuropeptide (RFamide) was discovered and found to inhibit the hypothalamic gonadotropin secretion in quail [1]. Based on its inhibitory nature in gonadotropin release, the neuropeptide was named as gonadotropin inhibitory hormone (GnIH).

This was the first instance where any hypothalamic neuropeptides was evidenced to actively inhibit the release of anterior pituitary gonadotropin. Subsequently, the orthologues of GnIH were also been found in other vertebrates including chicken, sparrow and starling, bovine, Siberian hamster, rat, macaque and human [2,3,4,5,6,7]. **In mammals, GnIH orthologues are RFRP-1, RFRP-2 and RFRP-3 out of which RFRP-3 is functionally similar to the avian GnIH by inhibiting LH release in mammals. Interestingly, GnIH has remained highly conserved among vertebrates, from teleost fish to humans.**



(SIKPSAYLPLRFamide) with RFamide (Arg-Phe-NH<sub>2</sub>) at C- terminal.

**Classification**

RFamide peptides in vertebrates include GnIH group, the neuropeptide FF (NPFF) group, the prolactin-releasing peptide (PrRP) group, the kisspeptin (kiss1 and kiss2) group, and the pyroglutamylated RFamide peptide/26RFamide peptide (QRFP/26RFa) group.

The GnIH is a dodecapeptide structure (12 amino acid) Ser-Ile-Lys-Pro-Ser-Ala-Tyr-Leu-Pro-Leu-Arg-Phe-NH<sub>2</sub>

**Distribution**

In birds, cell bodies and terminals of GnIH neurons are located in the paraventricular nucleus (PVN) and median eminence (ME), respectively [1]. By using a polyclonal antibody developed against avian GnIH, immunoreactive cells were detected in the diencephalon, pons and medulla of the mouse brain with major abundance in the dorsomedial hypothalamic nucleus whereas, its localization was also observed in the posterior hypothalamus between the ventromedial nucleus and the dorsomedial hypothalamic nucleus [8]. GnIH/RFRP immunoreactive cells have been found to be concentrated in the dorsomedial nucleus of the hypothalamus in hamsters & rats [9]. In ovine brain, in situ hybridization revealed primary localization of GnIH cells in the ventral region of the paraventricular nucleus and the dorsomedial nucleus [10]. The primary localization of GnIH in the dorsomedial nucleus in numerous species might provide more insight towards the potential role of this neuropeptide in the regulation of energy balance.

**GnIH receptors**

In birds, GnIH acts via GnIH receptors known as GPR147, a member of the G-protein-coupled receptor (GPCR) superfamily which is also called NPFF receptor 1 (NPFF1) [11]. In mammals, a specific receptor for GnIH (RFRP) was identified which was identical to GPR147 and known as OT7T022. The receptor has now been identified in many mammalian species including the rat, sheep, and human [12]. GnIH receptor (GnIH-R) is expressed at a high level in the hypothalamus, with moderate levels of expression in the thalamus, mid-brain, medulla, eye and testis. In the sheep brain, predominant expression of GnIH-R is found in the suprachiasmatic, supraoptic nucleus and periventricular nuclei, and the pars tuberalis. Mouse GnIH after binding to its receptor GnIH-R, reduces GnRH-stimulated gonadotropin secretion by specifically interfering with GnRH actions via a AC/cAMP/PKA-dependent ERK pathway [13]. As GnIH receptor is expressed in gonadotropes in the anterior pituitary, GnIH can directly act on pituitary gonadotropes to reduce gonadotropin release in

birds [2,6,14]. GPR147 couples to Gai protein that inhibits cAMP production in the cell [15].



**Mode of action**

GnIH plays an important role in both inhibition and release of GnRH secretion. It inhibits GnRH-induced gonadotropin subunit, LHβ, FSHβ, and common α gene transcriptions. The inhibitors of adenylate cyclase (AC)/cAMP/protein kinase A (PKA) pathway, inhibitor of AC and inhibitor of PKA, effectively inhibited GnRH-stimulated gonadotropin expressions. On the other hand, the inhibitor of protein kinase C (PKC) did not inhibit GnRH stimulated gonadotropin expressions. Accordingly, mouse GnIH may inhibit GnRH induced

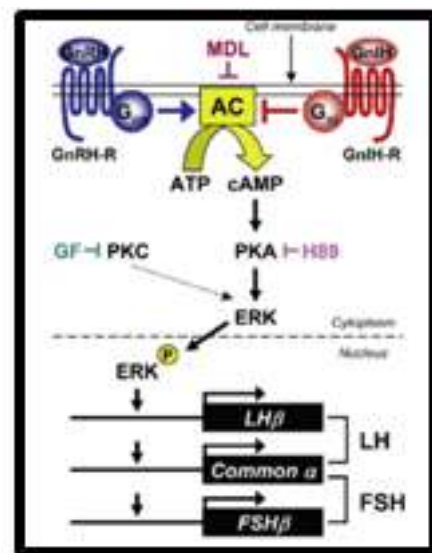


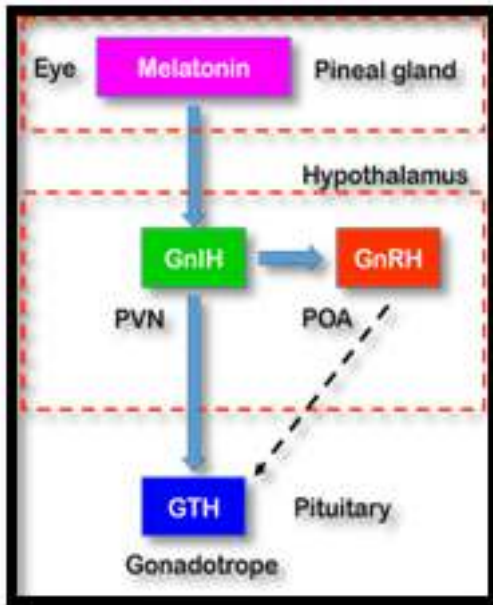
Image source- Ubuka et al. [16]

gonadotropin subunit gene transcriptions by inhibiting AC/cAMP/PKA dependent ERK activation in LβT2 cells. Thus GnIH displays two modes of action- (a) GnIH inhibits GnRH-induced LH release (in vitro) and (b) Inhibits synthesis of LH-β and FSH-β subunits within the pituitary gland of quail and chickens (both in vivo and in vitro).

**Effect of melatonin on GnIH expression and release**

Terminals of GnIH neurons are localized not only in the median eminence but also GnRH neurons in the preoptic area (POA). GnIH receptor (GnIH-R) is expressed in gonadotropes in the pituitary and GnRH neurons in the POA. Thus, GnIH acts directly on gonadotropes in the pituitary via GnIH-R to inhibit gonadotropin (GTH) release and synthesis. GnIH also acts on GnRH neurons in the POA to

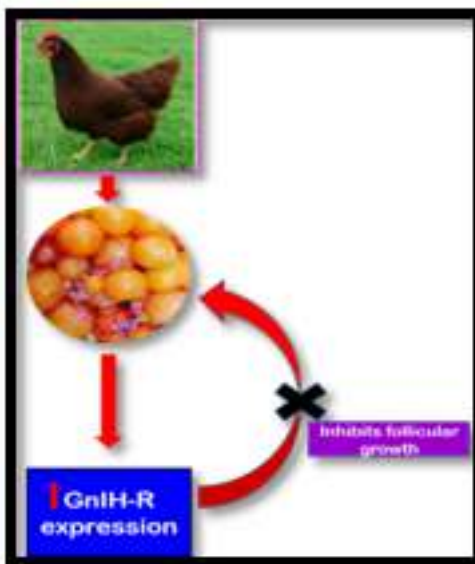
inhibit GnRH release. Melatonin originating from the pineal gland and eyes induces GnIH expression and release in GnIH neurons. Melatonin receptor is expressed in GnIH neurons. Thus, melatonin acts directly on GnIH neurons via melatonin receptor to induce GnIH expression and release.



The proposed mechanisms of GnIH actions on gonadotropin synthesis and release and melatonin action on GnIH expression and release

**Effect of GnIH and GnIH-R in gonads**

Maddineni et al., [17] have characterized the expression of GnIH-R mRNA in the chicken ovary which revealed that GnIHR may exert an inhibitory



effect on ovarian follicular development. GnIH-R mRNA expression was found in the chicken testis and in the ovary in the cal and granulosa cell layers.

The expression pattern of GnIH-R also varies in chickens which are in different growth phase. GnIH-R mRNA quantity was significantly decreased in sexually mature chicken ovaries versus ovaries of sexually immature chickens.

A decrease in ovarian GnIH-R mRNA due to sexual maturation or by estradiol and / or progesterone treatment might implicate an inhibitory role for GnIH-R in ovarian follicular development.

**GnIH action in brain and pituitary to regulate reproduction and reproductive behaviors**

GnIH (RFRP) neurons in the brain project their axons to GnRH-I neurons as well as to the median eminence (ME). GnIH receptor (GnIH-R; GPR147) is expressed on GnRH-I neurons as well as on gonadotropes. GnIH may thus inhibit gonadotropin synthesis and release by inhibiting the activity of GnRH-I neurons as well as directly inhibiting the pituitary gonadotrope. GnIH (RFRP) neurons may also regulate GnRH-I neurons by regulating the activity of kisspeptin (Kiss) neurons that project to GnRH-I neurons. There are also reports showing that GnIH (RFRP) neurons project their axons to GnRH-II, dopamine, pro-opiomelanocortin (POMC), neuropeptide Y, orexin, melanin-concentrating hormone (MCH), corticotrophin-releasing hormone (CRH) and oxytocin neurons in the brain. GnIH (RFRP) inhibits reproductive behaviors of birds and mammals by possibly acting within the brain. The expression of GnIH (RFRP) is regulated by melatonin, stress, and estradiol-17β

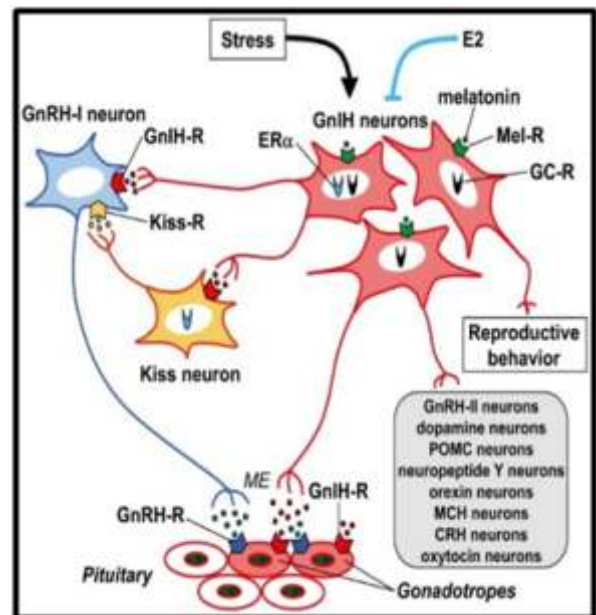


Image source- Ubuka et al., [18].

(E2). Expressions of melatonin receptor (Mel-R), glucocorticoid receptor (GC-R), or estrogen receptor  $\alpha$  (ER $\alpha$ ) in GnIH (RFRP) neurons were shown in several species. These mechanisms of action of GnIH (RFRP) on gonadotropin secretion or regulatory mechanism of GnIH (RFRP) expression may vary between species, sexes, and developmental stages.

**Changes in GnIH expression from birth to adult stage**

Study in mice showed that RFRP-3 fibers are nearly absent before or at birth. By 2<sup>nd</sup> - 3<sup>rd</sup> week post natal period, RFRP3 neuron increases significantly in posterior hypothalamic neuron (PHN), paraventricular nucleus (PVN), lateral hypothalamic area(LHA) and in Arcuate nucleus. RFRP-3 expression level of newborn as well as adult mice by in situ hybridization revealed that RFRP cell number as well as RFRP quantity per cell also significantly differs between two stages. At neonatal stage there were numerous RFRP neurons detected, but in adulthood, the number of detectable neurons expressing RFRP was found to be less. However, the relative amount of RFRP mRNA per neuron was higher in adults. This dramatic change in RFRP mRNA levels per cell demonstrated that while RFRP cell number is decreasing during postnatal development, each RFRP cell is producing, on an average, more RFRP mRNA as the animal ages [19].

**GnIH studies in livestock**

**In sheep**

Out of all domestic livestock species, sheep have been taken as a model to study RFRP-3/GnIH expression in brain as well as its relationship with photoperiodicity. RFRP expression in the ependymal cells and *pars tuberalis* (PT) was highly

photoperiodic, with levels being negligible in animals held on short photoperiod but consistently high under long photoperiod. Therefore, it was suggested that ependymal cells, as well as PT interplay, might be important in seasonal timing system[20].

The expression pattern of kisspeptin and GnIH neurons also varies from nonbreeding to breeding season. It has been identified that the number of kisspeptin terminal contacts to GnRH neurons is higher during the breeding season. Also, the terminal contacts from RFRP cells to GnRH neurons is higher during the nonbreeding season. These kisspeptin and GnIH expression might imply their important role in seasonal breeding regulation in sheep[10]. Apart from the effect on seasonal timing and breeding regulation in sheep, RFRP-3 is an inhibitor of gonadotropin subunit gene expression in ovine pituitary gonadotropes, strongly suggesting that the peptide reduces synthesis of the gonadotropins as well as secretion. Therefore RFRP-3 might act as a hypophysiotropic peptide[21]. Also, RFRP-3 neurons project to hypothalamic regions and cells involved in the regulation of energy balance and reproduction in the ovine brain [22].

**In cow**

Work done in cattle for RFRP expression and its effect on gonadotropin secretion is meager. But it has been shown that bovine C-terminal octapeptide of RFRP-3 (RFRP-3-8) suppressed LH secretion from cultured anterior pituitary cells, as well as LH pulse frequency in castrated male calves [23].

**In pig**

Study in pig demonstrated that GnIH immune reactive (GnIH-ir) neurons were widely distributed in the pig CNS, but the number and size of the

**GnIH in different species with their physiological functions**

Animal	Name	Physiological function	Refs
Human	RFRP-1	Stimulation of prolactin release (i.c.v.)	[15]
Bovine	RFRP-3	Inhibition of GnRH-elicited gonadotropin release (in vitro)	[23]
Ovine	RFRP-3	Inhibition of GnRH-elicited gonadotropin release (in vitro) Inhibition of gonadotropin release (i.v.)	[26]
Quail	GnIH	Inhibition of gonadotropin release (in vitro) Inhibition of gonadotropin synthesis and release (i.p.)	[1] [19]
Chicken	GnIH GnIH-RP- 1 & GnIH-RP-2	Inhibition of gonadotropin synthesis and release (in vitro) Stimulation of feeding behavior (i.c.v.)	[27] [28]

GnIHir neurons varied and exhibited diverse morphology. In the peripheral organs, GnIH immunoreactive cells were observed in the respiratory tract, alimentary tract, endocrine organs, genitourinary tract and lymphatic organs. GnIH mRNA was highly expressed in the CNS, with the highest expression in the hypothalamus. In the peripheral organs, high GnIH mRNA levels were detected in the testis, while no GnIH expression was observed in the liver, lungs and heart. These results demonstrated that GnIH might act as a multifunctional neuropeptide involved in many physiological actions such as reproduction, feeding, stress and behaviours [24]. Studies also identified the specific GnIH receptor GPR147 in pigs and role of GnIH/GPR147 in the regulation of gonadotropin secretion at the level of the brain and ovary [25].

### Conclusion

- GnIH are dodecapeptide structures with RFamide C terminal
- GnIH acts through G-protein coupled receptor which sends an inhibitory signal to GnRH neurons
- GnIH acts on the pituitary and within the brain and modulates the reproductive axis as well as reproductive behaviors
- GnIH controls feeding behavior in vertebrates, such as birds and mammals

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## New Insights into the Diagnostic and Treatment Approaches for Subclinical Endometritis in Cattle

Nilendu Paul, Sunil Kumar Mohapatra and Abhishek Paul  
Theriogenology Laboratory, ICAR- NDRI, Adugod, Bengaluru, Karnataka.

### Introduction

Parturition and post-partum period is a matter of great concern to the dam as well as for the offspring. There is a high risk of getting uterine infection during the late gestation, parturition and early post-partum period. Microbial contamination during parturition is very common in dairy cattle, which has profound detrimental effect on general as well as reproductive health with a negative impact on production. Most of the animals (80-100%) have bacterial infection in their uterus within first two weeks after calving. Although host innate immune responses progressively eliminate the microbes, up to 40% of animals still have a bacterial infection three weeks after calving (1). Uterine involution, uterine immunity, load of microorganisms and its pathogenicity decide the fate of post-partum uterus. If the uterus does not return to its normal non-pregnant size at proposed period of time and the cleanliness of the calving pan is also not maintained satisfactorily, then there is likelihood to develop various post-partum diseases like puerperal metritis, clinical metritis, clinical endometritis, subclinical endometritis and pyometra (2). Out of these above mentioned diseases, subclinical endometritis is a potential threat as it remains undiagnosed by naked eyes of even a skilled veterinarian. The term subclinical endometritis

is defined as an inflammation of the inner lining of uterus (Endometrium) without clinical signs of endometritis seen externally such as vaginal discharge (2) causing decreased reproductive performance, increased feed intake per lactation, increased number of services per conception and increased culling rate (1).

### Post-partum Period in Cow

Post-partum period is the time period for complete uterine involution or the interval between parturition and completion of uterine involution (3). Under normal condition, the post-partum uterus reduces back to its normal size within a time period of 3 weeks whereas functional resumption requires 6 weeks. The degree of contraction of post-partum uterus is more during first few days after delivery along with clear lochial discharge which is necessary to cleanse the uterus. PGF<sub>2</sub> $\alpha$  is likely to be the most important contributor of uterine size reduction during immediate post-partum period.

### Causes of Subclinical Endometritis

Endometritis and subclinical endometritis are caused by a number of microorganisms, including virus, bacteria and fungus. The microorganisms commonly

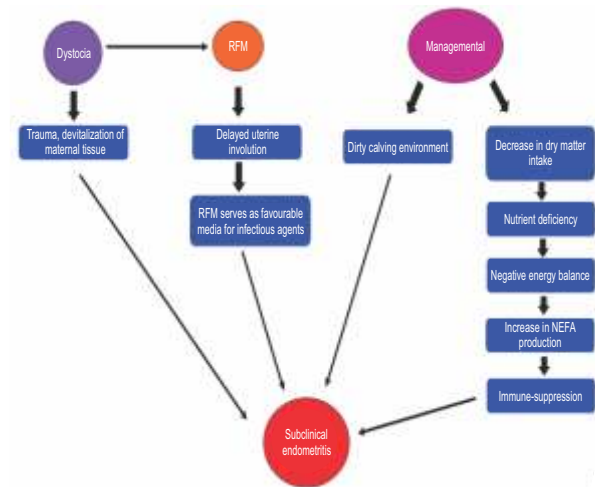
enter the vagina during the mating process, calving process or during forceful traction to relieve dystocia. Thus the infection sets in inside uterus. Uterine defense mechanism plays an important role in preventing disease process. Establishment of infectious agent and onset of disease process takes place when load of micro-organisms and their pathogenicity overwhelms uterine defense mechanism (4). Although endometritis and subclinical endometritis

are non-life-threatening condition but it severely impairs the future reproductive performance of the affected animals and also disturbs the embryo survival after successful fertilization incurring a significant economic loss to the animal owner or to the dairy industries. The most common infectious agents, which cause endometritis and subclinical endometritis, have been listed in the table below.

**GnIH in different species with their physiological functions**

Causative Organism	Type of Infectious Agent	Effects
<i>Campylobacter fetus</i>	Gram –ve bacteria	Abortion, mild endometritis, muco-purulent exudate accumulation inside uterine lumen
<i>Escherichia coli (E. coli)</i>	Gram –ve bacteria	Opportunistic infection, abortion, mild endometritis, negative effect on reproductive health, reduces fertility potential
<i>Fusobacterium necrophorum</i>	Gram –ve bacteria (obligate anaerobe)	Mild endometritis, abortion, secretes toxic leukotoxin protein which modulates host immune function
<i>Arcanobacterpyogenes</i>	Gram +ve bacteria	Endometritis facilitates growth of <i>F. necrophorum</i>
<i>Bovine Herpes Virus -4 (BOHV-4)</i>	ds DNA virus	Endometritis, vulvovaginitis, and associated abortion

**Table 1: Primary Causative Agents of Endometritis in Cows**



**Fig. 1 Predisposing Factors of Subclinical Endometritis**

**Effect of Subclinical Endometritis on Fertility of Dairy Cattle**

Inflammation of the uterus due to Subclinical Endometritis(SCE) has profound negative impact on reproductive performance.

Irritation of the endometrium may cause premature release of prostaglandin and lysis of the corpus luteum thus resulting in shorter inter estrus interval.

Affected animals show prolonged days to first service and reduced first service conception rate.

SCE severely impairs the economic growth of dairy industry or dairy farmer by increased feed intake of animal per lactation, reduced milk yield and increased culling rate.

Cows with SCE have slower growth of dominant follicles, lower peripheral plasma estradiol or progesterone concentrations and are less likely to ovulate.

More number of services (~2.4) is required for conception for cows that are positive for SCE at the 4th week postpartum, whereas, less number of services (~1.7) is required for conception for cows negative for SCE.

## Diagnostic Approaches for Subclinical Endometritis

Although many methods are available to diagnose uterine infection and clinical endometritis, only few of them may be used for diagnosing SCE. To diagnose SCE in herd level and for rapid screening, a cow side test is to be employed which is currently unavailable. The most recent and accurate techniques available for diagnosing Subclinical Endometritis are mentioned here under:

### 1. Lavage Technique

The uterine body is lavaged by infusing 60ml of 0.9% sodium chloride solution (Isotonic) in to the uterine body with a syringe attached to disposable plastic infusion rod. The uterus was gently massaged per rectum and then retracted to recover the fluid. As much fluid as possible was recovered by negative pressure aspiration into the syringe and transferred to a 50 ml centrifuge tube without preservatives. Uterine lavage samples were brought to the laboratory within 2 hours and centrifuged at 500 revolutions per minute for 5 minutes. A drop of sediment was smeared on to a clean microscopic slide and air-dried. All slides were fixed with methanol and stained with giemsa stain and examined under a microscope at 400x magnification by counting 80-100 cells to determine the percentage of neutrophils (PMN). If greater than 5% of the cells are neutrophils per 400x microscope power field, it was categorized as subclinical endometritis.

### 2. Cytobrush Technique

Cytobrush is a less harmful technique for the endometrium than the uterine lavage, since the fluid in uterine lavage may produce endometrial irritation. The normal cytobrush handle was cut to approximately 3 cm in length, threaded on to a 65cm solid stainless steel rod, and placed in a stainless steel tube 50 cm on length and 5mm in diameter for passage through the cervix. Endometrial cytology samples were collected by rotating the cytobrush three times in a clockwise direction while in contact with the uterine wall. Slides were prepared by rolling the cytobrush on to a clean glass microscopic slide. Slides were brought to laboratory within 2 hours and stained with modified Giemsa stain. The cytobrush technique yielded sample might represent the inflammatory nature of the endometrium, compared with the uterine lavage technique, which represents diluted sample of luminal contents. Thus cytobrush technique is a more consistent and reliable method than the lavage method to obtain endometrial cytology samples from postpartum dairy cows (3).

### 3. Leukocyte Esterase Test

Presently many studies have been conducted on detecting leukocyte esterase enzyme in uterine fluid using commercially available urine analysis strips. Polymorph nuclear neutrophils are the first line of defense and are recruited to the uterus in response to inflammation. Granules in leukocytes contain esterase, which when in contact with leukocyte

esterase (LE) strips, catalyze a chemical reaction and causes colour change of the strip. As the number of neutrophils increases during inflammation and the amount of LE enzyme is positively correlated with the number leukocyte present in uterus, the change of strip colour is a sure sign of underlying inflammation. Once the cutoff value for LE enzyme in uterine fluid is validated on a large population the strip can prove useful as a cow side diagnostic tool for diagnosing SCE in a herd.

### 4. Acute Phase Proteins and Inflammatory Cytokines Estimation

Acute phase proteins (APPs) play an important role in different stages of the inflammatory response, and thus may serve as markers of various types of diseases in cattle. Out of many acute phase proteins occurring in bovine, haptoglobin (Hp) and serum amyloid A (SAA) are the positive indicators for uterine infection cows as level of SAA, Hp was found to be significantly higher in serum as well as in uterine washings of cows with endometritis and subclinical endometritis.

Key inflammatory cytokines such as IL-6, IL-10 and TNF- $\alpha$  level also increases significantly in cows with subclinical endometritis as compared to normal healthy cows. Therefore, assessing the level of cytokines and acute phase proteins in serum and uterine washings can be a useful diagnostic tool for identifying sub clinical endometritis in dairy cows.

### Treatment Approaches for Subclinical Endometritis

For treating SCE, a veterinarian should take the decision for treating the case and also should consider the cost of treatment regime. Subclinical endometritis can be treated by prostaglandin therapy through intra muscular injection of PGF $2\alpha$  (Cloprostenol 500 mg) or by intrauterine antibiotic therapy (Cephapirin) at 20–33 days PP to improve the reproductive performance. As different types of infectious agents involved in uterine infections, selecting a specific drug is of primary urgency. Ideally, identification of the infectious agent and drug sensitivity is done. Subsequent treatment with an approved drug is the most practical, economical and efficient approach. However, there are no recommended doses on most drug labels for intrauterine therapy. The following are some of the recent and efficient treatment approaches for subclinical endometritis.

#### 1. Alternative Enzyme Therapy

As an alternative to antibiotic therapy, use of proteolytic enzymes are gaining popularity due to the fact that antibiotics require frequent administration and its residue pass through milk of dairy cattle which might affect the quality of consumed milk. Therefore, recently researches are going on to use proteolytic enzymes as a component for treating SCE. Generally, a mixture of proteolytic enzymes namely chymotrypsin, trypsin and papain are used which have fibrinolytic as well as proteolytic effect on the damaged endometrium and degrades the necrotic tissue if present. Also it is

important in boosting the cellular immunity as well as controlling the microbial growth inside uterus. The enzyme mixture is available as readymade ointment form (e.g. Mastivexym) or the crude form can be made manually. Single intrauterine treatment is given at spontaneous estrus. There is report on improvement in pregnancy rate in dairy cattle treated with the enzyme mixture as compared to those animals that didn't get any treatment.

Trypsin, chymotrypsin and papain have the capacity to split proteins, fat bonds and have immune-regulatory effect. For example, papain works as a cysteine-protease, similar to bacterial cysteine-proteases from gram-negative anaerobes, on the CD14 molecule of macrophages and monocytes and raises up their level of efficacy as the instigator of acute phase reaction. Administration of proteolytic enzymes results in better pregnancy rates as compared to *E. coli* LPS and levamisol(5).

## 2. Injection of Prostaglandin

Treatment with PGF<sub>2</sub>α is only beneficial when responsive CL is present in one of the ovaries. Lysis of the CL will eventually bring most of the animal in estrogenic phase and subsequently the contaminant is eliminated from the uterus due to the uterine contraction and phagocytosis by the increased number of PMNs. In endometric cows with no palpable CL, prostaglandin treatment was found to be ineffective (6). Therefore, the role of PGF<sub>2</sub>α in the therapy of uterine infections are by luteolysis of CL, improvement of contractility, elimination of immune suppressive effect of progesterone and direct stimulation of the function of immune cells due to its pro-inflammatory action in endometrium (7). Due to growing concern of developing antimicrobial resistance by using antibiotics, PGF<sub>2</sub>α might prove to be effective as an alternative therapeutic method to treat subclinical endometritis and other uterine infections.

## 3. *E. coli* Lipopolysaccharide (*E. coli* LPS)

A single intrauterine infusion of 100 µg *E. coli* lipopolysaccharide (*E. coli* LPS) stimulate uterine defense mechanism by bringing a population of neutrophils from systemic circulation to the uterine lumen within 6 hrs of administration and thereby clears the infection and restores the fertility (8). *E. coli* LPS has been found to be most effective in controlling uterine infections as compared to other immunomodulators like lysozyme and oyster glycogen.

## Conclusion

Clinical and subclinical endometritis are common in high producing dairy cattle, which delay the onset of ovarian cyclic activity after parturition, extend luteal phases and reduce conception rates. Uterine bacterial infections impair the function of the hypothalamo-pituitary-gonadal axis and directly perturb steroidogenesis by affecting ovarian granulosa cells – providing mechanisms to explain the association between uterine disease and anovulatory anoestrus. If cows ovulate, the corpus luteum secretes less

progesterone in diseased than normal animals. Long luteal phases associated with endometritis are probably caused by a switch in endometrial prostaglandin production from PGF to PGE. Finally, the endometrial damage associated with bacterial or viral infections will further reduce the chance of conception. Understanding the mechanism of uterine disease should lead to new and better treatments and/or preventatives. Future approaches will need to address how pathogens infect the endometrium, how the endometrium responds to infection and how to regulate or limit the inflammation in the endometrium and the ovary.

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**Fig. 4. Showing lacerated teat that requires correction by suturing in a buffalo**

## Surgical Affections of Udder and Teats in Large Ruminants

Sharun Khan, Manjusha K M, Amitha Banu S, Haritha C V, Rohit Kumar, A M Pawde and Amarpal  
Division of Surgery, Division of Pharmacology and Toxicology, ICAR-IVRI, Izatnagar, Bareilly, Uttar Pradesh

### Abstract

*Udder is one of the most important organs of bovine as it is directly related to productivity and the farmer's economy. The anatomical peculiarities and rich vascular supply predisposes the udder to various disease conditions. The disease conditions of the udder can be categorized broadly as conditions affecting teat orifices, teat cistern, teat surface and the udder. Radiography can be used to locate the lesions of udder and teat while ultrasonography facilitates the imaging of internal structures. The udder and teat affections may not be life threatening to the animal but may affect its productive life. Many affections of teat and udder can be cured by undertaking surgical interventions but they must be carried out under complete aseptic conditions to prevent further complications.*

### Introduction

The anatomical abnormalities, enlargement of udder and teats during parturition, improper milking methods, repeated injury to teat mucosa, suckling injury caused by calf and paralysis resulting from metabolic disturbances during parturition make the udder vulnerable to various infections (1). The various disease conditions of the udder can be categorized according to their location as conditions of teat orifices, teat cistern, teat surface and the udder in general. Majority of the problems of teat and udder originate from poor management. However, even in well managed farm operations, accidents can occur where teats and udder may be

damaged due to improper flooring, agricultural equipment, fence wire, thorns, dog bite, horn thrust, kicking by other animal, self-inflicted injury, crushing injuries and injury caused by the feet of other animals. The anatomical position of udder in bovine and caprine make udder, particularly teats, vulnerable to traumatic affections (2). The suspensory ligament of udder often gets weakened and the udder occupies a position that is prone to major injuries.

Injuries to teat are common in dairy cattle and can be divided into two categories viz. external and internal injuries. When compared with other frequently occurring diseases, these injuries often

**Teat surface affections**

- Supernumerary teat
- Sloughed teat
- Teat papilloma and Fibropapilloma
- Teat wounds



**Teat cistern affections**

- Teat fistula
- Dilated teat cistern
- Teat polyp
- Webbed teat

**Teat orifice affections**

- Imperforate teat
- Contracted teat orifice
- Enlarged teat orifice

**Udder affections**

- Hypermastia
- Udder wounds
- Suppurative mastitis
- Gangrenous mastitis

result in premature culling of the affected cows (3). Various diseases and anomalies of udder and teat can affect the epithelial surface of the udder and teat, teat cistern and streak canal. Anomalies of the epithelial surface of the teat include supernumerary teats, fused teats, lacerations, teat fistula, papilloma and warts. Surgical affections of teat cistern include lactolith, polyps, teat spider and local or diffused obstruction. Conditions affecting the teat streak include contracted sphincter, enlarged teat orifice, rupture and inversion of the canal mucosa and occlusion of teat orifice (4).

Obstruction of teat canal is of common occurrence and is also of economic importance in milch cows.

This may be due to inflammation, growth, membranous partition and presence of lacteal calculi in the teat canal. Obstruction to the teat canal due to the teat affections is common in lactating animals and occur very often as a sequel to recurrent mastitis and probably due to faulty milking practices. Surgical interventions by using teat knives, bistouries and teat dilators, which are often recommended, may lead to further fibrosis resulting in complete obstruction of the teat canal. The teat pathologies that require surgical intervention include supernumerary, conjoined teat, agenesis of the streak canal, tight streak canal, obstruction in the area of the rosette of Furstenberg, milk stones, obstruction in the area of the teat cistern and /or the annular ring and fibrosis of the gland sinus (5). The

present review is aimed to discuss in brief about the anatomical structure and common surgical affections of udder and teat in large ruminants.

**Anatomy of the Udder**

In bovines, udder is a structural unit comprising four quarters; two fore and rear quarters. Each quarter corresponds to a functional mammary gland and bears one principle teat. A prominent median intermammary groove generally marks the division of the udder into right and left halves. A transverse groove separates the fore and hind quarters of each half externally. The udder is suspended by strong fascial sheets that surround and encloses the gland substance and extends inward to fuse with the connective tissue framework that permeates the entire region.

The internal structure of the udder consists of gland parenchyma and connective tissue intermingled in each other. Each gland develops around a branching duct system. The functional unit of the glandular tissue is the alveoli. Alveoli are microscopic, spherical structures lined by a single layer of cuboidal to columnar epithelial cells and smooth cells. A cluster of alveoli encapsulated by connective tissue sheet with their ducts constitutes a lobule. A group of lobules surrounded by a septum of connective tissue forms a lobe. The terminal ducts unite further to form intra – lobular ducts. These ducts unite successively to form large interlobular ducts, intra-lobar and inter-lobar ducts. The

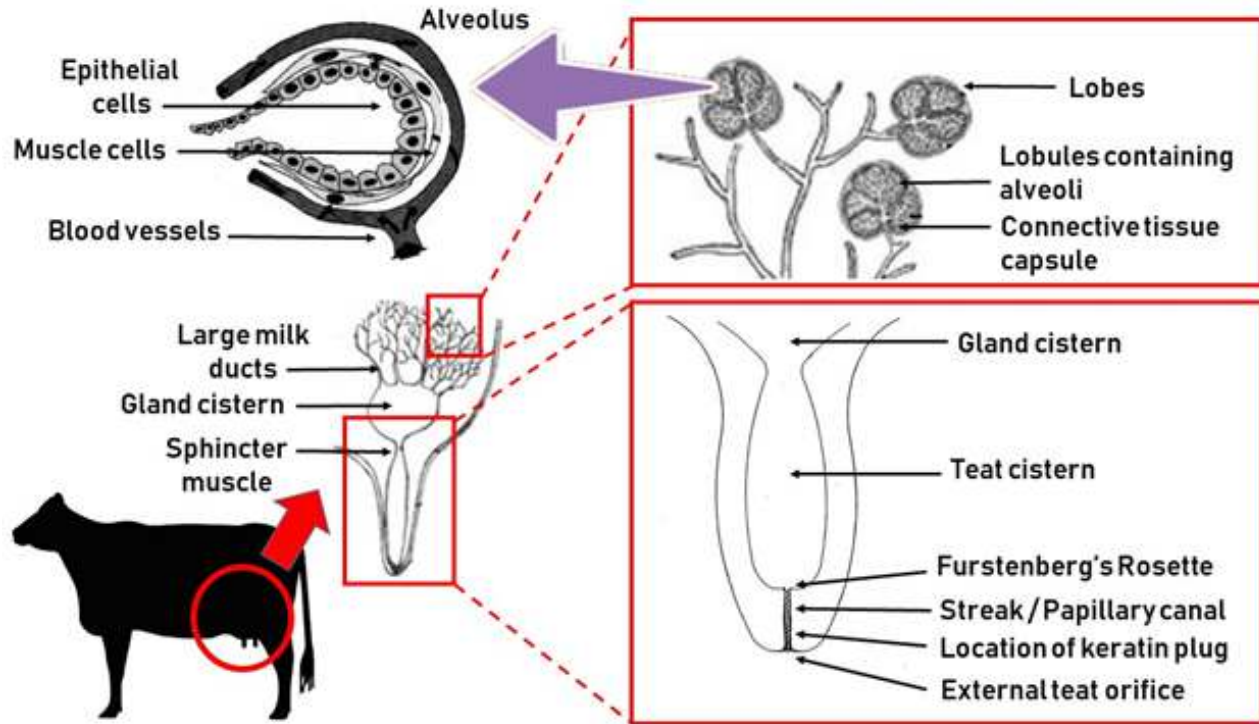


Fig. 1. Illustrating the anatomy of mammary gland in cattle

inter-lobar ducts open into a large basin called lactiferous sinus which empties into a large cavity called as gland cistern which further continues as teat cistern.

The wall of teat cistern is continuous with the exterior through a narrow tube known as streak canal which is the main barrier against infection. At the junction of teat cistern and streak canal, the mucosa of the teat canal is arranged in group of radial folds called the "Rosette of Furstenberg". At the end of the teat, the streak canal is surrounded by sphincter composed of smooth muscle fibres. The blood supply to udder is by the external pudental artery. Two posterior mammary lymph nodes located on the caudal aspect of the mammary gland receive the afferents from the posterior part of the gland. Udder receives multiple innervations from lumbar and sacral spinal nerves.

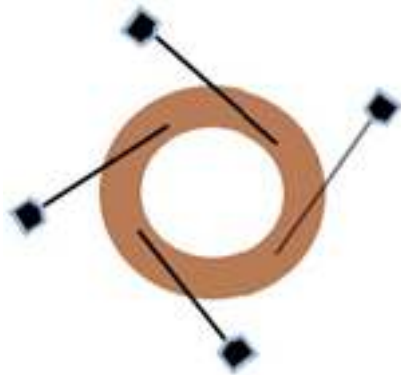
**Imaging Techniques of Udder and Teat**

Ultrasonography (USG) is a non-invasive and effective method for the diagnosis of various physiological and pathological conditions of the reproductive organs of ruminants (6). Radiography helps to locate the lesions of the teat while ultrasonography can be used for imaging of internal structure of the teat. With the help of USG, it is possible to differentiate morphological structures such as the glandular parenchyma, the gland

cistern, the teat cistern and the teat canal (7). Ultrasonography is commonly used for the diagnosis of obstructive fibrosis and to evaluate the extent of injury (8). It is a non-invasive technique that does not require the use of ionizing radiation. It is also an efficient tool that can be used for the visualization of all internal structures of the mammary gland. Ultrasonographic imaging is specifically useful for the identification of the location of the obstructive fibrosis - which is often not achieved by physical examination alone. Obstructive fibrosis of the teat is one of the most important indications of performing ultrasonography (7). Teat endoscopy is an efficient diagnostic tool for covered teat injuries. It provides exact condition of the mucosa, intensity/grade and eventual duration of pathological changes. Teat endoscopy can give accurate diagnosis about the internal teat injuries within a short period of time. It can also be used for the precise diagnosis of milk flow disorders (9).

**Surgical Anaesthesia**

Adequate restraint and anesthesia are important prerequisites for surgical interventions of the bovine teat. Success depends on adoption of aseptic condition and standard surgical techniques. Intracanal anaesthesia involves injection of 5 ml of lignocaine hydrochloride into the streak canal for local analgesia. However, this type of anesthesia



**Fig. 2. Needle placement for ring block technique in cattle**

was found to be unsatisfactory for surgical intervention that required suturing (5). Ring block is considered to be effective in surgeries involving teats. Addition of vasoconstrictor drug to the local anesthetic should be avoided as it may result in ischaemic necrosis at the end of the teat (10).

Ring block anesthesia can also be combined with intracanal anesthesia to get superior result (**Fig. 2**). Depending on the temperament of the cow, sedation with Xylazine hydrochloride at the dose of 0.2 mg / kg body weight intramuscularly or 0.1 mg / kg body weight intravenously can be performed which is then followed by local anesthesia. Ring block anesthesia can be performed by a circumferential injection of 20 ml of 2% lidocaine hydrochloride solution at the base of the teat in the area of annular ring avoiding the intravenous injection. This regional block won't interfere with the process of wound healing, and will produce adequate analgesia for all teat surgeries. Surgical management of teat affections can be performed in lateral recumbency with inverted 'V' shaped lignocaine infiltration and by using a proximal tourniquet.

### Teat Orifice Affections

#### Imperforate Teat

It is the occlusion of teat orifice which can be either congenital or acquired in nature. It is a developmental anomaly in the first calver or may be an acquired conditions due to trauma to the teat tip. The occlusion is relieved by inserting an 18 gauge hypodermic needle into the teat cistern under local anaesthesia until milk starts coming out. The obstruction of the orifice can also be cleared by the insertion of a sterilized milk siphon or a suitable trocar and cannula, the latter being left in position for some time to regain desired patency to its original size and for expulsion of contents of the udder.

#### Contracted Teat Orifice / Hard Milker

The Condition is characterised by small streams of milk upon greater pressure and prolonged milking time due to narrowing of streak canal. It can be congenital or acquired due to trauma. Overdeveloped spinchter muscles, reduced diameter of streak canal and inflammation as a result of trauma may cause difficulty in milking. The repair of hard milker teat can be performed by either conservative therapy, not milking the gland for 3–5 days and draining it with a cannula to allow the inflammation to subside or surgically via blind resection of the obstructing tissue through the streak canal (11).The condition can also be corrected by inserting stoll teat bistoury, litchy teat knife and ringed teat slitler with a quick thrush pushed into the lumen of the teat canal. Larson's teat canula is used to maintain the desired opening and can be left to remain for 5-7 days. Extra damage to the surrounding should be avoided to prevent further contraction of sphincter due to scar tissue

#### Enlarged Teat Orifice/ Free Milker / Leaky Teats / Incompetent Sphincter

This condition occurs as a result of traumatized sphincter with consequent leakage of milk all the time. Its treatment is indicated in only chronic cases as fibrosis of granulation tissue may correct the condition automatically. It can be treated by injecting minute amount (0.1 ml) of sterile mineral oil or lugol's iodine on the periphery of the teat orifice to reduce the size. Over correction may lead to contracted sphincter.

#### Black Spot

Black spot is seen as a necrotic dermatitis around the teat sphincter. The condition mainly occur due to traumas from over milking, improper vacuum of milking machine and wet teat exposed to cold weather just after milking. The treatment done by gentle removal of scabs after softening with mild antiseptic solution, application of anti-inflammatory cream, intramammary antibiotic for three days, with application of teat bougie to prevent further stenosis by scabs (12).

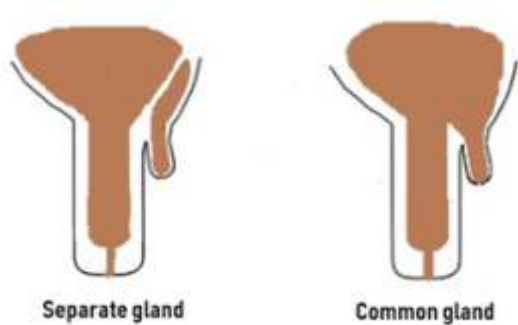
#### Teat Cistern Affections

##### Lactolith / Milk Stone / Lacteal Calculi

It occurs due to accumulation of mineral deposits. Usually seen as concretions and rarely as an organized calculus. It will move freely in the teat canal and at times get lodged in the orifice causing obstruction. Small lactolith can be retrieved by gentle manipulation of the teat sphincter with a haemostat while moving the stone distally.

##### Teat Fistula

It is an abnormal opening that connects the teat



**Fig. 3. Supernumerary teat with separate gland (Left) and with common gland (Right)**

cistern to exterior surface of the teat. Milk will eject out through this abnormal opening in addition to the normal opening (4). The size of fistula varies and smaller ones are difficult to locate. It can be congenital or acquired. Acquired case of teat fistula usually occurs as complication of teat laceration. There is no separate teat canal or teat sphincter in teat fistula. If there is concurrent occurrence of teat fistula along with mastitis or oedematous udder wounds, these conditions should be corrected primarily and then only proceed for teat fistula repair. The condition needs immediate attention in lactating animals which need surgical correction. Fine fistulas can be corrected using needle point cautery but larger ones need to be corrected under local anaesthetics. Treatment of choice for teat fistula is surgical correction. In surgical repair, mucosa, connective tissue and subcutis, followed by skin have to be apposed separately.

**Teat Polyp**

It is a pedunculated pea sized growth attached to the wall of teat cistern that interferes with the milk flow. It can be treated surgically after teat infusion anaesthesia. A Hug's teat tumor extractor, Cornell teat curette or alligator forceps is passed through the sphincter to sever the stalk.

**Webbed Teat**

A conjoined teat or webbed teat is defined as the supernumerary teat attached to the side of a primary teat. Insufficient development and function of the streak canal and teat sphincter cause the increased incidence of mastitis (5). Webbed teat was presented as a functional extra teat which attached to teat sinus and ranged from distinct extra teat to an elevation area on teat wall with an opening discharging milk which usually is confused with teat fistula (13). Surgical correction of webbed/Siamese teat is essential to prevent milk loss and mastitis (5).

**Teat Surface Affections**

**Supernumerary Teat / Accessory Teat**

This is an inherited defect. The accessory teats may

be found in the caudal region independently or as fused with the normal teat (Fig.3). They can be functional or non-functional. They are removed when the udder is under developed i.e. at 1-9 months of age or during dry period. The condition is corrected by resecting the accessory teat at its base after crushing. The line of resection should be cranio-caudal than transverse so that the scar merges with the normal udder folds.

**Teat Lacerations**

This is a very common condition. If the lumen is not involved in the wound, normal healing can take place while the teat is allowed to be milked. The factors determining the prognosis and recovery include teat orifice involvement, wound direction, involvement of sinus, amount of skin loss and degree of infection. If the lumen of the teat is involved, the condition is treated as teat fistula (Fig.4). Sutured wound should be protected by an adhesive elastic bandage.

**Teat Papilloma and Fibropapilloma**

These are single or multiple abnormal projections that originate from the skin surface of the teat. Unless the affected teat becomes ulcerated or cracked, it will not interfere with milking. A tight ligature at the base of the wart will occlude the blood supply leading to its sloughing. In case of multiple warts, autoimmunization is found to be useful.

**Udder Affections**

**Udder Wounds**

Accidental wounds to udder and teat presents real problem to diary practitioners. Many are flap wounds with irregular shapes and depth of penetration. Wounds penetrating the milk cistern are skin flap wounds with V-shaped or L-shaped configurations (14). Cleaner wounds can be sutured successfully after debridement. The suture materials should not be buried inside as it can act as nidus for further infections. Contusions or hematoma of the udder are visible as soft tissue swellings either in the cranial or caudal region of the udder as a result of injury. Haematoma should not be incised or drained unless they are infected or enlarged to avoid the possibility of shock and haemorrhage. Subcutaneous abscess of the udder secondary to teat injury and chronic mastitis can occur. It should be drained and cleaned using antiseptic solutions until complete healing occurs

**Suppurative Mastitis**

Inflammatory condition of mammary gland is termed as mastitis. Following trauma or poor management conditions of udder, bacteria invade the udder and mastitis results. Mastitis can also be caused by virus and fungi. Mastitis caused by pus

producing organism is commonly known as suppurative mastitis. It can be acute or chronic. Most probable underlying cause is poor hygienic condition. Un-noticed acute case usually progresses to chronic condition with abscess formation. Prognosis of the condition is determined primarily by time of presentation and treatment protocol. Early identification and prompt treatment minimize the udder damage and production loss. Parenteral antibiotics combined with teat infusion usually produce desirable result. In complicated cases, where medical management fails, unilateral or bilateral mastectomy is recommended (15).

### Gangrenous Mastitis

Gangrenous mastitis is per acute or acute form of mastitis caused mainly by infectious agents like *Staphylococcus aureus*, *Clostridium perfringens* and *Escherichia coli* in cattle. It is characterised by classical signs of inflammation with bluish discoloration which then progresses to necrosis of the udder. The affected area is cold to touch with crepitating sound and fluid exudates. The toxins released from bacteria cause ulceration, and sloughing of udder epithelium which then lead to toxæmia with fatal consequences. The histopathology lesions are characterised by progressive swelling with vascular degeneration, ulceration and erosion of the epithelium (16). If treated early, animal can be saved from fatal toxæmic conditions. The safe and effective treatment includes partial mastectomy of the affected quarter (17). Amputation of complete mammary gland can also be done considering the general health status of the animal (15). Administration of antimicrobials and fluids helps to eliminate the toxins.

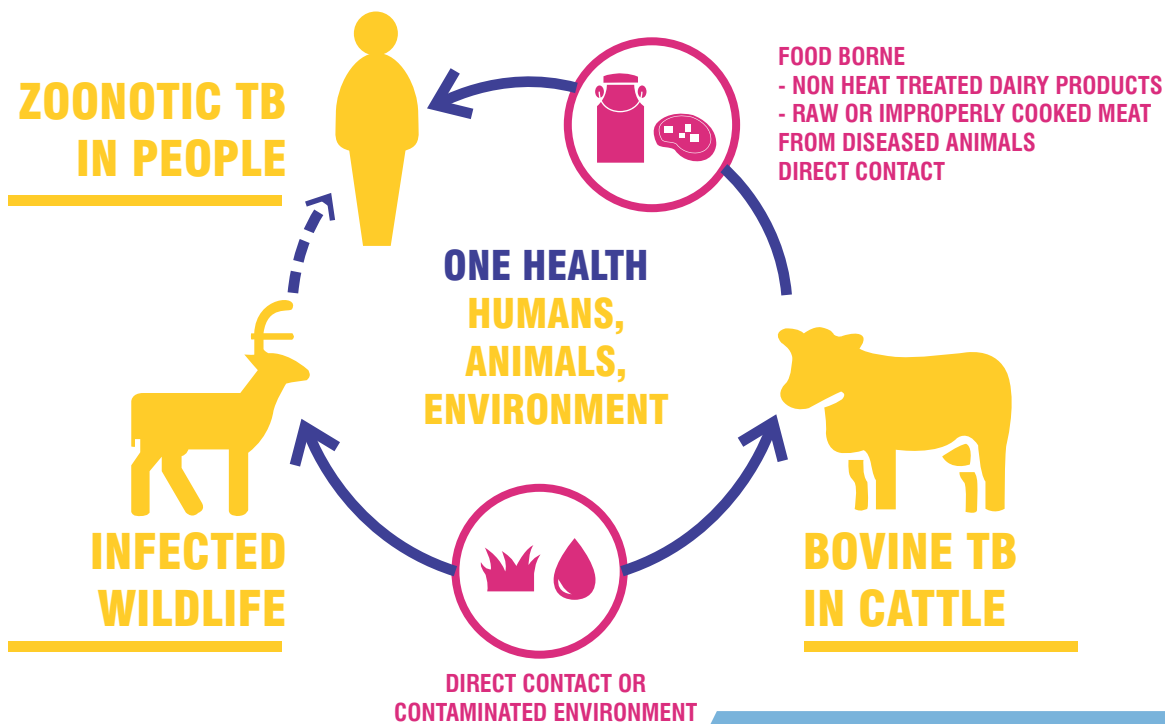
### Conclusion

A surgeon needs to have adequate knowledge of the anatomical features of the udder to undertake any surgical interventions for correcting various conditions. Mostly various problems associated with the udder and teat occur in late gestation or after parturition causing production losses. Ultrasonography is a helpful tool to diagnose and confirm different affections of teat and udder such as, teat Sphincter fibrosis, teat obstruction, blind teat, spider teat and inflammation of the teat in cattle. Post-operative complications mostly encountered are mastitis, reduction in milk yield, and further contamination. So strict asepsis should always be maintained while performing teat surgeries.

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## Zoonotic Tuberculosis

Smita and Sanjay Shakya

Dept. of Vet. Public Health & Epidemiology, College of Veterinary Science & A.H, CKVV , Anjora, Durg, Chhattisgarh.

### Introduction

Tuberculosis (TB) remains the leading cause of death worldwide and affecting more than 9 million people every year. Although *Mycobacterium tuberculosis* is the most common cause of human TB, unknown proportions of TB cases are considered to be attributable to *Mycobacterium bovis* infection, which is also termed Bovine TB (1). The infection currently poses a major concern in human populations in developing countries, as humans and animals share the same microenvironment. It has been estimated that zoonotic transmission of *M. bovis* is responsible for 10–15% of new human TB cases in developing countries. Multidrug resistant tuberculosis (MDR-TB) constitutes a major public health problem and represents a severe obstacle to the control of TB, particularly in countries in which the prevalence of this disease remains high. In 2017, an estimated 558 000 new cases of MDR/RR (Rifampicin resistant)-TB emerged globally and caused 230000 deaths. Around 50% of the global burden of MDR-TB is concentrated in India and China, followed by Russia (2). About 8.5% of MDR-TB cases constitute extensively drug-resistant TB (XDR-TB).

### Tuberculosis and The *Mycobacterium Tuberculosis* Complex

TB is caused by a group of phylogenetically closely related bacteria, collectively known as the *Mycobacterium tuberculosis* complex (MTBC). TB in

humans is primarily caused by *M. tuberculosis* and *Mycobacterium africanum*, a phylogenetic variant of MTBC limited to West Africa. In addition, several animal-adapted members of MTBC exist, which affect a range of wild and domestic animal species (3). These include *M. bovis* (a pathogen of cattle), *Mycobacterium caprae* (sheep and goats), *Mycobacterium microti* (voles) and *Mycobacterium pinnipedii* (seals and sea lions). *Mycobacterium bovis* used to be a significant cause of human TB, primarily in children who consumed raw milk. However, *M. bovis* infections in humans decreased markedly following the introduction of pasteurization of milk and meat hygiene practices. Moreover, *M. bovis* does not easily transmit between humans and, similarly, while *M. tuberculosis* has been isolated from various animal species, including cattle. There is currently no evidence of animal-to-animal transmission of *M. tuberculosis* or *M. africanum*. Hence, different members of MTBC appear to be best adapted to their particular host species. One particular member of MTBC deserves special mention: *Mycobacterium canettii*, which is part of the so-called 'smooth TB bacilli'. *Mycobacterium canettii* differs in many ways from classical MTBC. This organism produces smooth and shiny colonies on solid growth media, which are distinct from the rough colony morphology characteristic of classical MTBC (4).

### Epidemiology

Tuberculosis (TB) is chronic disease in nature

affecting a wide range of mammals that include: humans and cattle, deer, llamas and other wild animals. All species including humans with various age groups are susceptible. The bacteria primarily affect the cattle and other domestic and wild animals as well as human beings. Disease is found throughout the world but more prevalent in Africa, parts of Asia

known as tubercles. In many animals the course of the infection is chronic and signs may be absent, even in advanced cases when many organs may be involved. Subclinical signs include weakness, dyspnea, anorexia, emaciation, enlargement of lymph nodes and cough, particularly with advanced tuberculosis (5).

**Table1. Indian and Global Estimates of TB Burden**

Indicator	India	Global
Incidence of TB (including HIV)	27,90,000	1,04,00,000
Mortality due to TB (Excluding HIV)	4,23,000	13,00,000
Incidence of MDR-TB/RR	1,47,000	6,01,000
Incidence of HIV- TB	87,000	10,30,000
Mortality due to HIV- TB co - morbidity	12,000	3,74,000

(India TB Report 2018, Ministry of Health & Family welfare)

and America (5). The prevalence of disease is high in the tropical and sub-tropical countries. In many parts of the world, badgers, brush-tail opossums, wild boars, deer and other wildlife species constitute a wildlife reservoir of the pathogen (6). Thus, the natural movement of these reservoir animals increases the spread of the disease to domestic animals and thereby, its public health impact.

**Modes of Transmission**

Transmission of *M. bovis* can occur between animals, from animals to humans and vice versa and rarely, between humans. *M. bovis* infection is transmissible from cattle to humans directly by aerogenous route and through direct contact with material contaminated with nose and mouth secretions from an infected herd of cattle. Individuals, at risk are persons in contact with potentially infected animals such as veterinarians, abattoir workers, meat inspectors, autopsy personnel, farmers, milkers, animal keepers (as well as those in the zoo), animal dealers, laboratory personnel and owners of potential tuberculous pets (e.g. monkeys). Indirectly, man acquires the disease from animal sources by ingestion of meat and meat products from slaughtered infected cattle and consumption of unpasteurized infected milk (6). Ingestion of unpasteurized contaminated milk products poses a greater risk than ingestion of infected meat products because badly infected carcasses are condemned; parts of carcasses that are processed as meat products are inspected and thoroughly cooked. Thorough cooking at 170°F for 30 min, removes virtually all risk of infection.

**Clinical Presentation**

**In Animals**

Bovine TB is a chronic debilitating disease usually characterized by formation of nodular granulomas

**In Humans**

*M. bovis* infection in humans has similar clinical forms as those caused by *M. tuberculosis*. Common clinical manifestation of *M. bovis* infection in man is associated with the extra-pulmonary form of the disease (6). Cervical lymphadenopathy, intestinal lesions, chronic skin tuberculosis, and other non-pulmonary forms are particularly common. Infection due to *M. bovis* in humans usually has a prolonged course and symptoms generally takes months or years to appear. Sometimes, the bacteria remain dormant in the host without causing diseases. The common clinical signs of zoonotic TB include loss of appetite, diarrhea, weight loss, intermittent fever, intermittent hacking cough, large prominent lymph nodes, weakness, etc. Young children infected with *M. bovis* typically have abdominal infections and older patients suffer from swollen and sometimes ulcerated lymph glands in the neck. Pulmonary disease is more common in people with reactivated infections. The symptoms may include fever, cough, chest pain, cavitation, and hemoptysis. The pulmonary form of tuberculosis occurs less frequently and is usually occupationally related.

**Diagnosis**

**Human Tuberculosis is Diagnosed by**

Mantoux Tuberculin Skin test (TST), Chest Radiograph, Sputum smear microscopy for demonstration of acid fast bacilli (7), and by other molecular diagnostic test. Xpert MTB/RIF is a rapid test recommended by WHO as initial diagnostic test. This test detects *M. tuberculosis* complex within 2 hours, with an assay sensitivity that is much higher than that of smear microscopy. Also, this molecular assay has the potential to improve the performance of national tuberculosis programs and is currently being implemented in district-level laboratories in 67 countries with a high prevalence of tuberculosis (8).

### Animal Tuberculosis is Diagnosed by

Acid-fast Staining to detect acid fast bacilli (AFB) in clinical specimens by Ziehl-Neelsen (ZN) or fluorescent staining. It is a cost-effective tool for diagnosing BTB and to monitor the progress of treatment especially in developing countries. The stained slides are observed with an ordinary light microscope for the presence of acid-fast bacilli, which appear as red, colloidal or bacillary cells 1 - 3 microns in length occurring singly or in clumps. The isolation of mycobacterium can be done by culturing on the egg-based media known as Lowenstein-Jensen (LJ) medium. The luxuriant growth of *M. tuberculosis* on glycerol containing media, giving the characteristic 'rough, tough and buff' colonies is known as eugenic while the growth of *M. avium* on media containing glycerol is also described as eugenic whereas *M. bovis* has sparse, thin growth on glycerol containing media that is called dysgenic, however, grows well on pyruvate-containing media without glycerol.

**Tuberculin Testing** is performed to screen the positive animals which involves the intradermal injection of bovine tuberculin purified protein derivative (PPD) and the subsequent detection of swelling (delayed hypersensitivity) at the site of injection 72 hours later. The tuberculin test is usually performed on the mid-neck, but the test can also be performed in the caudal fold of the tail. The skin of the neck is more sensitive to tuberculin than the skin of the caudal fold.

**PCR** has been widely used for the detection of *M. tuberculosis* complex in clinical samples (mainly sputum) in human cases and has recently been used for the diagnosis of tuberculosis in animals. The real time PCR determines the status of infection in cattle for bovine tuberculosis as compared to IFN gamma mRNA in blood culture. Another useful diagnostic method for bovine tuberculosis in cattle is RT-PCR.

**Gamma-interferon assay** is an in vitro laboratory based blood test detecting specific cell-mediated immune responses by circulating lymphocytes (9).

Molecular Typing methods for *M. tuberculosis* Complex includes two groups (10). The first group is genomic methods for DNA and includes RFLP IS6110, polymorphic GCrch sequence (PGRS) analysis and pulsed-field gel electrophoresis (PFGE). The second method applies DNA-specific sequence amplification methods using polymerase chain reaction (PCR) which include, spoligotyping (spacer oligonucleotide typing), ligation-mediated PCR (LM-PCR), double repetitive element PCR (DRE-PCR). WHO recommended molecular methods, LPA and Xpert MTB/RIF assay, are commonly used for the diagnosis.

*M. bovis* and *M. tuberculosis* can be differentiated by a multiplex-polymerase chain reaction (PCR) assay based on one-step amplification and detection of two different mycobacterial genomic fragments differentiating *Mycobacterium bovis* and

*Mycobacterium tuberculosis* (11). The oligonucleotide primers were chosen from a 500-bp genomic fragment which is well conserved in *M. bovis* and the *pncA* gene (based on *M. tuberculosis*-specific nucleotide polymorphism, a cytosine residue at position 169), specific for *M. tuberculosis*.

### Diagnosis of Resistance to Anti-TB drugs

Laboratory methods of diagnosing resistance to anti-TB drugs may be classified as phenotypic (based on culture growth in the presence of drugs) or genotypic (i.e. identification of the presence of mutations that confer resistance).

Phenotypic methods, also referred to as drug susceptibility tests, can be performed in solid or liquid media. Liquid medium, using the BACTEC MGIT 960 system, significantly reduces the time involved in detecting *M. tuberculosis* to two weeks and after 1–2 weeks the results of the susceptibility test are available. The greatest disadvantage of the liquid medium technique is its high cost. The accuracy of susceptibility testing varies in accordance with the drug tested, accuracy being greater in the case of rifampicin and isoniazid and poorer in the case of ethambutol and streptomycin (12).

The genotypic methods, also referred to as molecular methods, are based on the detection of the mutations that confer resistance to anti-TB drugs. Many genotypic methods have been developed over recent years in response to the urgent need to be able to detect resistance to anti-TB drugs rapidly. The molecular tests most commonly used worldwide are the GenoType MTBDR plus, the INNO-LiPA Rif.TB, and the Xpert MTB/RIF assays.

### Treatment

DOTS (Directly Observed Treatment, Short Course) is a standard 6-9 month course of 4 antimicrobial drugs that are provided with information, supervision and support to the patient by a health worker or trained volunteer. These drugs are Isoniazid (INH), Rifampicin (Rif), Pyrazinamide (PZA) and Ethambutol (EMB).

Mycobacterium that are resistant to at least rifampicin, one of the most effective anti-TB medicines need second-line treatment similar to MDR-TB patients and are referred to as rifampicin resistant (RR-TB). Multidrug-resistant TB (MDR-TB) is caused by bacteria that do not respond to isoniazid and rifampicin, the two most powerful, first-line anti-TB drugs, are treatable and curable by using second-line drugs and requires extensive chemotherapy (up to 2 years of treatment). In 2016, WHO approved the use of a short, standardized regimen for MDR-TB patients who do not have strains that are resistant to second-line TB medicines (2). This regimen takes 9–12 months.

Extensively drug-resistant TB (XDR-TB) do not respond to the most effective second-line anti-TB

drugs and involves resistance to the two most powerful anti-TB drugs, isoniazid and rifampicin. They are resistant to fluoroquinolones (such as levofloxacin or moxifloxacin) and to at least one of the three injectable second-line drugs (amikacin, capreomycin or kanamycin) and need to be put on longer MDR-TB regimens to which one of the new drugs (bedaquiline and delamanid) may be added.

### Prevention and Control

- To maintain and reinforce the policy of boiling/pasteurization of milk and dairy products.
- Protecting workers in animal related occupations, especially the meat industry, from airborne transmission (13).
- To strengthen directly observed therapy, short course (DOTS) in tuberculosis control programs.
- Specific hygiene rules for foods of animal origin.
- Meat inspection system should be strengthened.
- All animals entering the food chain should be subjected to ante-mortem and post-mortem inspection.
- Screening by Tuberculin test for early recognition of preclinical infection in animals. intended for food production and early removal of infected animals from the herd.
- Segregation and phased slaughter of reactor animals.
- Reduce contact between domestic livestock and wild ruminants especially during grazing.

### Revised National TB Control Programme (RNTCP)

The National TB Programme (NTP) was launched by the Government of India in 1962 in the form of District TB Centre model involved with BCG vaccination and TB treatment (14). Thereafter revitalized NTP as Revised National TB Control Programme (RNTCP), was started in 1993. DOTS was officially launched as the RNTCP strategy in 1997 and by the end of 2005, the entire country was covered under the programme. RNTCP has also released a 'National strategic plan for tuberculosis 2017-2025' (NSP) for the control and elimination of TB in India by 2025.

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## Tyzzer's Disease: A review

Poorna Chandhar I, Madhu Swamy, Yamini Verma and Amita Dubey

Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Jabalpur, Madhya Pradesh

### Abstract

Tyzzer's disease is a fatal enteric disease of laboratory animals caused by *Clostridium piliforme*, a gram negative filamentous rod-shaped bacterium. They belong to the family Clostridiaceae. They are obligate intracellular spore forming bacterium with vegetative form ranging from 8-40  $\mu\text{m}$  in length. The important virulence factors produced by organism are cytotoxins which have high molecular weight, non-protease, temperature sensitive, protein molecules that are cytotoxic to mammalian cells. Affected animals show varied clinical signs ranging from mild anorexia to diarrhoea. Lesions are usually found in lower parts of intestine (Ileum, caecum and proximal colon), liver, and less frequently in heart of the affected animals. Although, considered as one of the important bacterial disease of laboratory animals, tyzzer's disease also affects and causes mortality in foals and birds.

### Etiology

Tyzzer's disease is a fatal enteric disease of laboratory animals. It was first described in mice in 1917 and later by 1965 in laboratory rabbits. Tyzzer described this disease in laboratory mice as intracellular bacteria. The causative agent was assigned to *Clostridium piliforme* on the basis of 16sRNA analysis in 1993 (1).

*Clostridium piliforme* is a gram negative filamentous rod-shaped bacterium. They belong to the family Clostridiaceae. They are obligate intracellular spore forming bacteria with vegetative form ranging from 8-40 $\mu\text{m}$  in length. They are motile by means of *peritrichous flagella* (2). Their spores are highly resistant to formalin (3). They are usually seen as slender bacillary organisms, apparently living within

liver cells bordering necrotic foci (4).

Tyzzer's disease frequently occurs in rodents, lagomorphs, foals and birds. Also occurs sporadically in other domesticated and wild animals like muskrats, cottontail rabbits, coyotes, dogs, snowleopards, lesser panda, greyfox, racoon, cattle, marsupials and white-tailed deer (5). Foals are acutely infected at 1-5 weeks of age and sometimes found dead on pastures with no clinical signs (2).

### Transmission

Transmission is through ingestion of spores from the environment (bedding, food, faeces of the infected animal).

The organisms form spores that can remain

infectious for at least a year in contaminated bedding. Spores are shed into faeces for 1-2 weeks post-infection.

Stress factors like overcrowding, change in environmental conditions and immune suppression can contribute to the outbreaks in laboratory animals. High protein diet predisposes animal to the disease (2). Zoonotic infection with *Clostridium piliforme* was reported in patients with immunosuppression (6).

### Pathogenesis

The important virulence factor cytotoxins which are higher molecular weight, non-protease, temperature sensitive, protein molecule. These cytotoxin affects the mammalian cells (2). B-cell deficient animals are especially susceptible to disease (7).

The organism after ingestion colonizes the apical surface of epithelial cells of intestine through phagocyte-like mechanism. Inside the cell they escape phagosome and replicate within the cytoplasm and nucleus of the host cell. Later, they destroy the host cell and get released into circulation. Through the portal vein, the bacteria reach the liver, resulting in bacteraemia which leads to spread of organism to all other tissues including heart.

### Clinical Signs

Most infections are subclinical and various environmental stressors may precipitate the clinical disease. Clinical signs most commonly occur in suckling and weaning rodents (7).

#### • Acute Disease

The disease is most commonly seen in suckling and weaning animals. They show rough hair coat, lethargy and greatly distended abdomen (8). Watery to pasty diarrhoea and perianal faecal staining can be appreciated in the affected animals.

#### • Subclinical Disease

In subclinical infections the signs are mild. Subclinical infections can develop into acute disease with stress factors like overcrowding, change in environmental conditions and immunosuppression (2).

### Gross Pathology

Lesions are usually found in lower parts of intestine (Ileum, caecum and proximal colon), liver, and less frequently in heart. Enlarged liver with multiple grey to white foci scattered throughout the mass are noted. These foci coalesce in severe cases. In intestine varying degrees of serosal edema with or without obvious haemorrhages can be noted. Small intestine may contain scanty ingesta whereas

caecum may contain abundant watery material. Severe lesions like necrotizing ileitis, typhilitis and colitis can be noted in severe cases. Mesenteric lymph node will be enlarged. In heart of affected animal, white streaks can be appreciated within myocardium.

### Microscopic Pathology

General degree of inflammation in response to the necrosis can be found in various affected organs. In acute cases necrotic foci surrounded by minimal primarily neutrophilic inflammation can be noticed in liver. In chronic cases foci may become mineralised or fibrotic. Intestine with single cell necrosis of luminal enterocytes can be noticed in acute cases. Lamina propria and serosal membrane may be oedematous and contains a mild neutrophilic infiltrate. In chronic cases extensive necrosis and lymphocytic inflammation along with necrotic foci at intestinal muscular layers can be noticed, especially in ileum. Hyperplasia of crypts and epithelium can be noticed during healing and repair of organ. In heart mild inflammation with small necrotic foci can be appreciated (2).

### Diagnosis

#### 1) Direct Microscopic Examination

Intracellular bacteria are found in viable cells adjacent to necrotic foci in an arrangement of "Bundle of sticks" or "Pick up sticks" (3). Stains like Haematoxylin and Eosin, stain the organism faintly so Silver stain like Warthin-Starry stains or Dieseris are used to enhance detection of these bacteria. Liver smears stained with giemsa also reveal the organism (8).

#### Serological Assays

Complement fixation test, indirect immunofluorescence assays and ELISA are commonly used (2).

#### 2) Cultivation of Organism

Direct cultivation of organism in media is mostly unsuccessful, hence cultivation in tissues or cell lines are carried out (4).

#### 3) Molecular Diagnosis

PCR on faecal samples can help determining the infected animal (9). PCR test to detect presence of *Clostridium piliforme* DNA by amplification of specific sequence associated with *Clostridium piliforme* 10sRNA in heart and liver samples from infected animals can be carried out (10).

Commercial services for screening faecal samples for presence of organism by 16s ribosomal analysis are also available (11).

Immuno assay is still regarded as the fundamental

screening tool for presence of *Clostridium piliforme*, with confirmatory diagnosis using PCR or microscopic examination.

### Differential Diagnosis

Tyzzer's disease is commonly differentiated from disease caused by organisms like *Francisella tularensis*, *Yersenia pestis*, *Yersenia pseudotuberculosis*, *Yersenia enterocolitis* which exhibits similar pathological lesions to that of Tyzzer's disease.

### Prevention and Control

- Decontamination of feed, bedding and water supplies.
- Infected spores can be inactivated by heating for 30min @ 80° C by routine autoclaving of associated fomites. Sodium hypochlorite or peracetic acid can be used to inactivate the spores.
- In endemic areas, depopulation with subsequent replication of *Clostridium piliforme* free animals is recommended.
- Tetracycline, Oxytetracycline, penicillin are the most efficient antibiotics against *Clostridium piliforme* (2).

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## Laminitis in Horses- A Case Study

M Akil, A K Soni, R K Bagherwal and R Singh

College of Veterinary Science and A.H., Mhow, Indore, Madhya Pradesh.

### Abstract

*Laminitis is a painful, destructive condition of lamina resulting from number of causations, commonly found in horses and ponies. It is manifested by partial weight bearing, stiffness and lameness. Two cases in mares were presented at T.V.C.C. MHOW with history of stiffness and partial weight bearing on forelimbs since one month. On palpation, percussion and radiographic examination of short pastern and coffin region it was diagnosed as laminitis. The horses were treated with phenylbutazone as drug of choice, along with supportive treatment for five consecutive days, both cases recovered successfully.*

### Introduction

Equine laminitis is a prevalent disease that affects thousands of equids of all shapes and sizes throughout the year. Despite laminitis commonly being thought of as only affecting ponies, in fact, horses are commonly affected too. It is potentially disastrous sequel of many equine diseases such as grain overload, colitis, endotoxaemia, and septic metritis (1).

The lamellae of the inner hoof wall, which normally suspend the pedal bone from the inner surface of the hoof capsule, fail and results in laminitis which causes deterioration of the lamellae and this affects movement of the pedal bone due to the degeneration of connections (2). The blood supply

of the foot is based on a delicate micro vascular system deep in the corium. Disturbances in this system have a direct influence on the dermal laminae that interdigitate with the horny laminae, when this blood circulation is disturbed, the normal alignment of foot also affected, resulting in characteristic lameness (3).

Therapeutic use of non-steroidal anti-inflammatory drugs (NSAIDs), such as acetylsalicylate, flunixin meglumine, phenylbutazone, have analgesic, antipyretic, and anti-inflammatory effects due to their ability to inhibit the synthesis of prostaglandins and provide relief to the animal. However, the benefits of NSAIDs outweigh their risks. As long as affected horses are stall rested and exercise is restricted, NSAIDs are beneficial in the treatment of

laminitis (4).

**Case History and Clinical Observations**

Two cases of horses with history of stiffness and partial weight bearing on forelimbs since one month were presented at T.V.C.C., Mhow. On palpation and percussion pain at short pastern and coffin joint region was observed. On basis of above clinical findings, it was tentatively diagnosed as laminitis and for confirmatory diagnosis it was referred for radiographic examination.



**Case No.1. Fig: Shifting of weight / Affected joint**

**Radiographic Examination**

Impression of muscle inflammation and slight enlargement of pastern and coffin joint was observed and it was diagnosed as laminitis.



**Treatment**

After confirmation, treatment was started with non-steroidal anti-inflammatory drug (NSAIDs) Phenylbutazone @ 2.2 mg/kg b. wt. intra-muscular for 5 days as a drug of choice. Along with this chlorpheniramine maleate @ 50 mg total dose I/M, Dexamethasone @ 30 mg total dose I/M and for supportive treatment multivitamins (Vit-B1, B6, B12) @ 15 ml I/M was given for five consecutive days once a day.

Oral preparation of serratio peptidase (75 mg) 2 bolus once daily orally, nervine tonic (neuroquick) 100 ml orally bid and diclofenac sodium spray was also prescribed for enriching the treatment and quick recovery. Both the mares showed good sign of recovery day by day and recovered successfully.



**Fig: Showing recovery of animal after treatment (Case No.1)**

**Results and Discussion**

Laminitis is a complicated disease of which the origin is not clearly understood. Achieving and maintaining a slim body condition is crucial, as overweight predisposes to laminitis. Regardless of etiology, laminitis ultimately results in deterioration of lamellae tissue. However, etiology affects pathogenesis and therefore determines the degree

of lameness.

Both cases of laminitis were recovered successfully and considered that an early diagnosis always have good prognosis. It is also concluded that Phenylbutazone may be used as drug of choice for treatment. Along with this supportive care may be provided by giving nervine tonic, anti-histaminic, oral and topical preparations which can helped to cure the disease quickly.

Usually acute laminitis has good prognosis but if it is not treated initially, then it can become chronic leading to less chances of recovery.

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## Kendrapara Sheep (*KujiMendha*)

Subhransu Mohan Nanda and Bhabesh Chandra Das

College of Veterinary Science and Animal Husbandry, Bhubaneswar, Odisha.

### Origin

Kendrapara sheep is predominantly found in the Kendrapara and in some parts of Jagatsinghpur district of Odisha. The state of Odisha is located in eastern India. It is surrounded by the states of West Bengal to the north-east, Jharkhand to the north, Chhattisgarh to the west and north-west, and Andhra Pradesh to the south. Odisha has 485 kilometres (301 mi) of coastline along the Bay of Bengal on its east, from Balasore to Ganjam. It is the 9th largest state by area and 11th largest by population. The State of Odisha is located on the eastern coast of India at 17° 49' North - 22° 34' North Latitude - 81° 29' East - 87° 29' East Longitude. Kendrapara district of Odisha is located at 20.50°N 86.42°E. It has an average elevation of 13 m (43 ft). It is surrounded by Bhadrak, Jajpur, Cuttack and Jagatsinghpur districts and Bay of Bengal in the east. The river Chitroptala (a branch of Mahanadi) is flowing out side of Kendrapara district. The other rivers of Kendrapara are Luna, Karandia, Gobari, Brahamani, Birupa, Kani, Baitarani, Kharasrota and Paika. This district has 9 blocks. Kendrapara is 58 km from Cuttack. This area receives heavy rainfall owing to the coastline. The total sheep population of Odisha accounts for

15,811,29 in number as per the 19<sup>th</sup> livestock census. This includes many indigenous breeds which have a marked significance in boosting the economy of the state. There are a variety of local sheep germplasm found in the state of Odisha. Some of them include Ganjam sheep, Koraput sheep, Machkund strain, Dharamagarh strain, Kathagarh-Dasmantpur strain, Erka sheep, Bolangir / Dharmagarh sheep, Chotanagapuri sheep etc. Apart from them, there is the famous and the most unique breed “KujiMendha” or “Kendrapara Sheep”.

### Characteristics of Kendrapara Sheep

Kendrapara sheep is known locally as “KujiMendha”. The National Bureau of Animal Genetic Resources (NBAGR) has conferred rare and singular species genetic recognition tag to this threatened breed (Breed Reg No INDIA\_SHEEP\_1500\_Kendrapara\_14042). This is the 42<sup>nd</sup> registered sheep breed of India. These sheep are fast breeders giving multiple births. Sheep in other parts of Odisha are not known to give multiple births. This characteristic makes them unique. They carry FecB mutation gene responsible for multiple births such as twins and triplets. This

characteristic makes the sheep distinct from other sheep of the Country. Kendrapara sheep was identified as the second in India and sixth in the world to carry FecB mutation gene. These animals are dwarf in built with the body covered with coarse hair. These sheep are mostly brown in colour. Head, face, belly and legs are bare. Rest of the body is covered with non-lustrous hair. These animals have horizontal ears; short, straight and drooping tail. Button type horns are found in few males. The average adult sheep weighs around 18-20 kg. The

Kendrapara ewe normally lambs twice in a year with the first lambing starts at the age of one year. The estimated population of the sheep in the state is around 1,23,000. They are primarily reared by small and marginal farmers for mutton purpose. They are well adapted to high ambient temperature, high humidity and heavy rain. The Government of Odisha through Kendrapara sheep breeder societies has taken number of steps to conserve the germplasm.

<b>Colour</b>	Mostly brown.
<b>Head</b>	Bare head.
<b>Horns</b>	Button type horns found in some males. Generally absent.
<b>Ears</b>	Horizontal.
<b>Tail</b>	Short, straight and drooping.
<b>Skin</b>	Covered with coarse hairs.
<b>Body weight</b>	Average adult weight is around 18-20 kg.
<b>Birth</b>	80% give birth to twins and 10% to triplets
<b>Lambings</b>	2 in a year.
<b>Height</b>	Dwarf in built.





## Kankrej Cattle: The Pride of Gujarat

B S Rathod, M P Patel, Y M Gami, A B Chaudhari and H H Panchasara  
Livestock Research Station, SDAU, Sardarkrushinagar, Gujarat.

### Introduction

Kankrej cattle, one of the oldest breeds of cattle as evident from wall pictures, statues and images from Sindhu culture, Haddappa and Mohenjo-daro cultures (large humped cattle with large half-moon shaped horn and a well-developed dewlap) domesticated by livestock keepers/farmers mainly for agricultural purposes, milk, hides and draught. Where and when precisely this domestication started is not clearly documented historically, but it is thought that cattle were probably first domesticated in India about 8500 years ago.

Our country is very rich reservoir of genetic diversity and possess some of the best breeds of cattle and buffaloes in the world. Cattle population of 191.00 million of India accounts for 17 per cent of the total world population of cattle (1). The best indigenous germ plasm of milch, draught and dual-purpose animals account for 22-25 per cent of the Indian cattle population, while 7-10 per cent of the cattle population is cross-bred. Among indigenous cattle breeds 75-79 per cent population is non-descript. Most of the indigenous breeds of cattle excel in draught capacity. Kankrej is an important dual-purpose cattle breed of India. The population of Kankrej breed is 30.28 lakh, of which 19.45 lakh cattle are pure and 10.83 lakh are graded. It is well adopted in North Gujarat as compared to other cattle breeds. The breed has been named after a Kankrej tehsil in North Gujarat of India. Though the Kankrej breed was named after Kankrej tehsil of Banaskantha district, Gujarat but it is abundantly found in Bani tract of Kutch District, North Gujarat and part of Rajasthan adjoining to Gujarat. It is also known by the different names as Bannai, Nagar, Talabada, Wagad, Waged, Wagadiya, Vaghiyar,

Wadhia, Wadher and Wadia. It originated from the south-east desert of Kachchh, along the banks of the Banas and Saraswati rivers, which flows from east to west and drain in to the desert. The breeding tract is Banaskantha, Kachchh, Patan, Ahmedabad, Gandhinagar, Mehsana, Surendranagar in Gujarat, Pali and Sirohi districts of Rajasthan and Tharparker district in Pakistan.

It is reared for both milk production and agricultural purposes. The average milk yield is 1200-1600 kg per lactation. Bullocks are strong and hard-working. It is well adopted to agro-climatic conditions of Gujarat and Rajasthan and have immense draught power with good quantity of milk and higher fat in milk even in stress conditions. Bullocks are well known for "Sawai Chaal" or "Majestic Gait" i.e. where the hind limb reaches further than the fore limb while walking leads to faster transportation / ploughing. It is selected for draught purposes since several generations hence it developed as one of the heaviest cattle on the earth with well-built body and are known for beautiful look and large half-moon shape horn. Coordination between horn, neck, tail and leg helps in balancing while faster walking i.e. Sawai Chaal. Similar to all *Bos indicus* breeds of cattle, the Kankrej also exhibits environmental adaptability to tropical and subtropical conditions, disease resistance, insect tolerance, better longevity superiority in utilising poor-quality roughages and maternal ability and fertility, especially compared with *Bos taurus* and its crosses. The Kankrej cattle are very active and gentle without a disposition problem but nervous and very much furious to strangers.

This is a very important breed and hence has been exported and accepted widely to many countries like

Israel, Brazil, Latin American countries and southern states of the USA.

### Physical Characteristics

Kankrej is one of the heaviest breeds of indigenous cattle whose coat colour varies from greyish white to steel black, sometime silver white and iron greyish white coat is also evident. Newly born calves have rusty-red coloured poll extending up to forehead, ears, back and tail which disappears with the advancement in age (6 to 9 months). Fore-quarters, hump and hind-quarters remain slightly darker than the barrel particularly in males. The switch of the tail remains black in colour. The face looks short with slightly upturned nose. Muzzle remains widely placed and completely open. The strong lyre shaped/half-moon shaped horns remain covered with skin up to two three inches which is at higher point than other cattle breeds. Ears of this breed are very large, pendulous and open, gets upward, active while approached or excited. The legs are very strong, active, particularly shaped and well balanced with small, round and durable hoof. Hump is well developed and not so firm, seen diverged to mostly right lateral side but comparatively less developed in females. The dewlap is thin, well developed and pendulous, similarly the penile sheath also remains pendulous in males. The skin is slightly loose and of medium thickness with dark pigmentation and gets darker during cool season. The hair coat is very soft and short and gets thicker during cool season.

### Strength of the Kankrej Breed

These cattle are resistant to tick fever and they have very little incidence of contagious abortion (brucellosis), tuberculosis and John's disease. It has also been observed that the red body colour is recessive in this breed. Viral diseases, bacterial diseases, protozoan diseases, reproduction diseases and other problems are comparatively of lesser headache in this breed as compared to crossbred and other indigenous breeds. It possesses excellent heat tolerance and have ability to survive in very harsh environmental condition with poor quality roughages as well as better feed conversion efficiency. They are the excellent grazers and can cover more distance than any other breed for grazing. Overall mortality and calf mortality is also very less (1-1.5 %) as compared to other breeds.

### Utility of the Kankrej Breed

It is a dual-purpose breed of Gujarat mainly reared for milch and draught. The cows are moderate milkers and yield about 1000 to 1400 Kg of milk per lactation under stall feeding conditions and lesser in grazing condition. Requires lesser concentrate and roughages (Dry matter requirement is less) as compared to other breeds. Bulls are powerful and fast having power rating of 0.7 horse power. It is excellent breed of cattle to be developed as milch breed through selective breeding or progeny testing. Because of its better utility compared to other breeds of cattle its population is highest and widely accepted by the farmers and ranchers as well as exported to other states of country like Tamilnadu, Kerala, Karnataka, Haryana, Uttar-Pradesh,

Maharashtra, Andhra Pradesh and Telangana. It has been also exported to a number of countries for pure breeding and cross-breeding.

### Scope for the improvement in Kankrej breed

The performance gap (almost double) between herd maintained at LRS, SDAU and animals maintained in the field indicates huge scope for the improvement in Kankrej breed at field level. It has very good potential in terms of milk production and reproduction, which is at par with the performance of milch breed and crossbreds. The highest recorded milk yield is 6198 litres/lactations (5415 SMY) adds further possibility/scope for the improvement (2).

### Brief success story of progeny testing program

The progeny testing program was started in 2009 with the aim to improve Kankrej cows maintained by farmers in the home tract. A total of 48 villages covering 5200 farmers, 6200 Kankrej cows were covered in the program. A total of 17098 inseminations were carried out, of which 8103 cows conceived and almost 2567 female progenies have born since inception. Total three sets of 8-9 bulls were inducted in the field. The first set was proven and daughters were producing almost 200 litres more than their dams. The breeding values of bull ranges from 50 litres to 245 litres (3). The use of proven bulls of first set will further improve the yield in farm and field to fasten the genetic gain. Few of the progressive farmers have started shifting from crossbred and buffalo to Kankrej breed for animal husbandry business.

### Why Kankrej?

Cow herding is not merely an economic activity, it is the symbol of an Indian culture, psyche and ethos. Contribution of livestock in agricultural and Indian economy has a special importance. Since time immemorial, cattle have proved their utility in agriculture, food, energy and transport sector etc. With the recent induction of two new breeds (Lakhimi and Dagari), the cattle genetic resources of India are represented by 43 indigenous breeds. It is truly established fact that betterment of Indian economy is meaningless without the wellbeing of cattle. In India, cow is called Kamdhenu due to its multi facet utility; milk production and its nutraceutical properties, fertilizing and insecticidal property of its dung and urine, draft power in agriculture and cultural values in the society. Right from the Vedic era till today, livestock improvement has been directed to meet changing demands. Initial thrust on cattle improvement was for draught hence, the most powerful breeds like Kankrej been developed over the years.

However, in recent past with the advancement and mechanization in agricultural operations and rural transport, the importance of animal draft power is reducing. It is apt clear that this situation is extending now to majority of small farm holdings in India. Consequently, there has been a major shift in utilities of cattle. The greatest victim of this paradigm shift in utility of cattle is indigenous breeds specifically draught breeds like Kankrej, as they were primarily used for draft purpose. This shift will have to be taken into account

while developing policies for improvement and conservation of cattle genetic resources of the country. Kankrej cattle is specially known for their unique characteristics of heat tolerance, disease resistance, majestic gait (Savai Chal) and ability to thrive under extreme nutritional stress, etc. In view of the stagnation or rather declining trend in the population of indigenous cattle, Central Government has taken initiative under "Rashtriya Gokul Mission" which aims to conserve, promote and develop indigenous breeds in a focused and scientific manner in their native tract.

We live in an ever-changing world where to fulfil our every growing demand, we are modifying the nature at a fast pace to suit our needs. But, interfering with nature can cause unpredictable results. The same applies to our food production system in general and dairy production system in particular. It has been found that there is huge difference between milk from exotic cows (Such as pure or cross breed which produce A1 Protein) and milk from cows of Indian Origin (Such as Gir and Kankrej which produce A2 Protein). A2 milk generally refers to a variety of cow's milk that mostly lacks a form of  $\beta$ -casein proteins called A1 and instead has mostly the A2 form. Milk of Indian cows contains A2 type proteins and moreover their milk contains important elements which helps to keep up fitness and vitality, helps in brain development and very useful in fighting dreadful diseases such as cancer, diabetes and heart disease. There seems to be only way to get goodness of milk is to GO DESI.

North Gujarat region is native tract of Kankrej cattle, which have adaptation to arid and semiarid climate. North and North-western Gujarat being drought prone area, face drought almost every alternate year. Due to lack of adequate feed and fodder, many old and unproductive cows are being poured to Panjarapoles. Where, practically no systematic genetic improvement efforts are being made to make them productive. Livestock Research Station, Sardarkrushinagar, SDAU was established in year 1978 with the mandate of conservation, genetic improvement and supply of superior germplasm of local indigenous breeds of north Gujarat. Efforts are in progress to establish and develop elite herds of Kankrej cattle with an optimum level of production & reproduction, through pure selective breeding. As a result of selective breeding, over last 40 years along with scientific feeding and better farm management this station has emerged out with the distinct animals of Kankrej cattle to be recognized as milch cattle breed. Livestock Research Station of SDAU has achieved a great milestone by improving production traits in Kankrej cattle as increase of 1397 liter (from 918 to 2315) in first lactation total yield (FLTY), 1707 liters (976 to 2683) in total lactation yield (TLY), 63 days (242 to 305) in lactation length (LL), 69 days' reduction (198 to 129) in dry period (DP). The reproductive performance such as age at first service (AFS) was reduced by 525 days (1364 to 839) age at first calving (AFC) by 290 days (1522 to 1232), service period (SP) by 40 days (155 to 115), calving interval (CI) by 10 days (445 to 435) and number of service per conception (NSPC) from 1.77 natural service to 1.68 in artificial insemination. The yield

of Kankrej in the beginning, was 977 liters with average lactation length of 225 days and age at first calving was 1522 days. These parameters were improved to the tune of almost standard for any dairy breed as 3408.9 liter (2683 milk in pail + 725.9 suckled by calf) standard lactation yield, 300 days' lactation length and 1218 days of age at first calving. Nearly 75% of the lactating cows are producing more than 2000 liters of milk during lactation. The wet average and herd average have shown the rise from 3.71 liter and 1.57 liter to 8.54 liter and 5.05 liter respectively (4, 5). Such achievements were not achieved in any other breeds of cattle in India. The highest record of 7251 litre (5415 SMY) milk production in one lactation is amazing and touching the standard of exotic breed such as H.F. and Jersey.

Besides this, Kankrej has remarkable ability to withstand the effect of environmental heat. The climate change has produced detrimental effect on Indian dairy cattle production and is indirectly contributing to economic losses. North Gujarat experiences about 6-9 stressful months in a year which is not congenial for optimum milk production; further, the Temperature Humidity Index (THI) ranges very high during monsoon. Various studies have indicated that in such climatic condition rate of decline in milk production is more pronounced in crossbred cattle as compared to indigenous cattle. The crossbred cattle perform better at 5-25° C temperature, while indigenous cattle feel comfortable even at a temperature of 38° C. Kankrej further have a potential to tolerate dry conditions and temperature up to 45° C. That is the reason Kankrej cattle has performed well as compared to buffalo and crossbred at station. The genetic improvement through rigorous selection is required for further improvement.

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## Congenital Disorders in Dogs

Ahlawat A R, Gamit V V and Odedra M D

College of Veterinary Science and A.H., Junagadh Agricultural University, Junagadh, Gujarat.

### Introduction

*Canis lupus familiaris* is considered to be the first domesticated animal. They are today the most morphologically diverse mammalian species, with a huge variation in size and shape (1), divided into more than 400 recognized breeds (2) and majority of the breeds are less than 200 years old.

Abnormalities of the body that are present at the time of birth are called congenital defects. Such anomalies may involve any organ system or any part of the body. Though certain defects may be quite obvious at birth, many may remain hidden for months, years, and some even lifelong. Congenital defects can occur for no known reason, can be inherited, can be caused by environmental factors, or can be a combination of any of these.

### Selective Breeding and Selection Pressure

Selective breeding has created more than 400 different breeds each representing genetic isolates with breed-specific morphological and behavioural characteristics. Frequency of common genetic disorders varies among breeds (3) and may be caused by random changes (i.e. genetic drift),

popular sire syndrome, selection for aesthetic traits linked on chromosomes to disease-liability genes, or anatomic or conformational aspects that can alter disease liability (3).

Dogs are second only to humans in the number of hereditary diseases identified in the population (4). Although no single registry maintains a record of genetic disease in dogs, it has been suggested that purebred dogs are more prone to genetic disorders than are mixed-breed dogs (5). Breeding practices and selection pressures used by breeders of purebred dogs have been implicated in the perceived high frequency of genetic disorders, whereas the random mating practices of mixed-breed dogs have been suggested to increase hybrid vigor (heterosis), resulting in healthier dogs (6).

Pedigree dogs are known to be at risk of a range of genetic disorders and a common assumption is that mixed breed dogs are healthier (7). Lesser popular breeds have a smaller gene pool (and thus less genetic variation) would be more likely to have a recessive disorder. Today over 600 genetic disorders have been described in dogs and that most of them are similar to human conditions, dogs have emerged as a clinically relevant model for

human inherited disorders.

**Hip Dysplasia**

Dysplasia' means abnormal growth. Hip dysplasia is one of the most common skeletal diseases seen in dogs. Hip dysplasia is a deformity of the hip joint (coxofemoral joint) that occurs during an animal's growth period.

**Breeds at Risk**

Large and giant breeds like German Shepherds, Rottweilers, Bulldogs, Great Danes, Saint Bernards, and Labrador Retrievers.

**Signs and Symptoms**

The development of hip dysplasia is determined by an interaction of genetic and environmental factors, though there is a complicated pattern of inheritance for this disorder, with multiple genes involved. Hip dysplasia is the failure of the hip joints to develop normally (known as malformation), gradually deteriorating and leading to loss of function of the hip joints. In this condition the ball of the femur cannot fit properly into the hip socket.

An affected dog may show absolutely no signs of this condition, whilst others may show severe signs. The signs of hip dysplasia include:

- Difficulty in rising or laying down and going up and down stairs.
- Inability to jump onto furniture or into a vehicle and reluctance to run or walk.
- "Bunny-hopping," or swaying gait.
- Narrow stance in the hind limbs (back legs unnaturally close together).
- Loss of muscle mass in thigh muscles.
- Enlargement of shoulder muscles due to more weight being exerted on front legs as dog tries to avoid weight on its hips.

**Treatment**

Depending on the degree of hip dysplasia, medical and sometimes surgical treatments are indicated. Medical therapy includes pain medications, physical therapy, cold laser treatments, and glucosamine chondroitin products. There are several surgical procedures available such as total hip replacement.

**Prevention and Management**

Maintaining your dog's ideal weight is one of the most important ways to reduce the clinical signs. Feeding a specifically formulated puppy food to puppies of high risk breeds so their growth is slower. The disease is inherited but not in a simple fashion

and there are many genes involved in the development of the condition. Therefore, it is very difficult to eradicate the disease absolutely and the best that can be achieved is a significant reduction in the disease levels by controlled breeding programs. Hip scoring should be considered as criteria for breeding programme, and breeders should choose breeding stock with hip scores around and ideally below the breed median score

**Epilepsy**

An epileptic seizure is "a transient occurrence of signs due to abnormal excessive or synchronous neuronal activity in the brain" (8). Epilepsy is a chronic condition that causes repeated seizures (which may be described by terms such as 'fits' or 'funny turns'), and is the most common chronic (long term) neurological disorder in dogs, a number of different underlying diseases and other factors can cause seizures leading to epilepsy. If no underlying cause can be found, the presumptive diagnosis for recurrent seizures is idiopathic (unknown basis) epilepsy.

**Breed at Risk**

German Shepherds, Beagles, Dachshunds, and Golden and Labrador Retrievers

**Signs and Symptoms**

- A seizure occurs when the cells in the brain become overly excited and exceed what is called a "seizure threshold".
- Dogs will typically stiffen and fall to the ground,
- Paddling' of the legs (as though he's treading water)
- Excessive salivation and drooling or foaming at the mouth
- Irregular seizure attacks that commence and finish suddenly
- Loss of consciousness and stiffness of the extremities
- Teeth chomping; chewing
- Temporary loss of vision
- Loss of control over bladder and bowels

**Treatment**

Epilepsy is managed with anticonvulsant medications. Depending on the dog, one drug or a combination of medications is used to control seizures. The goal of therapy is to manage seizures by decreasing their frequency and severity.

**Management**

The epileptic dogs may be kept in cool places and the dog should be kept away from pools. The hands should be kept away while the pet has an epileptic attack. One should manage the salt intake by the pet.

**Heart Disease**

Some types of congenital heart diseases in certain breeds have been shown to be heritable, or genetically passed down from the parents to the puppy. A congenital heart defect, or combination of defects, can occur as a malformation of any valve, chamber, or great vessel. A normal mitral valve functions as a one-way valve, which flows blood from the left atrium to the left ventricle. Deformities in the mitral valve cause it not to shut tightly, which results in the backflow of blood into the left atrium.

**Breed at Risk**

German Shepherds, Great Danes, Mastiffs and Toy breeds like Spaniels and Dachshunds.



**Signs and Symptoms**

- Heart murmur
- Hacking cough and Lack of stamina
- Fainting
- Weakness
- Dyspnea
- Heart failure
- Sudden death

Antiarrhythmic drugs can be used. However in advanced mitral valve dysplasia angiotensin converting enzyme (ACE) inhibitors to help lower blood pressure. Diuretics may help stimulate the kidneys to remove excess fluids from the body. Digitalis may be prescribed to help slow the heart rate and strengthen the heart's contraction. Vasodilator drugs can help the heart, to have a

better blood flow.

Management the patients that respond to the treatment plan will have an improved quality of life. The dog should be kept on low calorie diet and the diet should be modified to decrease the weight of the overweight dogs. The affected dog may be removed from the breeding programme.

**Degenerative Myelopathy**

Degenerative myelopathy (DM) is a slow progressive neurological condition caused by the deterioration of nerve fibers and their myelin sheath within the spinal cord.

**Breeds at Risk**

German Shepherd Water Spaniel, Bernese Mountain Dog, Boxer, Borzoi, Retriever, Kerry Blue Terrier, and Pug.

**Signs and Symptoms**

Age of onset of neurologic signs is usually 5 years or older with a mean age of 9 years in large dog breeds with DM(9). The failure of nerve signal transmission within the mid-to-lower spinal cord results in symptoms of the hind legs such as:

- Weakness and wobbliness
- Dragging of the hind feet
- Inability to stand
- Difficulty with urinating and defecating
- Paralysis

**Treatment**

Physiotherapy and principles of physical rehabilitation may improve the quality of life for the DM affected pet and pet owner. Overall, the long term prognosis of DM is poor, although it is hypothesized that DM is an immune-mediated neurodegenerative disease. Immunosuppressive therapies using corticosteroids have shown no long-term benefits in halting the progression of DM.

**Management**

Affected dogs do not experience pain from the nerve deterioration. As dogs lose the ability to support their hindquarters, it becomes increasingly difficult for them to urinate and defecate. Dogs with significant hind limb impairment can be greatly aided by the use of a harness or cart.

**Brachycephalic Syndrome**

"Brachy" means "shortened" and "cephalic" means "head". The skull bones of brachycephalic dogs are shortened in length, giving the face and nose a "pushed in" appearance. Due to the shorter bones

of the face and nose, the anatomy and relationship with the other soft tissue structures are altered, which may cause physical problems for the affected dog.

**Breeds at Risk**

Examples include the English bulldog, French bulldog, Boston terrier, Pug, Pekingese, Lhasa Apsos, Shih Tzus.

**Signs and Symptoms**

The average age of presentation for brachycephalic syndrome is 4 years. Clinical signs typically include

- Intolerance
- Heat intolerance
- Dyspnea
- Cyanosis and collapsing episodes
- Pet parents of these breeds become acclimated to their snoring and snorting sounds

**Management and Treatment**

Management in addition to breathing problems, the conformation of brachycephalic dogs also predisposes them to dental issues, skin issues and eye problems. Potential pet parents of these breeds should be aware that these dogs often need extensive maintenance care. Dogs with pronounced breathing difficulty or dogs that require surgery to correct airway obstruction should not be used for breeding.

**Conclusion**

Close cooperation among dog breeders, researchers, prospective purchasers, and purebred dog organizations at all levels is essential if genetically healthy dogs are to become a reality. Breeders should update themselves and be aware of all the genetic and phenotypic implications of breeding and should avoid breeding such males and females with these unwanted and undesirable characteristics. Dogs affected by genetic disorders should not be selected for breeding.

Prospective buyers should be made aware of the genetic diseases related to the breed they are considering. They should also ask a physical exam and test results or genetic histories for the animals they are planning to purchase.

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## Seizures in Dogs and Cats

Vishnurahav R B

University Veterinary Hospital and Teaching V C C, C V and A S, Mannuthy, Thrissur, Kerala.

### Abstract

*Seizures are an important clinical presentation in routine practice. This article describes the different causes, stages and clinical symptoms, diagnosis and treatment of seizures in dogs and cats. Idiopathic (unknown etiology), extra and intra cranial causes are the three important etiological factors of seizures in dogs. Based on the clinical signs of seizures, four different stages are classified as prodromal, aura, ictal and post ictal period. This article gives an important knowledge regarding the diagnosis and management of seizures in dogs and cats.*

### Introduction

A seizure is the clinical manifestation of excessive uncontrolled and hyper-synchronous electrical activity in the cerebral cortex. Seizures are the most commonly reported neurological disorders. The cerebral cortex is made up of neuronal cells, they communicate with each other via electrical activity. A sustained increase in muscle contraction lasting a few seconds to few minutes is called as tonic seizures (e.g. opening of the jaw, dorsal extension of the head and neck and extension of the limbs).

Clonic seizures are characterized by regularly repetitive, brief, involuntary muscle contractions (e.g. facial twitching, chomping of the jaws and

jerking movements of the neck and the limbs). Epilepsy is a chronic neurological condition characterized by recurrent seizures. The clinical features of seizures can be separated into four components: the prodrome, aura, ictal and postictal period (1,2).

### Prodrome

It is the period of time before the seizure begins, when the owner may report unusual behaviour such as agitation, attention seeking, whining and hiding. The prodrome may be barely noticeable in some animals and distinct enough to enable owners to accurately predict seizure onset in others.

**Aura**

It is the initial manifestation of the seizure, when animals exhibit stereotypical behavioural changes of sensory or motor activity (pacing, licking, swallowing), autonomic patterns (salivation, vomiting, urination) or abnormal behaviour (barking, attention seeking) for seconds to minutes before seizure onset.

**Ictal Period**

It is the actual period of seizure, when the animal exhibits a variety of signs that may include loss or derangement of consciousness, altered muscle tone, jaw chomping, salivation, and involuntary urination and defecation. This phase usually lasts only seconds to minutes.

**Postictal Period**

Postictal period immediately follows the seizure and can last from a few seconds to several hours, during which time the animal may exhibit abnormal behaviour, disorientation, altered thirst or appetite, somnolence, or blindness as well as defined sensory and motor neurological deficits. Epilepsy is a chronic neurologic condition characterized by recurrent seizures.

Seizures are classified mainly into two groups named focal or generalized seizures. Among these two types, generalized seizures being the most frequently seen. Stiffness noticed in all four legs and the dog lies down or falls before losing consciousness and convulsing. During convulsion

many dogs will salivate, urinate, defecate and vocalize.

Mostly duration of seizures will be few seconds to few minutes and some even longer. Focal seizures will affect one part of the body in milder form (one leg or part of the face). Any disturbance that either increases the electrical activity in the brain or decreases the control mechanisms that prevent seizures can cause these episodes. Seizures experienced by certain breeds of dogs - including Boxers, Yorkshire Terriers, Maltese and other terriers - are more likely to have an intracranial cause. Seizure disorders are classified according to their cause as being idiopathic, intracranial, or extra cranial in origin. Extra cranial causes like hypoglycemia, hypocalcemia, hepatic encephalopathy due to congenital shunt, parasitism and Lead and other toxicants are important differential diagnosis for the animals aged less than six months.

Dogs aged above six months to five years are suspected for intracranial primary epilepsy mostly idiopathic (70%) and intracranial secondary issues like trauma, inflammatory, vascular and neoplasia. Extra cranial causes like metabolic diseases, toxicity and cardiovascular diseases can also be suspected. Dogs aged more than five years are suspected for neoplasia, inflammatory and trauma of intracranial region. Metabolic diseases, cardiovascular and toxicities are also being suspected (1,2).

Idiopathic	Extra cranial (Outside of the brain)	Intra cranial (fore brain lesions)
<b>Primary epileptic seizures</b>	Toxins	Congenital malformations
	Metabolic diseases	Hydrocephalus
	Hypoglycemia	Neoplasia
	Liver disease	Inflammatory disease (Infectious)
	Hypocalcaemia	Granulomatous meningoencephalitis
	Hyperlipoproteinemia	Necrotizing encephalitis
	Hyperviscosity	Vascular disease
	Electrolyte disturbances (low Calcium)	Hemorrhage, Infarct
	Hyperosmolality	Metabolic storage diseases
	Severe uremia	Degenerative conditions

## Diagnosis

Diagnosis of extra cranial causes of seizures are relatively easy to identify with blood and urine tests. Diagnosis of intracranial causes of seizures need expert neurologist and advanced diagnostic techniques like MRI of the brain, and possibly a cerebrospinal fluid analysis (1,2).

Idiopathic epilepsy is a condition wherein the seizure threshold is decreased. This can be caused by intrinsic neurotransmitter imbalances, genetic mutations affecting ion channels, or other functional abnormalities. Epileptic foci contain cells with an intrinsic pattern of high spontaneous firing, leading to seizure activity. Idiopathic epilepsy has been shown to be inherited in a few dog breeds, and a familial basis for the condition is suspected in others.

Idiopathic epilepsy seizures with no known cause is the most common reason for recurrent seizures, and is the result of an inherent hyperexcitability of the neurons of the cerebral cortex. In general, dogs with idiopathic epilepsy are between one and five years of age when they have their first seizure, which is usually of the generalized type. In between seizures, they are completely normal, as are their neurological examinations(1,2).

## Treatment

In case of idiopathic epilepsy, first step is need to assess seizure frequency before starting treatment. Less than two seizures in six months does not require any treatment. If seizure frequency increases more than two in six months therapy is warranted (1,2).

Antiepileptic drug therapy should start with monotherapy. First drug of choice for single therapy is phenobarbital and its half life is 37 -73 hours. The best second choice drug is potassium bromide and some use it as first line drug of choice. Drugs used for monotherapy includes phenobarbital (2.5-3.5mg/kg PO q12h) or potassium bromide (20-30mg/kg PO q24h). If seizures are not controlled

after increasing phenobarbital to maximal tolerated concentrations, add potassium bromide to the phenobarbital. Phenytoin is a poor choice of drug in dogs and cats.

Second anticonvulsant might be added with above mentioned drugs included levetiracetam (20-30mg/kg PO q8-12h), zonisamide (5-10mg/kg PO q12h) and felbamate (15mg/kg PO q8h). In case of inadequate control of seizures, some other anticonvulsant drugs should be added. These drugs included gabapentin (10-20mg/kg PO q8h), topiramate (2-10mg/kg PO q12h) and pregabalin (3-4mg/kg PO q8h).

Diazepam (Valium) or midazolam, fast-acting drugs, will halt a seizure in progress. Repeat diazepam every ten minutes if effective (0.5 1mg/kg IV and 2mg/kg rectally). Diazepam can be administered maximum of four doses if necessary. Depending on the cause and the dog's response to treatment, longer-acting anti-seizure medications are also prescribed. Diazepam is a good first choice of drug in status epilepticus in cats (1).

## Conclusion

Client education is an important step to be carried out properly to explain that there is no complete cure for seizures. The aim of the treatment is mainly to reduce the frequency and intensity of the seizures to enhance the quality of life. Antiepileptic drug therapy starts with single drug followed by adding of another drug if animal did not respond to the first one. Add on therapy is carried out for the non-responsive seizures.

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## A Review of Bat Borne Zoonoses and Its Significance in Public Health

Abhishek Madan, Hariharan R, Ajay Kumar VJ and Bhanu Rekha V

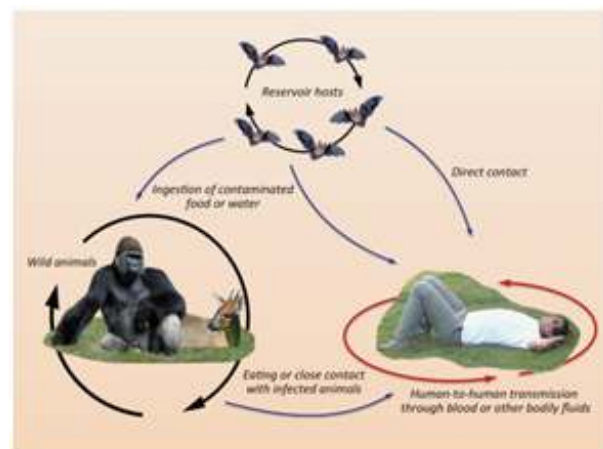
Department of Veterinary Public Health and Epidemiology, RGIVER, Kurumbapet, Puducherry.

### Introduction

Bats are considered as the second most abundant mammals in the world, next to rodents, with around 1300 species (1). Bats are found on all continents except Antarctica. They have variations in anatomy, their lifestyles and their roles in ecosystems. Bats fly with wings which range in span from 130 mm to 2 m (2). Bats of various species feed on insects, mammals, fish, blood, fruit and pollen making their feeding habits diverse. Most species use echolocation to navigate and to find prey. Myths about the roles of bats in ecosystems and their danger to other species as hosts of rabies virus have led to efforts to extirpate bat populations, with serious consequent effects on insect control and crop production, without coincidental reduction in the already low incidence of rabies virus transmission by bats (3). This is because bats play an important role in pollination. Their ability to fly long distances (up to 41,000 km has been reported), hibernate, live longer (up to 35 years in little brown bat), feed diversely and have a unique population structure make them potential hosts and reservoirs for transmission of zoonotic diseases especially viruses by spill-over mechanism. However, bacterial, fungal and rickettsial diseases which may be arthropod borne or transmitted individually by bats are of significance as well considering the emerging and re-emerging

scenarios of the diseases in the present years. The emergence of zoonotic diseases is a multi-dimensional event, involving many of the anthropologic factors like habitat destructions, change in land use patterns, change in human demography and behaviour; and the increase in encroachment to the wild life (4).

**Fig 1. Transmission Cycle of Diseases among Bats, Animals and Humans**



(FAO: Animal Production & Health manual, 2011)

**Viral Zoonoses**

Sixty-six viruses from 18 different families have been isolated from naturally infected bats worldwide. Some of the important zoonotic viruses have been discussed here.

**Lyssaviruses**

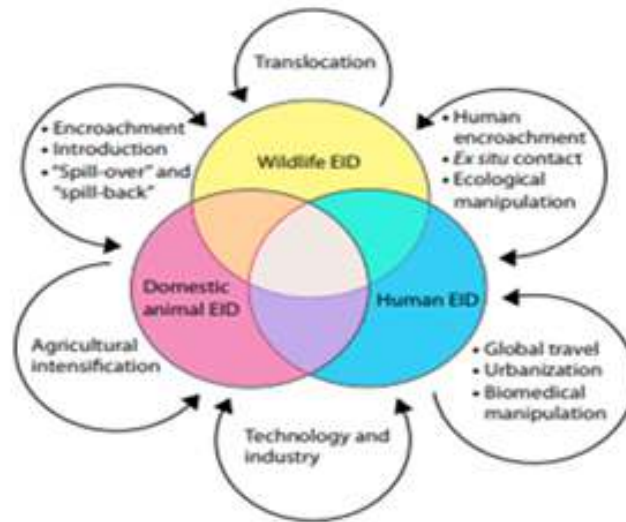
Rabies virus continues to be the most fatal disease transmittable by bats. The transmission is by vampire bats usually by aerosol route in caves wherein the air contains a high density of the virus. However other members of *Rhabdoviridae* such as *Lagos bat virus (LBV)*, *Duvenhage virus (DUVV)*, *European bat lyssaviruses type 1 and 2*, *Australian bat lyssavirus (ABLV)*, *Aravan virus (ARAV)*, *Khujand virus (KHUV)*, and *Irkut virus (IRKV)* which cause illness and signs similar to rabies are also transmitted by bats. One of the setbacks to canine rabies eradication program is the sylvatic cycle of virus transmission especially by bats to wild animals such as foxes and jackals. ABLV infections have been reported from Australia and Thailand indicating potential distance transmission due to flying ability of the bats. Diploid cell strain vaccine has been reported to be an effective prophylaxis against ABLV (5).

**Nipah and Hendra viruses**

*Henipaviruses* of the family *Paramyxoviridae* are also zoonotic pathogens of significance. *Nipah* and *Hendra viruses* are transmitted by *frugivorous* bats mainly called “flying foxes” of *Pteropus species*. The clinical signs of *Nipah* and *Hendra viruses* in humans continue to be acute febrile cases with encephalitis that leads to death. Bats act as reservoirs of the virus in their population. Both enzootic and epizootic patterns of the disease spill-over have been reported (6). Due to El Nino ecological effects the bats migrated to field near piggery and then caused pigs to be infected with the virus and also make them potential reservoirs. Culling of 1 million pigs was done to arrest the infection back then (7).

An outbreak in Kerala, India in May 2018 has also been linked to *frugivorous* bats. Man to man transmission has also occurred making it a re-emerging zoonotic pathogen (8). The control of *Nipah virus* involves culling of infected animals and controlling bat population but it is an uphill task to perform considering various transmission attributes of the bats.

**The host-parasite continuum: most emerging diseases exist in a host-parasite continuum among wildlife, domestic animal and human populations**



Source: Daszak, Cunningham and Hyatt, 2000

**Filoviruses**

*Marburg* and *Ebola viruses* belonging to *Filovirus* are also important zoonotic pathogens.

**Marburg virus:** First reported from African Green Monkeys which may have encountered fruit bats in Uganda in 1982. Roosting of bats is also reported to be a source of outbreak in Congo in 1998-2000.

**Ebola virus:** Since first outbreak in 1976 search for source of *Ebola virus* has kept researchers busy. Evidence of *frugivorous* bats as reservoirs has been proved by circumstantial evidence. The deadly haemorrhagic fever which has man to man transmission cycle possible has made this virus a dangerous zoonotic pathogen. The epidemic in

2016-2018 in Africa shattered social and economic security of countries like Guinea, Liberia and Sierra Leone. Also poor health care facilities in these countries are the reason for epidemic. As on March 2016 WHO has reported 28,610 suspected Ebola cases (9).

**SARS-CoV**

In 2002, *Severe Acute Respiratory Syndrome* (SARS) occurred in China and initial evidence suggested its association with wildlife meat industry. Palm civets were found to be main reservoir hosts by RT-PCR. Also, cave dwelling bats of *Rousettus sp.* showed serological evidence of antibodies to SARS-CoV indicating emergence of the virus from bats. Extensive epidemiological surveillance in southern China indicated Horse shoe bats of genus *Rhinolophus* were true reservoirs of the virus. Subsequent human to human infections may be due to adaptive mutations in the viral genome.

**MERS-CoV** – Middle-east respiratory syndrome corona virus.

This disease occurred in middle-east Arabian countries and Egyptian Tomb Bat (*Taphozous perforates*) seems to be the origin of the virus based on PCR test of the strain as it was found identical to that of MERS-CoV from humans. As of May 2018, 2220 lab cases with 79 associated deaths have been reported from Saudi Arabia making it a case fatality rate 38.6 per cent reinstating bat borne zoonotic potential of the virus (10).

**Other viruses**

Viruses such as *Menangle virus (Australia)*, *Tioman and Melaka virus (Malaysia)*, *Parainfluenza virus, Hantavirus, hepacivirus and Pegivirus* are zoonotic viruses which have potential to be transmitted by bats and it is necessary for further research to monitor spill over and emergence of these zoonotic diseases.

**Bacterial Zoonoses**

Unlike viruses, bacteria in bats and their putative threat to humans remain poorly studied. Here, some reports of bacteria responsible for common human and animal infections that have occasionally been detected in bats.

***Bartonella spp.***

Bartonellosis is a globally emerging zoonotic bacterial disease. *Bartonella sp.* is a Gram-negative bacterium transmitted through the bite of hematophagous arthropod vectors. Several species have been identified in bats. Recently, two species of *Bartonella*; *B. mayotimonensis* and *B. naantaliensis* were detected from both the peripheral blood of bats and in their ectoparasites,

suggesting that bats might be a source of the human bacterial pathogens. More recently, it has been reported the presence of closely related *Bartonella* genotypes in fruit bats and their associated bat flies in Madagascar, suggesting the transmission of a potentially zoonotic pathogen by bat fly vectors (11), (12).

***Pasteurella spp.***

*Pasteurella* is commonly spread among animals as part of the normal microbiota of the oral, nasopharyngeal and upper respiratory tract. This genus comprises opportunistic pathogen species that can cause endemic disease and are associated with epizootic outbreaks. In bats, various *Pasteurella* species mainly, *P. multocida* have been identified as the main pathogens of several localized and systemic infections. The predominant source of infections appears to be wounds caused by the bite of bats. However, a recent study from Wisconsin in USA reported for the first time an outbreak of acute pasteurellosis from *P. multocida* in wild bats without associated traumatic injuries (13).

***Leptospira sp.***

*Leptospira* has worldwide distribution and its transmission to humans is mainly through exposure to water contaminated with the urine of infected bats. Bacterium harbours in several wild and domestic hosts, colonizes their kidneys and it is eliminated in their urine. The presence of *Leptospira* in bats has been demonstrated in several studies. However, the potential role of bats in human leptospirosis is questionable. Bat exposure confirming bats as reservoirs of these bacteria has been provided in a case report (14).

Dense roosting behaviour of little bent-wing bats (*Miniopterus australis*) facilitating the transmission of virus among individuals



**Roosting behaviour of Bats**



**Wild Bat meat consumption  
(Possible source of infection to humans)**

**Enterobacteriaceae**

Several members of the *Enterobacteriaceae* family are responsible for a variety of human illnesses have been isolated from bats. A number of studies reported that *Salmonella* serotypes isolated from bats have similar characteristics to those found from livestock and humans, indicating that bats can be locally important in the epidemiology of salmonellosis in human and domestic livestock. Two of these serotypes, *S. Typhimurium* and *S. Enteritidis*, are a frequent cause of human and animal diseases. *Escherichia coli* strain has also been frequently isolated from bats. It is to emphasize the high percentage of multi drug resistance of these class of pathogens to several classes of antimicrobials that is a major and increasing global health care problem.

Antimicrobial resistance was also observed in domestic and wild animals, with an increased incidence of resistance in both pathogenic and endogenous bacteria. Resistant pathogens can then be transmitted to humans and bats can therefore contribute to the spreading of resistant bacteria. Several other genera – such as *Yersinia*, *Campylobacter*, *Vibrio* have been identified in bats, but their impact on these animals remains mostly unknown (15).

**Salmonellae**

*Salmonella* are important food and waterborne pathogens and the leading causes of the most widespread acute gastrointestinal illnesses around the globe. The organism has been detected in a wide range of host species such as mites, insects, crustaceans, mussels, fish, amphibians, reptiles, birds and mammals including wildlife animals. *Salmonellae* have been isolated in many species of bats in other countries. In the Philippines, there are 70 species of Philippine bats reported of which nine are considered as endemic (16).

Since *salmonellae* have been detected in a large variety of environment and host species including insects, these bats may have acquired these microorganisms in water and in their diet. This finding shows that Philippine bats may serve as potential reservoir and carrier of *Salmonella* organisms. The data also strongly indicates that bats may actively contribute in the dissemination of *salmonellae* into the environment through their guano. This currently makes Philippine bats as a potential threat to livestock and may pose a serious public health concern, since all serotypes of *Salmonella* are considered to be pathogenic to humans.

Family of bats	Bacterial species isolated	Source	Location	References
<b>Vespertilionidae</b>	<i>Campylobacter jejuni</i>	Faeces	the Netherlands	<i>Hazeleger et al. (2010)</i>
	<i>Clostridium perfringens</i>	Faeces	Czech Republic	<i>Hajkova and Pikula (2007)</i>
	<i>Listeria spp.</i>	Rectal swab	Poland	<i>Rozalska et al. (1998)</i>
	<i>Salmonella Enteritidis</i> <i>Salmonella Typhimurium</i>	Organ samples Organ samples	Germany UK	<i>Mu'hldorfer et al. (2011a)</i>
	<i>Shigella spp.</i> <i>Yersinia enterocolitica</i>	Rectal swab Organ samples	Poland Poland	<i>Ro'zalska et al. (1998)</i>

Family of bats	Bacterial species isolated	Source	Location	References
<b>Molossidae</b>	<i>Clostridium spp.</i>	Intestine	Togo	<i>Klite (1965a)</i>
	<i>Listeria spp.</i>	Intestine	Trinidad	<i>Ho¨ hne et. al., (1975)</i>
	<i>Salmonella spp.</i>	Intestine	Colombia	<i>Adesiyun et. al., (2009)</i>
<b>Mormopidae</b>	<i>Clostridium spp.</i>	Intestine	--	<i>Klite (1965a)</i>
<b>Desmodontinae</b>	<i>Salmonella Typhimurium</i>	Faeces	Brazil	<i>de Souza et. al., (2002)</i>
	<i>Shigella spp.</i>	Faeces	Brazil	
<b>Carolliinae</b>	<i>Clostridium spp.</i>	Intestine	--	<i>Klite (1965a)</i>
<b>Glossophaginae</b>	<i>Salmonella Typhimurium</i>	Faeces	Panama	<i>Klite (1965b)</i>
<b>Epomophorinae</b>	<i>Listeria monocytogenes</i>	Intestine	--	--
<b>Pteropodinae</b>	<i>Salmonella typhi</i>	Heart blood Organ samples	Madagascar	<i>Brygoo et. al., (1971)</i>
<b>Noctilionidae</b>	<i>Salmonella spp.</i>	Gastrointestinal tract	Trinidad	<i>Adesiyun et. al., (2009)</i>

**FUNGAL ZONOSSES**

***Histoplasma capsulatum***

*H. capsulatum* is a dimorphic pathogenic fungus of mammals, which causes pulmonary and systemic infections in humans and it is acquired via inhalation of the fungal spores. This microorganism is commonly found in soil associated with great amounts of birds' droppings or bats guano. Although bats are considered as the main reservoir and dispersers of this fungus in the environment, their role in spreading *H. Capsulatum* remains unclear. It has, however, been observed that subjects occupationally exposed to bat sites, such as miners, geologists or farmers who use bat guano as fertilizer, have high risk of infection and can develop severe clinical forms of histoplasmosis (17).

***Pseudogymnoascus destructans***

Although implications in human health for this microorganism are not known, it is important to write a few words on an emerging fungal disease, named WNS, responsible for the deaths of millions of bats in North America. It is caused by the psychrophilic (cold-loving) fungus *P. destructans* that infects the skin of bats – especially the wings – during the

winter months while they are in hibernation. Unlike other dermatophytes, which colonize the outer layer of the skin, *P. destructans* is able to invade the living tissue of the host causing the characteristic severe skin lesions. In addition, *P. destructans* increases the frequency of periodic arousals in bats, resulting in premature consumption of stored fat essential to survive the winter leading to death within 4 months of infection. Recently, it has been observed that bacteria of the *Pseudomonas* genus isolated from the skin of bats inhibit the growth of the fungus in vitro. Additional in vivo studies will tell us whether in the future they could be used as biological control agents to protect bats exposed to *P. destructans* (18).

**Conclusion and future perspective**

Emergence of new infectious diseases correlates with socioeconomic, environmental and ecological factors and are a major public health problem as well as an important burden on economies worldwide. Most of these are caused by zoonotic pathogens originating in wildlife and then spreading to humans. Bats are an important reservoir of several pathogenic agents, mainly viruses, and many of them have already caused disease outbreaks worldwide. The increasing rate of bat-

associated infections is also supported by an expanding overlap between bat and human habitats. Recently, to increase the knowledge of bat-associated viruses, a database has been constructed (<http://www.mgc.ac.cn/DBatVir>). DbatVir analyzes the virome diversity of bats as well as the ecological and epidemiological data to examine and track current and future bat-related transmissible diseases. To date, DbatVir has collected information on 5717 bat-associated animal viruses detected from 207 bat species in 77 different countries (update on 2 march 2016). Strategies on surveillance and monitoring of disease outbreaks in bat populations need to be further developed, in particular where bats and humans are in close contact. Extensive studies are also needed to improve our understanding of bat-human interactions to design new control measures in future. Furthermore, the identification of new human pathogens requires a continuous study to monitor the potential impact of these animals in their diffusion. One health approach to control and understand disease transmission and interaction between human and animal domains is necessary so as to provide sustainable time to time strategies to tackle emerging diseases of public health significance.

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## Biosecurity at Egg Processing Plant – Initiatives, Measures and Procedures

Naveen Z, Bindhu S and Sudheer K

Department of Livestock Products Technology, Sri Venkateswara Veterinary University, Tirupati and Veterinary Dispensary, Pincha, YSR Kadapa Dt., Andhra Pradesh.

### Abstract

*Biosecurity refers to programs intended to protect human, animal or even plant life. Biosecurity can be defined as a practice or set of programs that will limit or prevent the introduction and spread of diseases and prevent the contamination of production facilities. Biosecurity procedures include documentation and training, operational standards for facility, personnel etc. Free range production and High risk biosecurity operations are discussed.*

### Biosecurity

Biosecurity refers to those measures taken to prevent or control the introduction and spread of infectious agents to a flock. Such infectious agents, whether they cause clinical or subclinical disease, significantly reduce the productivity, profitability and long term financial viability of a poultry operation. Biosecurity is about managing risk to meet the objectives stated above. It is essential that a risk assessment be conducted for each enterprise to establish what level of risk exists in each phase of its operations and to identify and implement control measures appropriate to these levels of risk.

### Global Initiatives on Biosecurity

European Economic Community [EEC] commission decision (2005/734/EC) of 19 October, 2005 was adopted laying down “Biosecurity Measures” to reduce the risk of transmission of highly pathogenic avian influenza caused by *Influenza 'A' virus* of subtype H5N1 from birds living in the wild to poultry

and other captive birds and providing for an early detection system in areas at particular risk(1).

This decision was amended on 21 October, 2005 by adding article (2a) re-additional risk mitigating measures as follows

- a) The keeping of poultry in the open air is prohibited without undue delay; however, the competent authority may authorize the keeping of poultry in open air provided the poultry are provided with food and water indoors or under a shelter which sufficiently discourages the landing of wild birds and prevents contact by wild birds with the feed or water intended for poultry.
- b) Outdoor water reservoirs required for animal welfare reasons for certain poultry are sufficiently screened against wild waterfowl.
- c) The poultry is not provided with water from surface water reservoirs accessed by wild birds, unless such water was treated to ensure

inactivation of possible virus.

- d) The use of birds of the orders *Anseriformes* and *Charadriiformes* as decoy during bird-hunting is prohibited.
- e) Members States shall ensure that the collection of poultry and other birds on markets, shows, exhibitions and cultural events is prohibited(1).

The Association of Southeast Asian Nations (ASEAN) held a meeting represented by their Agricultural and Health Ministers on 28 January, 2004 in Bangkok. Recognizing the potential serious impact of *Avian Influenza* (AI) on global public health, livestock production, trade and economic development, decided to

- Commit to more stringent surveillance and effective response systems, improved research and development capabilities, and sharing of information and technology.
- Intensify national, regional and international efforts to tackle the outbreak of AI and future similar threats.
- Implement domestic measures to control AI having regard to the recommendations of **World Trade Organization [WTO]**, **World Organization for Animal Health [OIE]**, **World Health Organization [WHO]**, and **Food and Agriculture Organization of the United Nations [FAO]**.
- Work closely with OIE to strengthen guidelines on reporting and surveillance system.
- Promote rapid, transparent and accurate exchange of scientific information to provide early warning of potential outbreak, and consider creating a regional veterinary surveillance network to link it with human health surveillance mechanisms.
- Strengthen cooperation with regional and international organizations and joint research and development initiatives to reduce the hazards of epizootic outbreaks on human health, share best practices, devise counter measures, and develop effective, low-cost diagnostic test kits, vaccination and anti-viral drugs.
- Call for assistance and exchange of expertise to assist affected countries to enhance their epidemiological and laboratory capacity for prompt detection, monitoring, surveillance and controlling of the disease.
- Investigate options for designing more Biosecurity developments of the poultry sector for both small scale and commercial production.

**Enforcement of Biosecurity Measures(1)**

Having deliberated most literature on the subject of Biosecurity, one can accomplish the following

- Biosecurity is a relatively new concept. It has been emphasized in a meaningful way after the emergence of *Avian Influenza* in many parts of the world.
- Biosecurity was, until the emergence of *Avian Influenza*, used in conjunction with biosafety and bioterrorism and referred to measures to produce safe food for human consumption.
- FAO started addressing Biosecurity until recently but several years after the emergence of AI(2).
- If not for the highly pathogenic form of *Avian Influenza* (HPAI) not hit the poultry industry hard and HPAI not affect humans by making certain of them ill or die, neither the United Nations, represented by FAO, nor governments would have resorted to Biosecurity actions and measures to reduce the spread of this disease or ultimately other poultry diseases as well (3).

Biosecurity measures within the capacity of individual farmers or poultry companies, were implemented at varying degrees for a long time (4). These measures were taken in view of their economic benefits to such institutions. Benefits included avoidance of bacterial disease infections such as coryza, cholera, mycoplasmas, salmonellas, and reduction of exposure to viral diseases such as Newcastle and recently *avian influenza*. Even though the level of Biosecurity on any farm needs to be continuously upgraded and improved, certain Biosecurity measures cannot be imposed by individual farmers or poultry companies. Such issues need to be handled by authorities or governments.

It seems that governments, due to the complexities of issuing laws and acts, have so far not adopted certain Biosecurity measures that could certainly reduce disease transmission from one farm to another or from ranging birds to other ranging birds or commercial farms.

Therefore, in order to really reduce the risks of poultry disease transmission, particularly *Avian Influenza*, governments have to interfere by issuing acts and directives in the form of laws and enforce their implementation (5).

**Biosecurity Procedures**

**1. Documentation and Training(6)**

**Objective**

To ensure awareness and training of all production area employees in relevant biosecurity requirements.

- Each production facility must keep a copy of the Manual readily accessible to staff.
- Staff must be provided with training in the relevant parts of the Manual and such training is

to be recorded and shall be continuous.

## 2. Facility Operational Standards

### Objective

To limit and control access to poultry production areas by vehicles and people, and prevent as much as possible access by livestock, wild birds and other animals (including rodents).

- The production area must have a perimeter fence establishing a clearly defined biosecurity zone.
- If livestock graze the property, then the production area must have a stock proof fence. Grazing near sheds (i.e. on part of the production area as defined in this Manual) is only permitted where the grazing area is separated by a stock proof barrier from the area used by poultry, effectively preventing transmission of contaminants from grazing livestock to poultry, and the grazing area is not used for access to other parts of the production area. Drainage from livestock pastures or holding areas must not enter poultry enclosures or areas that can be accessed by poultry (e.g. through fences). In free-range egg operations where either dogs or alpacas are used to protect fowl in the range area from attack by foxes, feral dogs or birds of prey, these guard animals should be tested for freedom from salmonella by faecal microbiological culture prior to introduction and ideally re-tested annually.
- A sketch or map of the layout of the property, showing the production area, sheds, ranges, access roads and gates must be created and maintained and kept current. This must be readily accessible to all staff and visitors.
- The main entrance to the production area must be capable of being closed off to vehicle traffic (e.g. lockable gate which, where feasible, should be kept locked at all times) and must display appropriate signage including "Biosecure Area - No Entry Unless Authorised" or similar wording. In addition, signage must direct visitors to contact the producer before proceeding i.e. telephone number and/or enquire at house.
- There must be a parking area for vehicles not entering the production area. There must be a change area away from sheds with clean protective clothing and boots provided. Showering and changing into clean protective farm clothing is preferable, particularly for pullets that are susceptible to endemic poultry diseases until they have completed their vaccination program.
- Entry to sheds must only be made through entrances where a footbath exists containing a

suitable disinfectant used in accordance with company or manufacturer's instructions and changed regularly before the disinfectant deteriorates and loses effectiveness. There must be provision for scraping the soles of boots before dipping to ensure the sanitiser is making contact with the soles of the boots. Facilities for hand washing/ sanitation must also be placed at the entry of each shed. In free-range operations similar foot bath procedures should apply for access to the production area to avoid the possible introduction of offsite pathogens. While footbaths provide a degree of security in regard to the incursion of pathogens into the production area it is preferable to have the policy that requires a change of footwear at the boundary of the shed/range area. Each shed/ production facility should have its own footwear to change into.

- Dead bird storage and disposal methods must conform to applicable hygienic containment and environmental compliance requirements (7).
- All poultry housing must be designed and maintained so as to prevent the entry of wild birds and limit the access of vermin as far as is practical. The control of wild birds has limitations in free-range operations.
- **Free-range landscape** - trees, shrubs and other range amenities should be selected to minimise the risk of attracting the types of wild birds that are a high biosecurity risk, particularly in free-range operations. The area around sheds must be kept free from debris and vegetation, and should be mown regularly to discourage wild birds, insects and rodents which are potential disease vectors. Vegetation buffers for environmental compliance should not be compromised. Trees may be used as shelter belts, along fence lines and on free-range premises to provide shade and provide poultry with some protection from unfavourable ambient conditions and flying predators.
- **Drainage** - The production area should be adequately drained to prevent accumulation and stagnation of water likely to attract water fowl, especially in the areas around sheds and range areas. Standing water may also increase the presence of insects which can act as significant disease vectors. A range management plan should be implemented to manage pot-holes or water pooling after heavy rain falls.
- An appropriate vermin control strategy and plan must be developed and implemented, including rodents, foxes, and wild dogs and cats.
- A baiting program for rodents must be implemented where a risk assessment deems this necessary (live rodents, droppings, nests).

- Drinking water for poultry, as well as cooling water (fogging or cooling pads) used in poultry sheds, must meet appropriate water standards (8). Water that does not meet the standard must be effectively treated to ensure that the standard is met. All surface water (dam, river, channel, rainwater catchment, etc.) must be effectively treated and sanitised before being used as drinking, cleaning or cooling water for poultry.
- Treated and sanitised water supply must be kept in a closed system from the point of treatment to its time of utilisation for drinking water or cooling.
- Only pullets and/or laying fowl are to be kept in the production area and no other avian species (including aviary birds and pet birds).
- While not a preferable practice, if more than one commercially produced avian species is kept in the production area, the species must be housed and managed separately, with suitable internal biosecurity arrangements for each species as well as the overall property boundary biosecurity for the entire site (9). Shared equipment must be cleaned and disinfected between uses. The risk of increased endemic disease should be considered as an increased risk assessment in such mixed operations. As domestic species of waterfowl can be asymptomatic carriers of AI, they should never be housed on sites where other types of commercial poultry species are present.
- Feeding systems must, wherever possible, be closed to ensure that feed in silos and feed delivery systems are protected from access and contamination by wild birds and rodents. Feed spills outside the shed must be cleaned up without delay to prevent the attraction of wild birds and vermin.
- Where bird weighing is practised, it must be carried out using the production area's own weighing frames and scales. Company service personnel can use their own scales provided that they are cleaned and disinfected when moved between production areas.

### 3. Personnel Standards and Procedures

#### Objective

To minimise the risk of introducing or spreading a disease or contaminant through vehicle and/or people movement, including staff (including production, service and grading floor personnel), contractors, suppliers and other service personnel, visitors and family members and to document such movements to facilitate tracing in case of a concern. To minimise the risk of introduction of disease or contaminants by production personnel.

### 4. Operational Standards

Water Supply, Vermin Baiting, Cleaning, Ground, Amenities Maintenance and Record Keeping.

### 5. Grading Floor and Egg Processing Specific Additional Biosecurity Requirements

Egg grading floors, Egg processing floors, EAD traceability

### Free-Range Production Operations

This applies to caged, barn and free-range operations. It is recognised that free-range birds will potentially have increased exposure to some avian pathogens. Diseases such as internal and external parasites, fowl cholera and miliary hepatitis (Spotty Liver) are more commonly recognised in laying poultry farmed under extensive conditions. While it is difficult to apply standard hygiene practices to free-range areas the basic biosecurity principles of preventing the introduction of disease by controlling movement of livestock, equipment and personnel still apply. The use of enhanced vaccination programs and strategic prophylactic medications are a useful tool to limit disease build up on free-range operations. Increased exposure to wild birds is considered a biosecurity risk and most importantly to waterfowl, particularly wild ducks belonging to the Order Anseriformes (includes the Wood duck, Chestnut Teal, Freckled duck, Black duck and Whistling duck). It is important for the free-range area not to have environmental and amenity factors that attract congregations of large numbers of wild birds or surface water for ducks. Wild water fowl surveillance identifies that most ducks have at some stage been exposed to *avian influenza* (AI) and more importantly at any one time a small percentage of these are shedding virus in their faeces that can contaminate surface water and pastures. Some AI of the H5 and H7 subtypes which have in the past caused EAD outbreaks in the egg industry. In all cases there was evidence of an association between wild ducks and contamination of drinking and/or cooling water or direct physical contact. Control programs that reduce and eliminate the presence of wild waterfowl on free-range areas should be a priority consideration for all free-range operators. This will involve the absence, or elimination, of water catchments and other surface water within and in the vicinity of the free-range area, the netting of retention dams, the destruction of wild water fowl where and when legislation permits and the use of aversion programs. Good fencing is required around freerange farms to prevent entry of animals such as foxes. In many situations, however, fencing alone is insufficient to stop such intrusions; therefore, some free-range enterprises keep specially trained dogs or guard animals such as alpacas with the chickens to reduce predation by foxes and birds of prey.

## High Risk Biosecurity Operations

### Objective

To enhance biosecurity protection by strategically assessing movements to protect the property from the increased threat of a disease being introduced from the outside in the face of a suspected outbreak of an emergency disease or a serious endemic disease (6).

### 1. Action Plan for Suspected Emergency Animal Disease [EAD]

It is imperative for all egg producers and handlers to be aware that there is potential for an EAD to occur at any time and thus producers must be proactive at all times to ensure biosecurity procedures are in place that will prohibit the entry into the poultry operation of an emergency or serious endemic disease. For an EAD, the level of biosecurity at all times must be optimal because the infection will occur before clinical signs are observed and thus there will be a period of potential "silent" spread prior to any industry awareness of the EAD. This is an important concept for all horizontal contacts (egg producers, transporters, clean out and vaccination crews) who are potential spreaders of an EAD while not aware of its presence. The preventive activity level should be of high awareness at all times and not just during a suspect EAD or after an outbreak notification.

- Each producer must establish and document clear guidelines regarding the circumstances when an EAD alert should be raised (e.g. an unusual increase in mortality or drop in production), and who must be informed. The action plan must also clearly state that, if an alert is raised, movement of birds, eggs and egg products, disposables, equipment and personnel from (and onto) the suspect property must immediately cease and/or be strictly controlled. For other farms and properties which are close horizontal contacts, movements must have a risk-based assessment.
- The frequency of monitoring of mortality and production variations is to be increased and enhanced across the operation including in contact properties.
- Senior management or the operation's veterinarian must be immediately notified who will assess the situation to consider or rule out an EAD. The directions given regarding biosecurity, livestock and product movements must be strictly followed and all other relevant personnel made aware of them.
- In the event of a suspect EAD being notified the state Chief Veterinary Officer becomes the responsible entity with the legislative authority to implement livestock movement controls and enforced quarantine.

### 2. Standard Operating Procedures (SOPs)

SOPs will be available for any specific outbreak of an EAD from Animal Health.

### Conclusion

*Avian Influenza* (AI) is continuing to spread in the world, even though at a slower rate since the use of effective vaccines, especially in the countries where compensation is not possible and hence stamping-out fails, and where rural and backyard, non-vaccinated, poultry exists on a large scale.

Biosecurity is a well proven means of checking the spread of this as well as other contagious poultry diseases. However, Biosecurity measures have so far, been implemented by farmers at their own consent and will. Mandatory Biosecurity measures may have been imposed in the context of biosafety and food security. Since AI is a threat to humans, and since the benefits of Biosecurity exceed avoidance of exposure to AI to improve poultry performance, enforcing Biosecurity measures on poultry farms and related facilities, such as hatcheries and slaughter houses, should be seriously considered and adopted by FAO and OIE.

In turn, FAO and OIE should persuade all governments of the world to adopt the same Biosecurity measures and enforce them by appropriate legislations and laws.

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## How Milk and Urine of Indigenous Cow Breeds are Useful

Akhilesh Pandey and Yogita Pandey

Department of A.G.B. & V.A.N., College of Veterinary Science, Jabalpur, Madhya Pradesh.

### Introduction

Historically it is fact that humped cattle remains were found in Mohenjo-daro site of Indus Valley indicating their presence in India even before the arrival of Aryans. Now they are known as indigenous cattle, scientifically called as *Bos indicus* or Zebu cattle, mainly inhabitant of the Indian subcontinent. It is thought to be world's oldest domesticated cattle. Now a days, a lot of emphasis has been given on the medicinal use of cow urine in India. Recently the cow urine has been granted U.S. Patents (No. 6,896,907 and 6,410,059) for its medicinal properties, particularly for its use along with antibiotics for the control of bacterial infection and fight against cancers (1). Traditional medicinal practitioners of Buddhist culture in Myanmar are still using cow urine therapy for many ailments with encouraging results. The customs practiced have roots which date back to the Buddhist era over 2500 years old. The growing awareness among the general public about the side effects of allopathic medicine is making them to use cow urine therapy/panchagavyachikitsa for few ailments wherein some benefits are derived. Though, the end user claims are many, scientific validation of those claims is required. However, scientific validation of panchgavya products is required for its worldwide acceptance and popularity in terms of agricultural, energy resource, nutritious and medicinal applications so as to exploit the optimal power of panchgavya for the service of mankind. Regardless of scientific validation, people are using and getting benefits of it. Like urine, milk of the indigenous breed show positive effect because of A2 beta-casein. It has no known negative effects on human health. In the past few thousand years, a

natural mutation occurred which has resulted in a proportion of cows of European breeds producing a casein variant called A1 beta-casein. Slowly, these protein variants became dominant in milk which producing A1 milk. The gene encoding beta-casein was changed such that the 67th amino acid in the 209 amino proteins was switched from proline to histidine. This new kind of beta-casein that was created is known as A1 beta-casein which is found in the milk of many crossbred cows such as Holstein, Jersey and Friesian (2). The bovine beta-casein gene is part of a cluster of 4 casein genes (alpha-S1 -casein, alpha-S2 -casein, beta-casein and kappa-casein) located on chromosome number six. Release of BCM-7 from the hydrolyzed raw or processed milk is related to the beta-casein A1 allele, irrespective of a lactation period.

### How exotic breeds cow milk is harmful?

#### 01. Relation of beta-casomorphin-7 to apnea in sudden infant death syndrome

*Sun et al.*, (2003) (3) reported that Sudden Infant Death Syndrome (SIDS) is the most common cause of death in infants. Beta casomorphin-7 absorbed through the gastrointestinal tract crosses blood-brain barrier (BBB) and inhibits the respiratory center in the brainstem leading to apnea and death of the infant.

#### 02. Efficacy of human beta-casein fragment (54-59) and its synthetic analogue compound 89/215 against *Leishmani adonovani* in hamsters

*Sharma et al.*, (2004) (4) reported that several rationally designed analogues of human beta-casein fragment (54-59) were evaluated for their ability to stimulate the non-specific resistance in hamsters

against *Leishmani adonovani* infection.

### 03. Beta-casomorphins-7 in infants on different type of feeding and different levels of psychomotor development

This study was conducted on a total of 90 infants, of which 37 were fed with breast milk and 53 were fed with formula containing cow milk (5). The observed data indicate that breast feeding has an advantage over artificial feeding for infants' development during the first year of life and support the hypothesis for bovine beta casomorphin work as a risk factor for delay in psychomotor development and other diseases such as autism.

### 04. Beta-casomorphins-7 alters $\mu$ -opioid receptor and dipeptidyl peptidase IV genes expression in children with atopic dermatitis

Atopic dermatitis (AD) is a chronic inflammatory skin disease with heterogeneous clinical phenotypes reflecting genetic predisposition and exposure to environmental factors(6). Reactions to food may play a significant role especially in young children. Milk proteins are particularly strong allergens and are additional source of bioactive peptides including  $\beta$ -casomorphin-7 (BCM7, Tyr-Pro-Phe-Pro-Gly-Pro-Ile). BCM-7 exerts its influence on nervous, digestive, and immune functions via the  $\mu$ -opioid receptor (MOR). Proline Dipeptidyl Peptidase IV (DPPIV) appears to be the primary degrading enzyme of BCM-7. Moreover, DPPIV is known to restrict activity of pro-inflammatory peptides. BCM7 is considered to modulate an immune response by affecting MOR and DPPIV genes expression provide an important diagnostic tool.

### 05. Beta-casomorphin (BCM) and human colonic lamina propria lymphocyte proliferation

Recently, modulation of the immune system by BCM-7 was also reported(7).. Investigated the in vitro effect of BCM-7 on the human mucosal immune response as represented by lamina propria lymphocyte (LPL) proliferation. Results show that BCM-7 significantly inhibited concanavalin A (ConA) stimulated LPL DNA synthesis. BCM -7also inhibited ornithine decarboxylase activity (ODC) in ConA-stimulated LPL. The anti-proliferative effect of BCM-7 was reversed by the opiate receptor antagonist, neloxone. Our results suggest that BCM may affect the human mucosal immune system, possibly via the opiate receptor.

### How much desi breed Cow Urine is beneficial for different purpose?

### What type of cows are suitable for collection of cow urine?

Jersey and Crossbred cow are not suitable and only the Indian traditional cows are suitable for collection of cow urine(8).

### Cow Urine Contents

Cow urine contents are 95% water, 2.5% urea and 2.5% minerals, salts, hormones, and enzymes(9)..It contains Iron, Calcium, Phosphorus, Carbonic acid, Potash,Nitrogen, Ammonia, Manganese, Sulphur, Phosphates, Potassium, Urea, Uric acid, Amino acids, Enzymes, Cytokine, Lactose etc.

Composition of urine varies according to the species, breed, season, physiological status, quality and quantity of water consumed along with few pathological conditions as in diabetes and ketosis(10).

### Anti-Diabetic Effect

Cow's urine mixture, a traditional remedy for convulsions, administered intra-peritoneally to fasting grey rabbits was found to produce marked depression of their plasma glucose. A similar effect on the plasma glucose was produced by a sample of cow's urine mixture given by the nasogastric route(11). These findings confirm that the mixture has hypoglycaemic effect when given both parenterally and orally, the latter being the usual mode of administration of this concoction.

Cow urine reduces the blood sugar level (12). The most probable mechanism of action is that it increases the glucose transport across cell membrane thus it increases peripheral glucose utilization. It increases glycogen synthesis from glucose, and also increases insulin release from beta cells of pancreas. Cow urine enhances sensitivity of insulin receptor, decreases insulin resistance, and decrease insulin absorption from intestine. Various herbal metabolites are present in cow urine which produces anti hyperglycaemic effect.

### Disinfectant Effect

Cow urine acts like a disinfectant and thus purifies atmosphere and improves the fertility of the land(13). Urine used as fertilizer is the best in cultivation analyses, owing to the availability of its nutrient which, at the same time combined with soil humidity, acts as an optimal environment for the micro fauna and the mineralization process.

### Anti-Microbial Effect

Copper has the power to destroy diseases and act as an antidote(14). Cytokines and amino acids might play a role in immune enhancement. Gomutra alone has got all such chemical properties, potentialities and constituents that are capable of removing all the ill effects and imbalances in the body. Cow urine contains various inorganic compounds including silver, Na-K ratio of 4:1 (36%:9% in dried urine), apart from 3% urea.

### Anthelmintic Activity

Cow urine shows marked anthelmintic activity by causing paralysis and death of worms in a dose

dependent manner.

### Anti-Inflammatory Property

Fresh cow urine also contains 50-100 mg oestrogens/100 ml; 20- 200 µg of cortico-steroids /100 ml and 0.05-0.15 mg of 17-keto-steroids/100 ml. Due to corticosteroid it shows anti-inflammatory property(15). Cow urine helped in wound healing in dogs on dorsal neck(16).

### Bio-Enhancement Effect

Bio enhancement has also been observed with other drugs viz. Ampicillin, Isoniazid, Clotrimazole, Cyanocobalamine etc. Bioenhancer activity has been found to reduce(17).

The bioenhancing property highlight the beneficial role of cow urine in treating bacterial infections and cancers and that cow urine enhances the efficacy and potency of therapeutic drugs the antibiotic dose per day and duration of treatment in tuberculosis patients(1).

### Antioxidant Effect

Volatile fatty acids are present in the cow urine which acts as antioxidant(12). Antioxidants protect cells against the effect of free radicals. Free radicals are produced due to exposure of tobacco, smoke and radiation. Free radicals may damage cell and cause heart and cancer disease. The free radicals cause damage to various tissues and attack enzymes, fat and proteins disrupting normal cell activities or cell membranes, producing a chain reaction of destruction leading to the ageing process of a person. By regular use of cow urine one can get the charm of a youth as it prevents free radicals formation. Cow urine efficiently repairs the damaged DNA, thus can be very effective for the cancer prevention and therapy, and can also reduce the spread of malignant cancers and help fighting tumors.

### Antioxidant Property

The redistilled cow's urine distillate (RCUD) possesses strong antigenotoxic and anticlastogenic properties against human polymorphonuclear leukocytes (HPNLs) and human peripheral lymphocytes (HLC) treated with manganese dioxide and hexavalent chromium (which induce DNA strand break, chromosomal aberration and micronucleus). This property is mainly due to the antioxidants present in RCUD (18).

### Antineoplastic Effects

The antineoplastic effects of cow urine may possibly be due to the antioxidant properties of vitamins A, E, C and the volatile fatty acids, both of which acted together to elicit a favorable response(19).

### Use in Cardiovascular Disorder

Wound Healing and Skin Disorder: The cow urine contains various components responsible for proper

working of CVS system. Kallikrein acts as a vasodilator and reduces the risk of hypertension. Enzyme Urokinase acts as a fibrinolytic agent, Ammonia maintains the structural integrity of the blood corpuscles, Nitrogen, sulfur, sodium and calcium components act as blood purifiers, while iron and erythropoietin stimulating factor maintain haemoglobin levels. Cow urine acts as a Diuretic so it is effective in hypertension(12).

### Blastogenic Activity

*Kumar et. al.*, (2004a) (20) and *Chauhan and Singh* (2001) (14) evaluated the blastogenic activity of lymphocytes and effect of in-vivo cow urine treatment on blastogenesis. The increase in lymphocyte proliferation activity was maximum during first two weeks of development. During developmental period cow urine enhanced the T- and B- cell blastogenesis by 1.81% and 2.21%, respectively.

### Diuretic Action

Diuretic action can be determined by urine output and sodium content of the blood before and after administration of the cow urine (12). The cow urine contains erythropoietin hormone so it is useful in cure of anaemia. Cow urine contains volatile salts these salts destroys acidity and reduce the pain in kidney, intestine and womb. The cow urine provides quick recovery of the wound.

### Multi Minerals Multi Vitamins Property

The biochemical estimation of cow urine has shown that it contains Sodium, Nitrogen, Sulphur, Vitamin A, B, C, D, E, Minerals, Manganese, Iron, Silicon, Chlorine, Magnesium, Citric, Succinic, Calcium salts, Phosphate, Lactose, Carboic Acid, Enzymes, Creatinine and Hormones(21).

Non-descript or our Desi cattle urine showed maximum concentration of zinc, potassium and calcium when compared to crossbred cattle(22).

### Pesticidal Activity

*Subedi and Vaidya* (2003) (23) evaluated cow urine and buffalo urine for the pesticidal activity against flea beetle, *Phyllotretanemorom* at three concentrations (20, 15 and 10%) and observed significant results at all concentrations

*Budhathoki* (1992) (24) reported that diluted cow urine applied on broad leaf mustard significantly reduces powdery mildew.

### Effect of Cow Urine on Fertility and Hatchability of Eggs

Cow urine has the capabilities to potentiate the egg production and can be used as feed additive for layer birds in order to get good quality eggs (25).

### Conversion of Urine into Bio-Wealth

*Golder et. al.*, (2007) (26) studied the conversion of urine into bio-wealth in the form of zooplankton.

Human urine was an excellent liquid waste followed by cow urine that can be used for the mass production of zooplankton and *Moinamicrura* required for larval and post larval rearing of commercial fishes.

#### Hepatoprotective Effect

Kamdhenu ark' has antagonistic effects against cadmium-induced liver toxicity and it also work as a bioenhancer of Zn(27).

Bramhighrita a formulation that belongs to the panchagavya class of Ayurvedic formulations, used in combination with herbs significantly reduced the levels of serum marker enzymes, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase and acid phosphatase elevated during carbon tetrachloride-induced hepatotoxicity (28).

#### Phagocytic and Lymphocyte Proliferation Activity

Cow urine enhances the phagocytic activity of macrophages and thus helpful against bacterial infections (29). It also facilitates the synthesis of interleukin-1 and interleukin-2 augments B & T-lymphocyte blastogenesis, IgA, IgM and IgG antibody titers.

#### Anti-Anemic Effect

The cow urine contains erythropoietin hormone so it is useful in cure of anaemia. Cow urine contains volatile salts these salts destroys acidity and reduce the pain in kidney, intestine and womb. The cow urine provides quick recovery of the wound(30).

#### Antiviral Activity

2 Phenyl Phenol constituent of urine works as antiviral and antimicrobial, for AIDS patient it is useful (12).

#### Adverse Effect of Cow Urine

Common side effects of urine therapy include diarrhea, itch, pain, fatigue, soreness of the shoulder, fever, etc which appears more frequently in patients suffering long term or more serious illnesses.

#### Conclusion

##### Desi breed cow milk

From the foregoing discussion it can be concluded that A1 beta-casein-derived Beta Casomorphin - 7(BCM-7) likely poses a health risk and should be minimized or eliminated as much as possible. So it can be concluded that we should not drink A1 cow milk, or eat products made from it. It seems that the populations that consume milk containing high levels of beta-casein A2 have a lower incidence of cardiovascular disease and type 1 diabetes. BCM-7 has also been suggested as a possible cause of sudden infant death syndrome. In addition, neurological disorders, such as autism and schizophrenia, seem to be associated with milk

consumption and a higher level of BCM-7. Therefore, careful attention should be paid to that protein polymorphism, and deeper research is needed to verify the range and nature of its interactions with the human gastrointestinal tract and whole organism. There is significant association between the A1A1, A1A2 and A2A2 genotype and milk volume, fat%, protein% and SNF%. In most of the cases it was found that milk volume, fat%, protein% and SNF% is comparatively more in the cow having A2A2 genotype.

#### Desi breed cow urine

Jersey and Crossbred cow are not suitable, only the Indian traditional cows are suitable for collection of cow urine. Cow urine contents are 95% water, 2.5% urea and 2.5% minerals, salts, hormones, and enzymes (9). It contains Iron, Calcium, Phosphorus, Carbonic acid, Potash, Nitrogen, Ammonia, Manganese, Sulphur, Phosphates, Potassium, Urea, Uric acid, Amino acids, Enzymes, Cytokine, Lactose etc. Composition of urine varies according to the species, breed, season, physiological status, quality and quantity of water consumed along with few pathological conditions as in diabetes and ketosis. Cow urine show various positive effect like anti-diabetic effect, anti-microbial effect, anthelmintic activity, anti-inflammatory property, anti-inflammatory property, bio-enhancement effect, diuretic action, multi minerals multi vitamins property, pesticidal activity, effect of cow urine of fertility and hatchability of eggs, conversion of urine into bio-wealth, hepatoprotective effect, phagocytic and lymphocyte proliferation activity, anti-anemic effect, antiviral activity. Sometimes cow urine also shows adverse effects and causes common side effects of urine therapy include diarrhea, itch, pain, fatigue, soreness of the shoulder, fever, etc which appears more frequently in patients suffering long term or more serious illnesses.

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Fresh colostrum collected



Frozen colostrum in a plastic bottle



Frozen colostrum in storage from a HF dairy cow

## Failure of Transfer of Colostral Immunoglobulins

Shashi Pradhan, KabitaRoy, P C Shukla and S Sunandha Devi

Department of Veterinary Medicine, College of V.S. and A.H., NDVSU, Jabalpur, Madhya Pradesh.

### Introduction

Transfer of passive immunity in the bovine neonate occurs solely through maternal colostrum. This is in contrast to humans where placental transfer of immunity via specific Fc receptors is the predominant mechanism. However, inadequate transfer of immunity is a commonly diagnosed problem affecting young stock. This is particularly the case in modern Holstein dairy herds, where large milk yields dilute the antibody concentration in the colostrum meaning relatively more must be consumed to have the same immunity transferred. Due to the lower yields and possibly other genetic reasons, this tends to be less of a problem in suckler herds.

Receiving sufficient colostrum and having adequate immunity is important to reduce the risk of pre and post weaning morbidity and mortality. Good calf health is fundamental to achieving adequate weight gains and reaching the optimal age at first calving.

### Inadequate absorption of immunoglobulin occurs because:

1. The neonate did not receive any colostrum.
2. The neonate did not receive enough colostrum.
3. The neonate did not absorb enough colostrum.
4. The quality of the colostrum was inadequate.

### Colostrum Quality Amount of IgG Depends on

- **Breed** - Antibody and milk fat are correlated, thus Jersey cows have the highest amount of immunoglobulin. Conversely, Holsteins have the lowest amount of immunoglobulin.
- **Nutrition** - It is especially important for cows during the dry period (last 2 months of pregnancy) to have the correct nutrition in order to produce immunoglobulin. There is also some evidence that trace element deficiencies during the dry period may affect

colostrum quality.

- **Season** - The lowest quality of colostrum is produced in the late winter months as this is correlated to the quality of forage available. High ambient temperatures may also have an adverse effect which is likely to be related to depressed dry matter intakes. In countries where this is a problem, heat abatement systems may help.
- **Stress** - Dams under increased stress produce colostrum of reduced quality.
- **Immune Status of the Dam** - Dams exposed to specific diseases mount an immune response and produce antibodies for that particular disease. These antibodies are then transferred to the neonate via colostrum. Dams may also be vaccinated which, if done during the colostrum production period (last 2 months of gestation), will also provide the neonate with some protection via passive transfer.
- **Milk yield** - Increased yield dilutes the amount of immunoglobulins in the colostrum. The volume of colostrum produced at the first milking may be used as a guide of colostrum quality.
- **Dry Period Length** - Excessively short dry periods, no dry period or mistakenly milking a cow during the dry period will have detrimental effects on colostrum quality.
- **Dripping from teats** - Poor teat confirmation may result in a poor teat seal and thus dripping before the calf has been born or sucked.
- **Parity** - Older cows have better quality colostrum as they have been exposed to more pathogens and may have had multiple vaccinations causing their colostrum to have a higher amount of immunoglobulin.
- **Mastitis** - A cow with clinical mastitis, or indeed any

other illness, should not be used as source of colostrum.

- **Pooling** - Cow's colostrum may be pooled to ease the calf management period. However, the larger volumes of lower quality colostrum tend to be relatively over-represented meaning this can be to the detriment of colostrum quality. Therefore, this practice is to be discouraged. It is also a risk for the spread of Johnes disease.
- **Time of collection** - The highest quality colostrum will be collected from the dam when milked within 1-2 hours of calving.

### Colostrum Quantity Depends Upon

**Amount the neonate suckles** - A strong neonate and good mothering from the dam increases the amount the neonate suckles. Udder conformation also influences the accessibility of the teats to the neonate. Dystocia may reduce mothering and produce a weak calf that potentially causes an increased time to the first suck and/or reducing the amount the neonate consumes.

- **Premature births** - If born early the dam has a shorter period of time for concentration of antibodies from the blood to the colostrum. It is more likely that the neonate will also be weaker and smaller, causing the amount of colostrum sucked to be reduced.

### Failure of Absorption from the Intestine

The amount of colostrum recommended is a minimum of 3 liters within 6 hours of birth. This equates to 8-10% of the neonates body weight. If the colostrum is of sufficient quality, this should provide approximately 100-200g of IgG in the first feed. Timing is important and is considered optimal within **the first four hours** before progressively declining towards what is referred to as "gut closure". The timing of the first feed can influence when this occurs and a delay can prolong the time before gut closure to approximately 36 hours. Therefore, the first feed is the most important in the uptake of colostrum derived passive immunity.

### Diagnosis

Failure of passive transfer can be a problem in the individual calf but more importantly can be a herd level problem which requires investigation. Diagnosing the problem can be done in a variety of ways each with their advantages and disadvantages. In all cases, calves should be sampled no earlier than 24 hours of age and up to a maximum of 7 days when the calf's own endogenously produced antibodies can distort the results.

### Prevention

After diagnosis, herd level prevention strategies should be based around the following areas

#### 1. Method and amount of colostrum administration

On a modern Holstein herd, leaving the calf to suckle from its dam is often inadequate to transfer sufficient passive immunity. Therefore, this process may be assisted by using a bottle or using a stomach tube. Farmers may need to be trained with the latter approach which is often preferred as it is more time efficient.

#### 2. Identification of high risk cows and calves

Calves at risk can be identified and action taken to ensure adequate colostrum intake.

#### 3. Colostrum storage

It is often useful for the farmer to store colostrum in the freezer for use when needed. Anecdotally, storage has been reported as satisfactory for up to 12 months. However, repeat freeze thawing should be minimized as this can have adverse effects on colostrum quality. Microwaving the colostrum as part of the thaw is not recommended due to protein denaturing. Thawing should therefore be done slowly by putting the bottles into warm water.

On harvesting, the colostrum should not be maintained at room temperature for prolonged periods due to bacterial growth occurring which may harbour infectious agents. Bacterial contamination can be minimized by good udder preparation prior to milking and using clean equipment. Freezing should be performed as soon as possible after collection. If the colostrum is not going to be frozen but kept for later use, it should be refrigerated and used within a few days.

It is important to select the dam carefully when deciding which colostrum to store. The cow should be healthy and in good condition. It is advisable to check the colostrum with a colostrometer before storage to try to ensure adequate quality. In herds with a history of Johnes disease, it may be worth testing the cow for infection, although due to the poor sensitivity of the test in clinically normal animals this is not a guarantee of uninfected colostrum.

#### 4. Colostrum quality

This can be done using a colostrometer.

#### 5. Colostrum supplements

A colostrum supplement is added to normal colostrum to increase the IgG content. It is important that products of this type are not used as the sole source of colostrum which would confer inadequate immunity to the neonate.

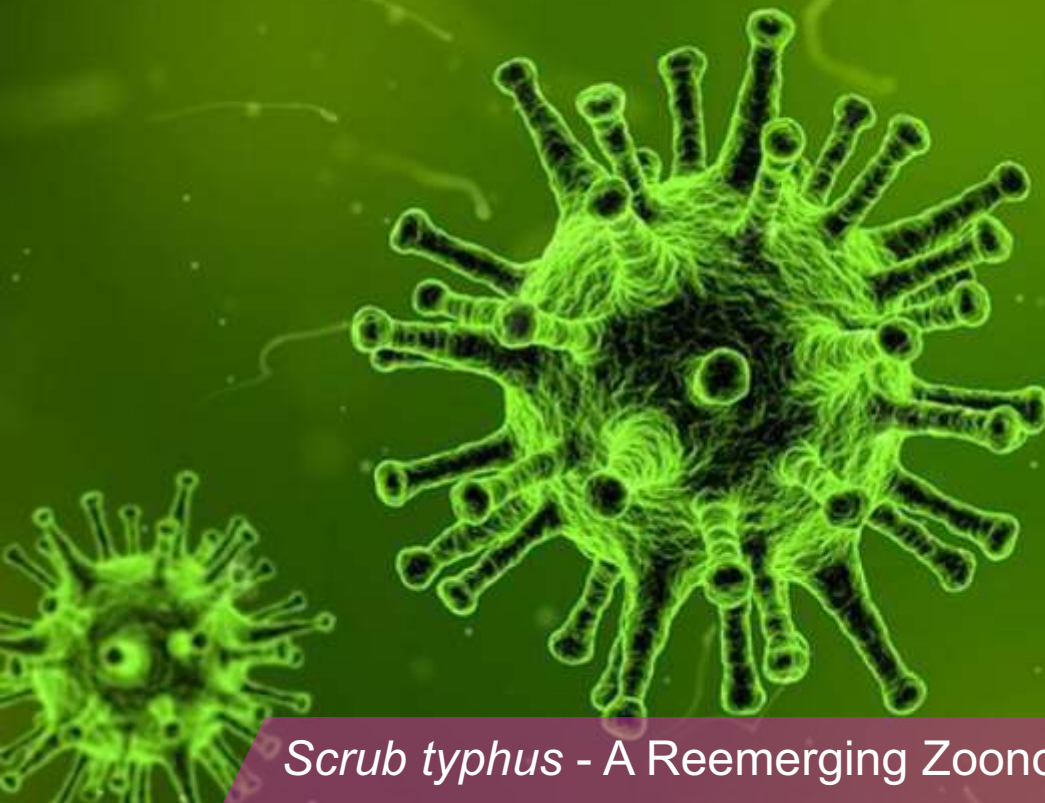
#### 6. Colostrum replacers

Colostrum replacers are designed to completely replace the need for normal colostrum from the dam. The advantages of these products are convenience and allowing some immunity to be conveyed to the calf when natural colostrum is not available. Their disadvantages are the cost and the lack of specific immunity to the pathogens present on the farm. They may be useful on particular farms affected with Johnes disease.

In situations where despite colostrum management practices being optimized, there is still evidence of high calf morbidity and mortality, other measures that may be more targeted to individual diseases may be more appropriate.

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## Scrub typhus - A Reemerging Zoonotic Threat in India

Indhu PrathibhaA, Bhanu Rekha V, Ajay Kumar V J and Nithya Quintoil M

Department of Veterinary Public Health & Epidemiology, Rajiv Gandhi Institute of Veterinary Education & Research, Puducherry.

### Abstract

*Scrub typhus is an acute febrile disease caused by Orientia tsutsugamushi which is transmitted to humans by the bite of the larva of lepto-trombidium mites (chiggers). One million people are affected globally every year. It is a reemerging zoonotic disease in India. The clinical manifestations are fever, headache, eschar and maculopapular rash. Scrub typhus can affect several body systems such as central nervous system, cardiovascular system, renal, respiratory, and gastrointestinal systems and results in complications which can be life threatening. Common laboratory findings are elevated liver transaminases, thrombocytopenia and leukocytosis. Mortality ranges from 1 to 50 percent. Scrub typhus is an under diagnosed disease in India. Effective diagnosis coupled with proper antibiotic treatment with doxycycline or azithromycin can reduce the mortality.*

### Introduction

*Scrub typhus* is a zoonotic rickettsial illness caused by *Orientia tsutsugamushi* which is a small gram-negative, obligate intracellular organism. In Japanese “Tsutsuga” means small and dangerous and “mushi” means insect or mite. *Orientia tsutsugamushi* is transmitted to humans by the bite of the larva of *leptotrombidium mites* (chiggers) which are often brilliantly colored and less than 1 mm in size. Infected chiggers are found particularly in areas of heavy scrub vegetation during the wet season. “Scrub” denotes the type of vegetation that harbors the vector and “Typhus” means fever with stupor. *Scrub typhus* is also known as tsutsugamushi disease or Chigger borne typhus. *Scrub typhus* was first described in Japan in 1899. *Scrub typhus* emerged out to be the most dreaded disease among the soldiers of the far east during the second World

War. In India there was an outbreak of this disease in military personnel during World War II in Assam and West Bengal, in the 1965 Indo-Pak war and resurged in 1990 (1). Gradually, the disease became prevalent in many parts of India emerging as a major cause of acute febrile illness (2). *Scrub typhus* is still an under-diagnosed disease in India (1).

### Etiology

*Scrub typhus* is caused by *Orientia tsutsugamushi* of the genera *Orientia* under the family *Rickettsiaceae*. It differs from the other members in its genetic makeup and in the composition of its cell wall structure which lacks lipopolysaccharide and peptidoglycan and does not have an outer slime layer. So far at least, eight serotypes of *Orientia tsutsugamushi* are recognized (3).

**Epidemiology**

*Scrub typhus* appears particularly to be distributed in the tsutsugamushi triangle (Figure 1). It is a region covering a very wide area of 13 million km<sup>2</sup> bound by Japan in the east, through China, the Philippines, tropical Australia in the south, and west through India, Pakistan, possibly to Tibet to Afghanistan, and southern parts of the USSR in the north. The disease is largely prevalent to southeastern and eastern parts of Asia; India, Pakistan, Indonesia, Maldives, Myanmar, Nepal, Sri Lanka, Thailand and other islands in the region (3). *Scrub typhus* is found only in areas with a suitable climate, plenty of moisture and scrub vegetation. It is increasingly reported that *scrub typhus* is not restricted to tsutsugamushi triangle. It threatens one billion people globally, and causes illness in one million people each year (4).



**Figure 1: Tsutsugamushi triangle (5)**

**Epidemiology in India**

*Scrub typhus* is prevalent in many parts of India. There have been outbreaks in areas located in the sub-Himalayan belt, from Jammu to Nagaland, Rajasthan, Himachal Pradesh, Sikkim and Darjeeling (West Bengal) during 2003-2004 and 2007. Field epidemiology studies indicate that the disease occurs all over India as cases are reported from Maharashtra, Tamil Nadu, Karnataka, Kerala, Himachal Pradesh, Jammu and Kashmir, Uttaranchal, Rajasthan, West Bengal, Bihar, Meghalaya, Nagaland and Puducherry. Outbreak occurs more frequently during the rainy season however in southern India outbreaks are reported during the cooler months of the year. The peak of the disease is between August and October (5).

**Host**

A number of small rodents, particularly wild rats, mice (Figure 2) and shrews (Figure 3) are the natural hosts for *Scrub typhus* as the rodents and acarines do not succumb to the disease. Field rodents and the vector mites act as a reservoir and between this two the infection perpetuates in nature (3). Mites act as the primary reservoirs for *O. tsutsugamushi*. Larvae of mites feed only once on a mammal host and remain infected throughout their life. In the life cycle of chigger mites, larva is the only infective stage (5).



**Figure 2: Mice**



**Figure 3: Shrew**

**Transmission**

*Orientia tsutsugamushi* is transmitted to humans by the bite of the larva of *leptotrombidium mites* (chiggers). *Leptotrombidium deliense* and *L. Akamushiare* present in most countries of the South-East Asia and they are endemic in certain geographical regions of India, Indonesia, Maldives, Myanmar, Nepal, Sri Lanka, and Thailand. The vector mite is mostly present in diverse ecological niches such as equatorial rain forests, semi deserts, and subarctic terrains in the Himalayan regions. These ecological patches are called mite islands and within this there may be a limited area of intense transmission called typhus islands(3). Humans are accidental hosts (5). Human beings are infected when they trespass into these mite islands and are bitten by the mite larvae (chiggers). No person to person transmission has been documented (6).

Chiggers usually feed on thin, tender or wrinkled skin (5). They use hair follicles and pores to gain entry into the skin and feed for 3–5 days using a styletome (feeding tube) to inject salivary secretions that lyse host tissue. This dissolved tissue is ingested by larval mites (7). *Orientia tsutsugamushi* has been found in the salivary glands of mites (5). *Orientia*

*tsutsugamushi* is maintained in trombiculid mites through transstadial and transovarial transmission (7). Outdoor workers, especially field workers in rural areas, have a higher risk of acquiring the disease. It is reported that rice fields are an under-appreciated location where the biting of mites and transmission of *O. tsutsugamushi* occurs in the endemic areas. Tropical weather provides stable and ideal conditions for transmission of the disease providing high temperature for optimal mite activity. In more temperate climates, the transmission of *Scrub typhus* is more seasonal due to the temporal activity of chiggers (5).

The Life cycle of mites (Figure 4) in the family *Trombiculidae* (*Acari: Trombidiformes*) consists of an egg, two six-legged stages: prelarva and larva, and four eight-legged stages: protonymph, deutonymph, tritonymph, and adult. The prelarval, proto- and tritonymphal stages are inactive and nonfeeding, while the deutonymph and adult stages are non-parasitic and generally feed on arthropod eggs or small arthropods. Larval mites, often called chiggers, are the only parasitic stage (7).

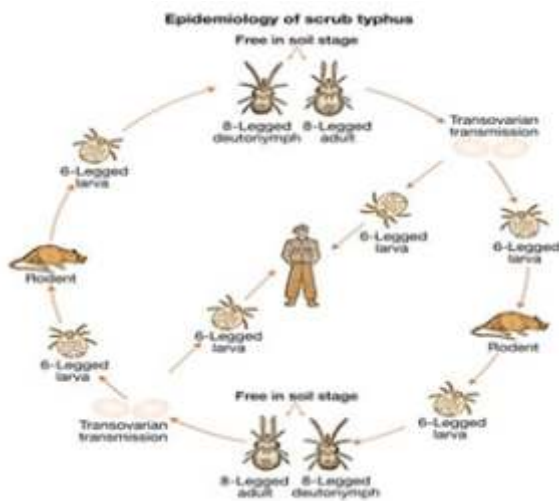


Figure 4: Transmission of *Scrub typhus*

**Pathophysiology**

The organism multiplies at the site of inoculation that progresses on necrosis and evolves into an eschar with regional lymphadenopathy. The pathophysiological hallmark of *scrub typhus* is disseminated vasculitis. *Orientia tsutsugamushi* invades endothelial cells to produce disseminated vasculitis and perivascular inflammatory lesions. Infection of the vascular endothelium results in vascular injury in several organs. The injury causes disseminated intravascular coagulation (DIC) with platelet consumption, vascular leak, pulmonary edema, shock, hepatic dysfunction and meningo-encephalitis (8). The organism downregulates the host defense mechanism by downregulating the GP-96 on the macrophages and

the endothelial cells, which plays a prime role in antigen presentation, functioning of the dendritic cells, antibody production, and cross-priming of the immune system. The immune response against *O. tsutsugamushi* is both humoral and cellular. Humoral immunity involves the production of strain-specific antibodies against the organism (3).

**Re-Emergence of *Scrub Typhus***

*Scrub typhus* was endemic in India in 1960 and 1970. However, in later years, the disease disappeared until recently, probably because of widespread use of insecticides to control other vector-borne diseases, empiric treatment of febrile illnesses with tetracyclines and chloramphenicol by medical practitioners, and changes in lifestyle (6). Nevertheless, now there seems to be a resurgence and reemergence of the disease in India. This resurgence may be attributed to changes in land use because of the continuously expanding Indian population, urbanization which leads to garbage accumulation and increase in rodent population, design of new enzyme immunoassays in the 1990s and their widespread use in the past two decades, increase in the numbers of pets which can serve as hosts and reservoirs for the vectors, reduced usage of chloramphenicol and doxycycline for febrile illnesses due to development of resistance and because of the apprehensions regarding side effects of tetracyclines and chloramphenicol, deforestation and rapid transport leading to displacement of vectors as well rodents from one place to another (9).

**Clinical Manifestations**

The infection can range from a mild, self-limiting disease to a fatal illness in 35-50% of cases, with multi-organ dysfunction, if not promptly diagnosed and appropriately treated (6). After a variable incubation period of 1 to 3 weeks, onset of disease is characterized by fever, headache, myalgia, cough, and gastrointestinal symptoms. Patients are often present with pyrexia of unknown origin (3). Fever (95% and 100%) and headache are the most common features among confirmed *Scrub typhus* patients (5).

The first sign of *Scrub typhus* includes a primary papular lesion (where the chigger has fed) which enlarges, undergoes central necrosis, and crusts to form a flat black eschar (Figure 5) or an ulcer with regional lymphadenopathy. It occurs prior to the onset of fever and other symptoms (5). The presentation of eschar varies among the *scrub typhus* patients depending on the geographic areas. Although the eschar is reported to be less frequently observed in South Asian patients than in East Asian or Caucasians (8) 55% of patients had an eschar in a recent study from South India. In males, eschars are primarily within 30 cm below the umbilicus, lower extremities and anterior chest. Head, neck anterior chest are the most prevalent areas in females.

Eschars are commonly present in the axillae of children in addition to the sites mentioned above (5). Unusual sites of eschar were reported to be in the cheek, ear lobe and dorsum of the feet (8). Five to eight days after the onset of fever, the symptoms gradually increase in severity and a macular (Figure 6) rash may appear on the trunk (10).



Figure 5: *Scrub typhus* eschar (Monthly newsletter, GOI, MAY-June, 2009)



Figure 6: Maculopapular rash (Monthly newsletter, GOI, MAY-June, 2009)

At the end of the 2nd week, systemic symptoms ensue mostly involving the central nervous system, cardiovascular system, renal, respiratory, and gastrointestinal systems and resulting in further complications (3). The lung is one of the main target organs for *Orientia*, leading to pulmonary complications of variable severity. Acute respiratory distress syndrome (ARDS) like picture and Interstitial pneumonia may occur in severe cases (5). Neurological manifestations include meningitis, meningoencephalitis or encephalitis, cerebral venous thrombosis, Guillain- Barre Syndrome,

transient Parkinsonism and myoclonus, opsoclonus, cerebellitis, transverse myelitis, polyneuropathy, facial palsy, abducens nerve palsy and bilateral optic neuritis (8). Severely ill patients can suffer gastrointestinal mucosal hemorrhage, multiple erosions and ulcers (5). Deafness, dysarthria, and dysphagia may occur, but are usually transient, although deafness can last for several months (3).

Common laboratory findings are proteinuria, elevation of creatinine, coagulopathy, elevation of liver enzymes and bilirubin - indicating hepatocellular damage, Leukopenia and thrombocytopenia with subsequent increase of white blood cell counts to normal levels. In children, leukocyte and platelet counts are usually within normal ranges, although thrombocytopenia and leukocytosis may also occur, Cerebrospinal fluid (CSF) examinations show a mild mononuclear pleocytosis with normal glucose levels (10) and elevated transaminases (6).

**Scrub typhus in Pregnancy**

*Scrub typhus* in pregnancy may be associated with increased foetal loss, preterm delivery and not fully grown gestational age infants. Vertical transmission from mothers with acute febrile illness during pregnancy is reported from transplacental infection and transmission in perinatal blood-borne infection during labor causing neonatal *scrub typhus* (10).

**Scrub Typhus in Animals**

*Orientia tsutsugamushi* was isolated from six species of mammals namely *Isoodon macrourus*, *Rattus fuscipes*, *Rattus conatus*, *Uromys caudimaculatus*, *Melomys cervinipes* and *Melomys lutillus*. Prevalence of *Scrub typhus* in rodents was moderately high in *Apodemus agrarius*. *Scrub typhus* has been reported in dogs with a seroprevalence of 21.3 per cent. Dogs are useful sentinels for *Orientia* species in regions of uncertain endemicity and distribution (11). The prevalence of the disease in other domestic animal needs to be explored.

**Diagnosis**

Laboratory methods for diagnosing rickettsial diseases including *Scrub typhus* are mainly based on serological tests and molecular assays (5). The success of a test in confirming the diagnosis of *scrub typhus* is dependent on the type of sample taken and the timing of the specimen (8).

**1. History and Clinical signs**-History of travel to endemic areas and exposure to mites and the presence of an eschar. Diagnosis by history is difficult because of the comparatively longer incubation period of 3 weeks. Presence of an eschar supports the diagnosis but it is variably present (12).

**2. Antibody Detection**

**A. Weil-Felix Agglutination test**- This is the oldest test in current use, cheapest and most easily available serological test. The Weil-Felix test has

a high specificity but a low sensitivity and is based on the detection of antibodies to various Proteus species which contain cross-reacting antigenic epitopes to antigens from members of the genus *Rickettsiae* with the exception of *Rickettsia akari*. The test is said to be positive when there is a titer of 1:320 or greater or a 4-fold rise in titer starting from 1:50 (3).

- B. Indirect Immunofluorescence Antibody assay-** This is a gold standard assay which detects the presence of *scrub typhus*-specific antibody bound to smears of *scrub typhus* antigen. This can confirm infection before their seroconversion. IFA is expensive, requires specialized laboratories and considerable training (3).
- C. Immuno Chromatographic Test (ICT) -** Detects antibodies against *O.tsutsugamushi*. It is a rapid diagnostic test which follows a simple protocol with no sophisticated equipments. It is field-deployable and attractive for use in rural areas where the use of diagnostics like ELISA and IFA may not be available (10).
- D. Indirect Immunoperoxidase Test-** Eliminates the expense of a fluorescent microscope in which fluorescein is replaced by peroxidase. This test has the advantage in that any rickettsial strain can be used as the antigen, either IgG or IgM antibodies can be titrated individually, gives a permanent preparation (for re-examination), and all cells (infected and uninfected) can be observed. It is easy, however the readings are subjective (10).
- E. Enzyme Linked Immuno Sorbent Assay-** ELISA can be used to detect both antigen and antibodies. ELISA using whole cell antigen and r56 from the Karp, Kato, and Gilliam strains of *O. Tsutsugamushi* were used and the final concentrations of antibodies were then measured by a Microplate Reader. This trivalent ELISA appears to be superior in terms of sensitivity, to the other ELISA tests, owing to the presence of large amounts of conserved and/or variable regions of the r56-kD protein from the three strains of *O. Tsutsugamushi*. IgM capture ELISA based on the capture of IgM antibodies in sera carries a high sensitivity and specificity. It is a reliable and useful diagnostic method for early detection of *Scrub typhus* and a large number of sera can be tested at a time (10).

**3. Culture -** The samples which can be collected for isolation are buffy coat of heparinized blood, defibrinated whole blood, triturated clot, plasma, necropsy tissue, skin biopsy, and arthropod. The various methods adopted to identify the rickettsial strains are embryonated chicken yolk sacs, cell culture in Vero cells, MRC 5 cells, BHK21, L929 mouse fibroblast cell monolayer in tube culture, shell-

vial assay, etc. Vero or L929 cells allow better and faster isolation of *Rickettsiae* while HEL or MRC5 cells prevent contact inhibition. Cell culture is time consuming (an average of 4 weeks) (3).

**4. Polymerase Chain Reaction (PCR)-** Molecular detection is possible from eschar samples, skin rash biopsies, lymph node biopsies and EDTA blood. Nested PCR technique can detect the disease as early as day 3 of the fever phase which is even before the appearance of antibody. Studies suggest that nested PCR in conjunction with IFA may serve as a rapid and reliable method for diagnosing *Scrub typhus* (3).

#### Treatment

Mortality in *Scrub typhus* ranges from 1 to 50 per cent. With effective diagnosis and treatment regimen the mortality due to *Scrub typhus* can be significantly reduced.

#### A) Uncomplicated Cases

##### Adults

(a) Doxycycline 200 mg/day in two divided doses for individuals above 45 kg for a duration of seven days

(b) Azithromycin 500 mg in a single dose for five days.

##### Children

(a) Doxycycline 4.5 mg/kg body weight/day in two divided doses for children below 45 kg.

(b) Azithromycin in the dose of 10 mg/kg body weight for five days.

##### Pregnant women

Azithromycin 500 mg in a single dose for five days. Azithromycin is the drug of choice in pregnant women, as doxycycline is contraindicated.

#### B) Complicated Cases

(a) Intravenous doxycycline 100 mg twice daily in 100 ml normal saline to be administered as infusion over half an hour initially followed by oral therapy to complete 7-15 days of therapy.

(b) Intravenous azithromycin in the dose of 500 mg intravenous in 250 ml normal saline over one hour once daily for 1-2 days followed by oral therapy to complete five days of therapy.

(c) Intravenous chloramphenicol 50-100 mg/kg/day 6-hourly doses to be administered as infusion over one hour initially followed by oral therapy to complete 7-15 days of therapy. Doxycycline and/or chloramphenicol resistant strains sensitive to azithromycin (13).

#### Prevention and Control

1. Control of mite vector in endemic areas by treating the ground and vegetation with residual insecticides such as chlorinated hydrocarbons (lindane, dieldrin, and chlordane) and destroying limited amounts of

local vegetation.

2. Avoiding human- mite contact during visit to an endemic area by

- Wearing protective clothing
- Impregnating clothes with miticide (e.g. benzyl benzoate)
- Applying a mite repellent (diethyltoluamide) to clothes taking care not to spray on underlying skin. Repellants should not be used on babies younger than 2 months of age.
- Treating clothes and gear with permethrin or purchasing permethrin-treated items. Permethrin kills chiggers and can be used to treat boots, clothing, and camping gear.
- Lathering with soap in a hot bath or shower will remove both attached and unattached chiggers.

3. Chemoprophylaxis - weekly administration of 200 mg doxycycline

4. Rodent control by minimizing the garbage and food wastes.

5. Vaccination- Initial efforts of preparation of vaccines with killed *O. Tsutsugamushi* were disappointing. This has been attributed to the diversity of the strains, lack of tolerability of live vaccines in volunteers due to absence of natural attenuated strains of the organism, and lack of achieving long-term heterologous protection even with irradiated strains.

### Conclusion

Currently, *Scrub typhus* outbreaks are being reported both within the known area of endemicity, as well as beyond the originally defined borders of the Tsutsugamushi Triangle. *Scrub typhus* is reemerging and is an under diagnosed disease in India. So the sensitization of health care professionals in endemic areas about *Scrub typhus* along with continued pursuit of diagnostic tools, vector control, enhanced therapeutics, and ultimately a broadly protective vaccine, will be key to the reduction of the disease burden associated with *O. tsutsugamushi* and other *Orientia species*.

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## Tips for Summer in Pet Management

**Prabhavathy Harikrishnan, Jayanthi M, Cecilia Joseph**  
 Madras Veterinary College, Vepery, Chennai, Tamil Nadu.

Human beings having sweat glands all over bodies that help to regulate body temperature, but dogs and cats only have a few in their feet and around their noses. Many animals rely on panting and external cooling to lose heat. Long thick hair coats can also predispose them to heat stroke. Because they're not able to cool themselves down as easily as us, we have to be extra careful to provide them with a cool, well-ventilated and shaded environment with access to clean fresh drinking water.

**Pets are highly susceptible to heat stress to overcome some of the tips for pet owners**

### 1. Provide plenty of water and shade

- Dehydration in dogs and cats is a common one. Signs of dehydration include dry gums and excessive drooling. Always has access to fresh, clean water inside the house. Switch over to a wet dog food during the hotter months to increase fluid intake. Dogs need constant access to clean, fresh drinking water. In hot weather, cold water can help keep dog even cooler. Add equal of parts ice and clean, fresh water to help keep dog cool and hydrated throughout the entire day.
- If keeping dog outside, make sure the bowl is in the shade. This will help keep the ice from melting too fast and keep the water cooler for longer.
- Check dog's bowl regularly to make sure pet still has water.

- Keep the pet in the shade. While dogs and cats like to sunbathe, direct sunlight can overheat them (especially dogs) and lead to heat stroke.

### 2. Fleas and tick control

Fleas and ticks thrive in the summertime. Dog's head and ears are mainly check for ticks. Ticks can transmit disease and embed into the skin within hours.

It is also important to be on the lookout for symptoms of fleas in dog. Flea often causes itching. Flea comb can help to find and remove fleas manually, and specially formulated flea-and-tick shampoos can help kill these parasites and repel them.

### 3. Brushing and Bathing

Bathe of dog more often, but it's important to stick to regular bathing schedule. Double-coated breeds should never be trimmed; other coat types can be trimmed for the summer, a good brushing schedule is more than enough to help keep dog cool because it prevents excess buildup of dead hair. Layers of pet's coat help to protect them from overheating and sunburn. Brushing cat more often to help remove loose fur can also prevent overheating.

### 4. Ear Care

Good ear care is extra important during warmer months. Humidity and heat can make dog's ears load with of yeast and bacteria, greater risk for ear infections. Make a point to clean dog's ears weekly,

and gently wipe dog's ears with a cotton ball after each swim.

**5. Paw care**

Pet Wax can be applied to dog's paw pads to prevent them from drying and cracking in the summertime. Paw wax can also keep dog's feet protected from hot sand on the beach and jagged rocks on hikes. If dog is hot, wiping paw pads with a cool, damp cloth will also help cool him down. Apply sunscreen to dog's skin if it has a thin coat.

**6. Cars**

Leaving pet in a car for a few minutes is no big deal, but it can quickly lead to heat stroke in dogs and cats. In bright sunshine, car acts like an oven, becoming much hotter inside than the outside air even. In fact, on a sunny 70 degree day, car can heat up to over 100 degrees within minutes. So, either take pet with you or leave him or her at home during shopping trips. Never, ever leave dog in the car;

**7. Keep your dog away from pet waste**

Intestinal parasites like hookworm, roundworm, and whipworm are transmitted through feces. If another dog with these parasites defecates and dog eats it, dog may become infected. Clean up waste from other animals in yard, but avoid coming into direct contact with waste.

**8. Exercise**

- Take walks during the cooler hours of the day
- When walking, try to stay off of hot surfaces (like asphalt) because it can burn dog's paws

**8. Swimming**

Good canine swimmers tend to be medium to large in size. Big chested breeds with short legs, such as the bulldog, are known to have a hard time staying afloat. And those with flat faces, such as the Pug, are prone to breathing problems that leave them easily fatigued. And very small dogs, such as Chihuahuas, can get overwhelmed by big waves or strong currents. Wash Your Dog After Swimming. Swimming is great exercise for dogs, and it's the perfect way to keep cool on those hot summer days.

**Heatstroke**

Heat stroke can be deadly if left untreated. Dog's body temperature exceeds 104 degrees Fahrenheit (40 degrees Celsius), it may be experiencing heat stroke.

- Signs of heat stroke include excessive and prolonged panting, collapse/lethargy, uncontrollable drooling, a deep red hue on the gums, increased heart rate, diarrhea (including bloody stool), and difficulty breathing.
- Elderly dogs and dogs with health problems may be more susceptible to heat stroke.
- Dog breeds with short noses, including Shih Tzus, Pekingese, bulldogs, boxers, and pugs, Boston terriers and Persian cats are particularly susceptible to heat stroke. Their short, squished noses make it difficult to expel heat while panting. Pets which are elderly, overweight and have heart or lung disease be kept in air-conditioned rooms as much as possible in the heat.



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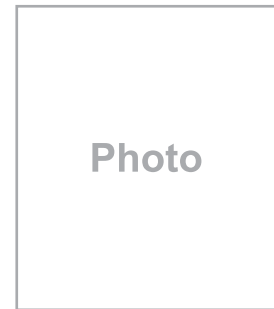
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