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NATIONAL DAIRY DEVELOPMENT BOARD ANAND GUJARAT

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ANIMAL HEALTH UPDATES

Animal Health Group

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Disease - Anaplasmosis and its control

Anaplasmosis is considered as one of the top ten economically important rickettsial diseases affecting ruminants in India.

Anaplasma species were **originally** regarded as protozoan parasites. The Family *Anaplasmataceae* (Order *Rickettsiales*) is now composed of four genera, *Anaplasma*, *Ehrlichia*, *Neorickettsia*, and *Wolbachia*.

Etiology

Outbreaks of bovine Anaplasmosis are due to infection with *Anaplasma marginale*.

Occurrence

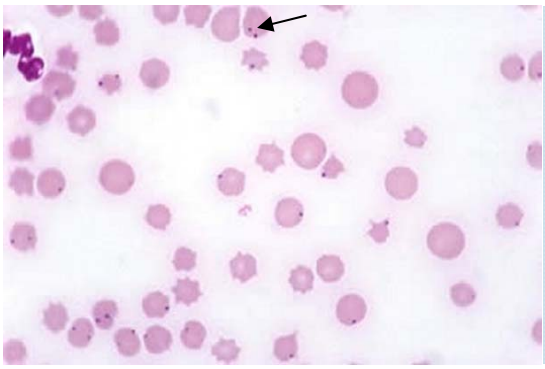
Anaplasmosis in cattle is common on all six continents. It is transmitted by a diverse group of biological and mechanical vectors. Infection in cattle is endemic in tropical and subtropical areas that support large populations of these vectors. Infection occurs more sporadically in temperate climate areas.

Distribution in India

A review of literature on the incidence of Anaplasmosis in India over the last four decades gives a varied range from **1 to 62%** in **cattle** and **5 to 33%** in **buffalo** in field and farm conditions. The details of the same are provided in the table.

Transmission

The source of infection is always the **blood** of an infected animal. Recovery from acute infection results in **persistent infection**



A. *marginale* infection in bovine blood with Wright-Giemsa stain. Intracellular organisms appear as basophilic, spherical inclusions that are generally located near the margin of erythrocytes (arrow).

Source: www.merckmanuals.com

No	Region	C	B	Source	Year	Location
1	Punjab and Haryana	60 %	33%	Gautam & Singh	1971	Field
2	Haryana	55%		Banerjee et.al	1977	Field
3	Punjab	13 to 47%	5 to 23%	Singh and Gill	1977	Field
4	Bihar	51%		Verma and Sinha	1983	Field
5	Bhopal	15%		Bhadang,S D	1988	Farm
6	MP	62%		Misraulia et. al	1988	Field
7	-	7%		Chitravel et.al	1998	Farm
8	Pantnagar	33%		Garg et. al	2004	Farm
9	Karnataka	1%		Muraleedharan et. al	2005	Farm
10	Punjab	48%		Sandhu,T S	2005	Field
11	Karnataka	7%		Harish et.al	2006	Field
12	Madras	8%	8%	Soundararajan and Rajavelu	2006	Field
13	Jaipur	11%		Godara and Sharma	2010	Field
14	Jaipur	11%		Godara et.al	2010	Field
15	Haryana	47%		Kumar and Sangwan	2010	Farm
16	Punjab	45%		Harkirat et.al.	2012	Field
17	Kerala	20%		Nair et. al	2013	-
18	Uttarakhand	34%		Rialch et.al	2013	-
19	Tamil Nadu	3%		Velusamy et. el	2014	Field

characterised by repetitive cycles of rickettsaemia. Persistent carriers are the **reservoir** for herd infection. The level of parasitaemia is often **too low** for detection by microscopy but can be detected by nucleic acid probe analysis.

Haematogenous insect transmission

Spread from animal to animal occurs chiefly by insect vectors. A variety of arthropods may act as vectors but significant natural vectors are ticks in the family **Ixodidae** (hard ticks) and flies in the family **Tabanidae**. Reviews based on careful study of reported transmission experiments list up to **19** different ticks as capable of transmitting *A. marginale* experimentally. **Male ticks** may be particularly important as vectors; they can become **persistently infected** and serve as a reservoir for infection.

Transmission cycle

The organism undergoes a complex developmental cycle in the gut cells of ticks and the final infective stage is present in the salivary gland. Transstadial transmission of the organism occurs in ticks but there is little evidence for transovarial transmission. **Intrastadial** transmission is significant with some species and transmission occurs as the ticks move from one host to another while they are engorging, including from cow to calf. **Tabanids** are efficient mechanical vectors and can transmit infection for **two hours** after feeding.

Iatrogenic transmission

Anaplasmosis may also be transmitted by **infected hypodermic needles,** castration, spaying or dehorning equipment, blood transfusion or by embryo transplants. It may vary with the virulence of the protozoan strain.

Transplacental transmission

Transplacental transmission has been reported and is usually associated with acute infection of the dam in the second or third trimester of gestation.

Risk factors

Age at infection

There is a **strong correlation** between age of cattle and severity of disease. Infection between six months to three years has increasing risk of clinical illness. Animals infected **after 3 years** of age are commonly affected by a **peracute fatal form of disease.**

Young calves below 6 months are susceptible to infection but seldom show clinical signs. They are much more **resistant** to disease (although not infection) than older cattle. After recovery from the acute phase of infection, cattle remain chronically infected **carriers** but are generally immune to further clinical disease. However, these chronically infected cattle may **relapse** when immunosuppressed or infected with other pathogens. **Carriers** serve as a **reservoir** for further transmission. Serious losses occur when mature cattle with no previous exposure are moved into endemic areas or under endemically unstable situations when transmission rates are insufficient to ensure that all cattle are infected before reaching the more susceptible adult age.

Geographic region

Clinical disease is rare in enzootic areas because the infection pressure is high and cattle are infected at an age when they are age resistant to clinical disease. The average age at which calves in enzootic areas become infected is **11 weeks.** Clinical disease occurs where there is **introduction of susceptible** animals into endemic areas or the **expansion of the vector population** into previously free areas or into the interface between endemic and non-endemic regions.

Breed

Bos indicus, Bos Taurus and their crosses have **equal susceptibility** to infection and show the same age susceptibility but under field conditions, *Bos indicus* are not commonly affected preferably because of their relative resistance to heavy tick infestation. Breeds with **black or red coat colour** have a higher risk of infection than those white coats in regions where biting flies are the insect vector. Dairy breeds may be at greater risk for iatrogenic transmission.

Nutritional status

Clinical disease is **less severe** in cattle on a **low plane** of nutrition. Exposure of infected, clinically normal animals to devitalizing environmental influences, particularly shortage of feed and the presence of other diseases may result in the development of acute Anaplasmosis.

Season

In temperate climates a seasonal occurrence of disease occurs in association with seasonal occurrence of the insect vectors.

Clinical findings

The most marked clinical signs of Anaplasmosis are anaemia and jaundice, the latter occurring late in the disease.

In cattle, the incubation period varies from 2-5 weeks. After the prepatent period, peracute, acute or chronic Anaplasmosis may follow. RBC count, PCV, and haemoglobin values are all severely reduced. Macrocytic anaemia with circulating reticulocytes may be present late in the disease.

Affected animals are often hyper excitable and tend to attack attendants just before death. Animals with per acute infections succumb within a few hours of the onset of clinical signs. Acutely infected animals lose condition rapidly. Inappetence, loss of coordination, breathlessness when exerted, and a rapid bounding pulse are usually evident in the late stages. The urine may be brown but, in contrast to babesiosis, hemoglobinuria **does not** occur. A **transient** febrile response, with the body temperature rarely exceeding 106°F (41°C) occurs at about the time of peak rickettsaemia. Mucous membranes appear pale and then yellow. Pregnant cows may abort. Surviving cattle convalesce over several

weeks during which hematologic parameters gradually return to normal. In case of surviving cattle, fertility is generally impaired. In convalescent bulls there may be depressed testicular function for several months.

Necropsy findings

1) Enlarged and congested spleen (splenomegaly) showing soft pulp.
2) Distended gall bladder with dark tarry bile.
3) Thin, watery blood, which clots poorly.
4) Enlarged, icteric liver, deep orange in colour and distended bile ducts
5) Lemon yellow carcass and connective tissue, sclera of the eye, tendons, pleura, peritoneum, and attachments of diaphragm.

Samples for post-mortem diagnosis

1) Clinical pathology: Blood smears from cut surface of ear; impression smears from internal organs (liver, kidney, heart and lungs) for Light Microscopy (LM) or Fluorescent Antibody Test (FAT). It is important that smears are well prepared and free from foreign matter.
2) Histology: Formalin fixed spleen, liver, bone marrow for LM.

Lemon yellow carcass

Diagnosis

Identification of the agent

Microscopic examination of blood or organ smears stained with Giemsa is the **most common** method of identifying Anaplasma in clinically affected animals. It can be done in any laboratory having an oil immersion microscope. In these smears, A. *marginale* appear as dense, rounded, intraerythrocytic bodies with most situated **on or near** the margin of the erythrocyte. A. *centrale* is similar in appearance, but most of the organisms are situated **away** from the margin of the erythrocyte. It can be difficult to differentiate A. *marginale* from A. *centrale* in a stained smear, particularly with **low levels** of rickettsaemia.

Icteric liver

Serological tests

A competitive enzyme-linked immunosorbent assay (C-ELISA) has been demonstrated to have good sensitivity in detecting **carrier animals**. Card agglutination is the next most frequently used assay. Cross reactivity between Anaplasma spp. can complicate interpretation of serological tests. In general, the C-ELISA has the **best** specificity, with cross-reactivity described between A. *marginale*, A. *centrale*, A. *phagocytophilum* and Ehrlichia spp.

Nucleic-acid-based tests

Nucleic acid based tests have been used experimentally, and are capable of detecting the presence of low-level infection in carrier cattle and tick vectors. A nested reaction is necessary to identify low-level carriers using conventional polymerase chain reaction (PCR). Recently, real-time PCR assays with analytical sensitivity equivalent to nested conventional PCR have been described.

Differential diagnosis

Differential diagnosis of Anaplasmosis should be made from *Babesiosis, Trypanosomiasis, Theileriosis, Leptospirosis & Bacillary haemoglobinuria.*

Treatment

Clinical cases

(1) Oxytetracycline : 6-10 mg/kg body weight (bw) daily for three days, or a single injection of long acting oxytetracycline at a dose of 20 mg/kg intramuscularly (I/m). The convalescent period is long. Concurrent administration of estradiol cypionate (14.3 mg/kg bw i/m) appears to improve the rate of recovery. Tetracycline treatment **may not eliminate** infection but immunity will persist.

(2) Imidocarb: 3mg/kg bw. It also does not interfere with the development of immunity.

(3) Blood transfusions are indicated in animals with a PCV less than 15%. Rough handling must be avoided.

Animals cleared off infection are susceptible to reinfection but are resistant to clinical disease for considerable periods.

Prophylaxis

Temporary protection in the face of an exposure risk can be achieved with a single i/m injection at 20 mg/kg bw of long acting tetracycline. The results generally are good except when cattle are exposed to infection during the 14 days prior to treatment. Prolonged protection can be achieved by administering 20 mg/kg bw of long acting tetracycline i/m every 28 days or by chlortetracycline in the feed at 1.1 mg /kg bw daily.

Elimination of carrier stage

(1) Four doses of long acting tetracycline at 3 day interval @ 20 mg/Kg body weight intramuscularly have **chances** of eliminating carrier stage.

(2) Imidocarb dipropionate @ 5mg/Kg given imtramuscularly in two doses at 14 day interval **may eliminate** carrier state.

Control

1. **Adopt** a proper tick and fly **control** programme in enzootic areas.
2. **Avoid** introduction of **carrier animals** into herds through prior serological screening.
3. **Avoid iatrogenic** transmission by proper disinfection of instruments /equipment after use on each animal.
4. **Limit** the introduction of animals that are **less than 2 years** of age and also induct them when the insect population is least numerous.
5. **Eliminate** carrier state by serological testing and culling of reactors or by treating them as outlined above.
6. **Manage outbreaks** by treating affected animals and providing prophylaxis to in-contact animals. Subsequently all exposed animals should be tested serologically and the reactors treated/ removed.
7. **Provide** prolonged treatment regimens to provide protect cattle in seasonal risk periods of transmission.
8. **Remove/re-treat** animals that are seropositive even six months after treatment (treatment failures).

Vaccination

Both live and killed vaccines are used in some countries to protect cattle against A. *marginale* infection. However, no vaccines are presently available in India.

Zoonotic potential

A. *marginale* infection has not been reported in humans and therefore is not zoonotic.

Sources

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Stand more-live more!

Eighteen studies reported during the past 16 years covering 8,00,000 people found that those who sat for more than 4 hours a day had a 46% increase in deaths from any cause when compared to people who sat for less than 2 hours. Other researchers have found that sitting for more than half the day, approximately doubles the risk of diabetes and cardiovascular problems. Overall, when you combine all causes of death and compare any group of sitters with those who are more active, sitters have a 50% greater likelihood of dying. Pat yourself on your back if you are reading this standing up!!

Scientific American, November 2014.

Significant animal diseases reported to OIE (Oct'–Dec'14)

No	Disease Outbreak	Countries reporting
1	Transmissible gastroenteritis (pigs)	Argentina
2	Highly path. avian influenza	China , Russia
3	Bluetongue	Croatia
4	Coronavirus (camels)	Iran
5	Caprine arthritis/encephalitis	Poland
6	African swine fever	Ukraine
7	Contagious agalactia(sheep & goat)	United Kingdom

Source: www.oie.int

'Plantibodies'- the new line of treatment

Doctors recently treated two Ebola patients successfully with an experimental drug ZMapp, a mixture of different antibodies made from tobacco plants. Plants do not have antibodies of their own, but they nonetheless have the cellular machinery to make these proteins. The process takes little over a month- a faster and cheaper means of manufacturing than using hamster ovary cells, which is the standard. Growing plants is relatively inexpensive. 'Plantibodies' in development include those designed to target HIV, herpes, cancer and rabies.

Scientific American, December 2014

CRISPR- The gene genie

Scientists have known how to alter the genomes of living organisms since 1970s. But many methods remained too difficult or costly to conduct. A new method called **CRISPR** (Clustered, Regularly Interspaced, Short Palindromic Repeats) could foment the genome editing evolution.

Based on immune defences of bacteria, it is faster, cheaper and easier than older techniques. CRISPR based treatments for diseases like HIV, Alzhiemer's and schizophrenia and already being explored.

Using CRISPR, scientists have been able to completely excise the integrated copy of HIV, converting infected cells to uninfected cells, a feat that could not be achieved despite huge strides in AIDS treatment.

Scientific American, December 2014.

WTO rules against India

WTO dispute panel has ruled that India's measure of blocking U.S. poultry imports citing bird flu fears was unsubstantiated and discriminatory and not based on international standards. The US filed the case in March 2012. India could however appeal against the ruling. The ruling could increase imports of poultry products from the US, although India could still restrict imports using other measures such as anti-dumping duties if US tries to sell at unfairly cheap prices. *Source : Reuters , October '14*

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