**APICULTURE**

Honeybees are indigenous to the Eurasian and African continents and were introduced to the Americas and Australia by European settlers. In India the genus Apis has the following species: the western honey bee, Apis mellifera, the eastern honey bee, Apis cerana indica, the rock bee, Apis dorsata and the small bush bee Apis florea.

**The Dwarf Honeybee or Bush Bee (Apis florea)**

This species is considered the most primitive honey bee species and is also the smallest. Apis florea is brownish and the basal part of abdomen is always red. There is another species, A. andreniformis which is darker in colour and the first abdominal segment is totally black.

**The Rock Bee (Apis dorsata)**

This is a large wild honey bee found in southern Asia, mainly in the forested areas. The workers are over 2 cm long and possess ferocious temperament. Hives are built in exposed places far above the ground, on the branches of trees or under the cliffs of rocks and also on the ceilings of ruins and abandoned buildings. The hive is made of a single vertical comb, sometimes more than a metre in length. During breeding season in March-April they swarm and migrate to different places in the forests looking for nesting sites.

**The Asiatic honey bee(Apis cerana indica)**

This is a medium sized honey bee found in southern Asia and all countries in the Himalayan Range, viz, Afghanistan to Indonesia and also in Japan, Malaysia and Thailand. Apis cerana indica is the subspecies still found in the wild in India, particularly in the Himalayan belt where it nests in tree holes and crevices of rocks. The species can be domesticated by farmers for honey production as it has gentle temperament and makes hive in enclosed spaces. Apis cerana is medium sized and has transverse stripes on abdomen. It is commonly found in Himalaya where temperate fruits bloom and provide abundant source of nectar. It can survive temperatures as low as 0ºC in winter.

**The Western honey bee or the European bee (Apis mellifera)**

This species is not indigenous to India but is introduced from USA and European countries in order to increase honey production in apiaries. The species is slightly larger in size and lighter in color and higher capacity to produce honey and hence is preferred by apiarists.

However, the species is also amenable to diseases such as the American foulbrood which sometimes destroys large number of colonies.

**LIFE CYCLE**

Queen is the largest caste that has pupal period of 16 days. Queens are reared in enlarged cells in which their larvae are fed exclusively on royal jelly. New queens can be raised by the worker bees anytime if the main queen dies. The virgin queen takes to nuptial flights for mating and then settles in the hive for laying eggs. The sterile worker bees clean the hive and feed the larvae during the first 10 days of their lives, after which they build comb cells in the hive. On 16 to 20th day, the worker makes honey out of the nectar brought by forager. After the 20th day, the worker leaves the hive and spends the rest of life as a forager and eventually dies as a water carrier.

Workers, drones and queen larvae are fed on royal jelly during the first 3 days of life, after which the worker larvae are fed on pollen and diluted honey, while those destined to develop into queens are fed on protein rich royal jelly.

Queens are reared in specialized large queen cells which are specially constructed for queen larvae and have a vertical orientation. When the old queen dies or becomes weak, the workers will construct emergency cells known as supersedure queen cells, which are larger and project from the comb.

Drones are genetically haploid males, which possess weak mouthparts and hence they cannot forage for nectar or pollen themselves and have to be fed by the workers. Drones fertilize the queen by mating in nuptial flight, after which drone dies. The drones are generally expelled from the hive and die of cold and starvation. The queen stores sperms in small sac-like organ called the spermatheca located in the queen’s abdomen.

**HONEY MAKING**

Honey making by bees is a specialized job in which they collect nectar from flowers. Nectar is a clear liquid containing 80% water and sugars. The worker bees collect nectar in crop and pollen on hind leg and then return to hive and unload it into the cell. The workers in hive then digest the raw nectar for about 30 minutes and regurgitate it into the cell. This is done several times to add enzymes and other materials into it. This honey is placed in empty cells of honeycomb to bring the water content to less than 20%. They fan the honey with their wings to bring down the water contents. Once ripe, the cells of the honeycomb are sealed with a wax cap.

Supersedure is a phenomenon in which old and ailing queen is replaced by a new one. As the queen ages or ails the output of queen substance pheromone is not produced. This signals workers to rear a new queen. The workers quickly detect the ailing queen or its inability to lay eggs and will then rear a new queen.

**PHEROMONES**

Chemical secretions or pheromones produced by the queen bind the colony together. Workers secrete pheromones from Nasanov gland which are inside the tip of abdomen. This helps the workers to identify the members of their colony when they group together or collect nectar or water. The colony pheromone is quickly recognized by the bees of the same colony because of its unique chemical composition.

When a queen flies to mate, her pheromones attract all the drones. Another pheromone, called queen substance is licked by workers from the queen’s body and passed to others. Queen substance also inhibits the ovaries of workers and renders them sterile.

The mandibular glands of workers also produce an alarm pheromone, which alerts the colony when it is threatened or attacked by a predator. Workers leave the sting on the body of victim which also produces a sting odor, which serves to attract other bees to the area for stinging,

**BEE BREAD**

Bee bread is a mixture of pollens collected by bees mixed with saliva and honey and contains most of the necessary nutrients required by the colony. Bee pollen contains about 25% proteins that carry about 18 amino acids. It also contains all essential vitamins, minerals, several enzymes, all essential fatty acids and carbohydrates. Bee pollen is low in calories and proves to be quite useful for activity enhancement. Bee bread provides energy to bees and enhances their performance.

**Beeswax:** Wax is secreted from the glands on abdominal segments 4-7. Beeswax is used by the honey bees to build honey comb. There are sternal wax plates on abdominal segments 4-7, which are located on the ventral side, from where it is removed as flakes and chewed with mandibles. Wax is softened with the secretion of mandibular glands to make it into a paste.

**Propolis**

This is a glue-like substance collected by bees from trees. The sticky resin is mixed with wax to make it into sticky glue which is used to construct the foundation of the comb strong. The bees also use propolis to seal cracks in their hive.

**Royal Jelly**

The royal jelly is a milky substance that is made of digested pollens and honey mixed with the secretion of pharyngeal gland of nursing bee. It is loaded with proteins, fats and all of the B vitamins.

**Bee Venom**

Honey bee venom contains Melittin, which is a potent anti-inflammatory agent. Adolapin is another strong anti-inflammatory substance. Apamin found in venom inhibits calcium-dependent potassium channels. Hyaluronidase, Phospholipase and Histamine are involved in the inflammatory response of venom. Venom also contains small amounts of the neurotransmitters such as Dopamine, Norepinephrine and Seratonin.

Bee venom is hemorrhagic in action. Apamine, melittin, phospholipase, hyaluronidase, inhibit the nervous system and stimulate heart and adrenal glands. Also present in venom are certain antibiotics.

**SERICULTURE**

Sericulture was first introduced into China by Hoshomin, the Queen of China. For a long time sericulture was considered to be a national secret by the Chinese and its industrial technique was not known in other countries. Later, it was introduced into Europe and Japan by smuggling the secrets from China through travelling monks.

According to some sources, sericulture was introduced in India about 400 years ago and the industry flourished as an agro-industry till 1857, with an annual production of a million kg of silk fibre.

According to Chinese legend, the technique of silk production using *Bombyx  mori* was invented at around 2,700 BC when prince Hoang-ti directed his wife Si-ling-chi to study the silkworm and explore the practicability of using its thread for textile. Si-ling-chi devised not only the technique of culturing silkworm but also the method of reeling the silk and making garments out of it.

She was later crowned as “The Goddess of Silk Worm”. Subsequently sericulture spread from China to other countries and silk became a precious commodity, highly sought after in all countries. In 139 BC the world’s longest highway that stretched from Eastern China to the Mediterranean Sea was opened, which was called “Silk Route” due to trade in silk.

**Five** species of silk worms are reared in India:

 1. ***Bombyx mori meridionalis,*** the **Mulberry silk worm** (Lepidoptera: Bombycidae), feeds on the leaves of mulberry (*Morus alba)* to produce the best quality silk fibre.

 2. ***Antherea paphia or Antherea mylitta,*** the **Tasar silk worm** (Lepidoptera: Saturnidae), feeds on *Terminalia tomentosa* that occurs in the jungles of Bihar, Madhya Pradesh, UP and Orissa.

3. ***Antherea royeli*** and ***Antherea perniyi,*** the **Oak tasar silk worms** (Lepidoptera: Saturnidae), feed on oak trees and were introduced from foreign countries.

 4. ***Antherea assama,*** the **Muga silk worm** (Lepidoptera: Saturnidae), is confined to the Brahmaputra Valley of India and produces the famous muga silk.

 5. ***Phylosamia ricini,*** the **Eri silkworm** (Lepidoptera: Saturnidae), which feeds on castor *(Ricinus communis)* is raised in Assam, Madhya Pradesh, Rajasthan and Orissa commercially.

**MULBERRY SILKWORM**

**Mulberry plantation**

Four Indian species of mulberry, namely, *Morus alba, M.indica, M. serrata* & *M.laevigata,* are cultivated as main food plants of silkworm. Different systems of plantations for mulberry are practiced in India. In India where the temperature ranges from 16oC to 31oC, mulberry silkworm can be reared throughout the year. Karnataka, where the temperature ranges from 16-31 degree centigrade, provides ideal climatic conditions for rearing mulberry silkworm throughout the year, whereas in West Bengal, the multivoltine silk-worm rearing is practised even under adverse temperature conditions. In Jammu & Kashmir univoltine variety of silkworm is cultured only once a year during May-June.

**LIFE CYCLE**

Eggs of *Bombyx mori* are small and hard, about the size of a pin head and resembling poppy seeds. The egg stage lasts 10 days; the larval stage lasts the longest, 25-30 days and the pupal stage takes 10 days. The larvae are white, 4-5 cm long and moult four times during their growth. At the end of the larval duration, the silkworm produces silk from its mouth to constructs a cocoon in some hidden and secure place. Healthy moths are allowed to copulate for 4 hrs, after which the female is consigned to a dark plastic ‘cellule’ for egg laying. She lays about 400 eggs in 24 hours.

Considering the various factors, such as the place of origin, voltinism (number of generations in a year), the colour of cocoons, the larval markings, the colour, shape and size of cocoons, the silkworms are classified into different breeds. The multivoltine races are reared in West Bengal and Karnataka due to optimum temperature conditions. Bivoltine races diapause in winter and hence can have only two generations in a year.

Univoltine and bivoltine races require more leaves than the multivoltine ones. However, the yield and quality of cocoons of the bivoltines are superior to those of multivoltines. The average annual yield of cocoons in India is as low as 150 kg under rain fed conditions and 400 kg under irrigated conditions The cocoons after cooking are reeled in hot water in different types of reeling machines. In India, 61 percent of the silk amounting to 1,320 tonnes is reeled on the country-type charkha.

**NON-MULBERRY SILK WORMS**

**TASAR SILK WORM**

Three species of *Antherea* are used for the extraction of tasar silk in India. They are *Antherea mylitta, A. perniyi* and *A. royeli*. Out of the total non-mulberry silk produced in India, about 400 tonnes is produced from *Antherea mylitta* in Madhya Pradesh, Orissa and Bihar. This silkworm feeds on *Terminalia tomentosa*and *Terminalia arjuna* found in the forests of central and north-eastern parts of India. The tasar silkworms is a wild species and hence cocoons are also collected by the tribal people from forests and silk is obtained. The first crop, usually called the seed crop is raised during May to July, whereas the commercial crop is raised during October-November.

The larvae are usually green in colour and moults four times before they complete their larval duration. However, yellow, blue and white larvae are also reported. At the end of the larval period, they spin a ring like structure around the twig and a long stalk from which the cocoon hangs. The cocoons are large and brown or yellow in colour. Moths emerge from the cocoons in June. To obtain silk, the cocoons are cooked in caustic potash and reeled to extract fibre and then spun to manufacture coarse thread.

The recent introduction of *Antherea perniyi*and*A. royeli* on oak trees in Manipur has opened up new opportunities for the production of superior quality tasar silk in India. The cocoons of *Antherea perniyi* can be easily reeled and fibre of superior quality can be obtained. *Antherea royeli*occurs in oak jungles of the sub-Himalayan region.

**MUGA SILK WORM**

The golden-yellow silk produced by *Antherea assama* is found only in the Brahmaputra Valley of India. This species of silkworm is semi-domesticated as the larvae which crawl down of trees at the end of their larval period are collected and allowed to spin cocoons in captivity. *Antherea assama* produces golden yellow silk that is of high quality which is expensive. The worms feed on Som (*Marchilus bombycina)* and Soalu *(Litsaea polyantha)* trees. At the end of the larval period, when the worms are ready to spin cocoons, they crawl down the tree in search of suitable places for making cocoons. To obtain silk, the cocoons are boiled in soap and soda solution and are reeled on a machine**.**The total production of muga silk in India is about 50 tonnes but there is plenty of scope for expansion of this industry.

A single Muga female moth lays 150-200 eggs after copulating with the male for 6-8 hrs. The larvae are yellowish with black markings on the body and have the habit of crawling down the trees in groups when all leaves are consumed on the trees and larvae have matured. If larvae have not matured and the leaves on the trees exhausted, they can be transferred to another tree. At the end of the larval period, when the worms are ready to spin the cocoons, they crawl down the tree in search of a suitable place for the construction of cocoons.

**Spinning of Cocoons**

The mature silkworms will come down from the trees to the base on ground in search of proper place for spinning of Cocoons. Therefore, bundles of semidried twigs are collected and the worms are placed on these twigs so that they make their cocoons.

**ERI SILK WORM**

The silk produced by *Philosamia ricini* is called **Eri silk**. It is grown in Assam and in the eastern parts of India. The heavy rainfall & humid atmosphere in these parts are conducive to eri culture. The food plants for *Philosamia ricini* is castor.   This silk worm is multivoltine and reared indoors.

The eggs are white and hatch in ten days. The hatched larvae are mounted on castor leaves in the rearing-houses and are allowed to grow by feeding on leaves. The worms moult four times during the larval period of 30-32 days. Eri silkworm is generally hardy and not easily susceptible to diseases. At the end of larval period, the larvae crawl in search of suitable places to spin cocoons.

 The cocoons of the eri silkworm cannot be reeled, as they are made up of several small fibres and hence the emergence of moths is allowed and the cocoons are spun like cotton to produce yarn. Approximately ninety tonnes of eri silk is produced in the country annually.

Recent efforts to rear tasar silkworms on oak plants in the sub-Himalayan range and in Manipur have contributed to the production of a significant quantity of quality tasar silk. It has also opened up new avenues for improving sericulture and also enhanced the employment potential in the tribal hilly areas.

**LAC CULTURE**

Members of two families of Hemiptera, namely, Lacciferidae and Tachardinidae secrete lac over their bodies for protection. **Lac Insect** belongs*Laccifer* of superfamily Coccoidea of order Hemiptera. In all 22 species have been recorded under the genus *Laccifer*in Indian subcontinent.

India is still being regarded as the principal lac producing country of the world. Burma went into lac trading since sixteenth century. Lac culture in China probably dates back to 4000 years and they use lac for dyeing silk and leather goods. India produces about 65% of the world’s total output. Bihar and Jharkhand account for 40% of India’s total production of lac.

**HOSTS**

Plants such as, *Zizyphus mauritiana, Z. jujuba, Butea monosperma, Schleichera oleosa, Acacia arabica, A catechu, Cajanus cajan, Ficus benghalensis, F. cunia,*and*F.* religiosa are common hosts of the lac insect *Laccifer (=Tachardia) lacca.*

**BIOLOGY**

*Laccifer lacca, (=Tachardia lacca)* is the commercially cultured lac insect. It is mainly cultured in India and Bangladesh on the host plants such as ber, *Zizyphusmauritiana*, palas, *Butea monosperma*and kusum, *Schleichera oleosa.*

Female insect is viviparous, producing about 1000 nymphs, deep red in colour with black eyes. The larvae settle down on a suitable place of the host plant gregariously. A day or two after settlement, the larvae start secreting lac all around the body except on the rostrum, spiracles and on the tip of abdomen. Thus it gets encased in a cell of lac which gradually increases in size along with the increase in size of the insect. The insect moults twice before reaching maturity. The male larvae produce elongated lac cells while the females produce oval cells

After the first moult larvae lose their legs, antennae and eyes and become bag-like. After the 3rd moult, the larvae pass on to a pseudo-pupal stage. Males emerge and copulate with the females and die. The female larvae never regain appendages and continue to remain under the lac cell, become adults and reproduce. As the lac insects remain close together, lac secretion from adjacent cells coalesces with each other and forms a continuous encrustation on the tree branch.

**LAC CULTIVATION**

Lac culture involves two important steps: (i) **inoculation**, and (ii) **cropping**. Inoculation can be carried out through artificial infection of tender branches by brood lac stick obtained form mature lac trees immediately after harvesting. In this process, the brood lac sticks are tied in bundles of 2 or 3 sticks on the branches of the host tree, allowing maximum contact with the branches.

There are four seasons of lac cultivation and according to the Hindi calendar, they have been named as **Kartiki, Aghani, Baisakhi,**and**Jethwi**. The crop period, from inoculation to harvesting, for **Kartiki**, ranges from July to November, for **Aghani**, from July to February, **Baisakhi**, from November to July, and **Jethwi**, from February to July.

When young shoots come up on branches, the brood sticks are tied adjacent to the growing tender branches in a way so that maximum contact between shoots takes place. Within a week or two the larvae emerge and settle down on tender shoots.

**PROCESSING OF LAC**

Lac encrustations are removed from the twigs of host plants by scraping. The raw lac thus obtained is known as scraped lac or stick lac. **Sticklac** is crushed into small grains, sieved, washed with mild alkaline water and dried. This semi-refined product, called **seed lac** or **grainlac** or **Chowrie**, which is further refined by a system of hot melting, filtration and stretching into thin sheets which are subsequently broken into brittle flakes called **shellac**.

Alternatively the purified lac resin can be in the form of circular discs called button lac. If a solvent process is used to purify the raw lac, de-waxed, decolorized lac can be obtained as the end product. The normally amber coloured resin can also be bleached with sodium hypochlorite to obtain bleached lac, which is white in colour. Bleached lac has specialised demand for coating medicinal tablets, confectioneries etc.

  India is the principal lac producing country of the world, producing approximately 18,000 metric tonnes of raw lac annually. About 85% of the country’s production is exported to various countries. The USA, Germany and Egypt are some of the major lac importing countries of the world.

**USES OF LAC**

The various applications of lac can be summarized as follows:

**Lac resin** is used in food processing industry; cosmetics and toiletries industry; varnish and printing industry; coating of fruits and vegetables; electrical industry; leather industry; adhesive industry; pharmaceutical industry; perfumery industry; miscellaneous applications.

**Lac dye (erythrolaccin)** has been used in India as a skin cosmetic and dye for wool and silk. In China it is a traditional dye for leather goods. The use of lac for dye has been supplanted by synthetic dyes. It is used in medicine to protect liver and to fight obesity.

Lac is used in food, confectionery and beverages industry and textile industry.

Lac wax is used in polishes for shoe, floor, car polishes etc. It is used in electric insulations, lamination of papers, hat proofing and coating of pictures and fossils.

Lac is used for manufacture of tailors chalks, crayons, bottle sealers, lipsticks, enamels, printing inks, gramophone records and in fireworks.

**NATURAL ENEMIES OF LAC**

**Predators:**

Two moth predators cause a lot of damage to lac.

1. ***Eublemma amabilis.***The larva is dirty white in colour and tunnels through the lac encrustation and feeds on larvae and adults. It pupates within the tunnel and adults after emerging lay their eggs near the lac encrustation.
2. ***Holcocera pulverea.***The damage by the brownish larva is similar to the above species. Pupa is slightly bigger and yellowish-brown.

**Parasites:**

The following insects are parasitic on lac insect.

*Paraecthrodryinus clavicornis; Erencyrtus dewitzi; Tachardiaephagus tachardiae; Eupelmus tachardiae; Tetrasticus purpurens.*

The above natural enemies can be controlled by maintaining healthy cultures and by enclosing the brood lac sticks in wire mesh before inoculation so that natural enemies are not able to emerge and cause re-infestation.

**CARP CULTURE**

Indian aquaculture has been growing at a fast pace over the last two decades, with freshwater aquaculture contributing over 95% of the production. The three Indian major carps, namely catla *(Catla catla),* rohu *(Labeo rohita)* and mrigal *(Cirrhinusmrigala)* contribute to the bulk of production amounting to about two million tonnes annually (FAO, 2003). Silver carp, grass carp and common carp formthe second important group for fish production. Average national production from pondfisheries has increased from 0.6 tonnes/ha/year in 1974 to 2.2 tonnes/ha/year in 2002 (Tripathi, 2003).

**COMPOSITE FISH CULTURE**

The three major carps cultured in India, namely, **catla***(Catla catla),***rohu***(Labeo rohita*) and **mrigal***(Cirrhinus mrigala),* contribute as much as 87 percent of the total Indian aquaculture production. Three exotic carps were also introduced, namely, silver carp *(Hypophthalmichthys molitrix);* grass carp *(Ctenopharyngodon idellus*) and common carp *(Cyprinus carpio)*. There are also several other medium and minor carp species, namely,*Labeo calbasu, L. fimbriatus, L. gonius, L. bata, L. ariza,Cirrhinus mrigala, Puntius sarana, Hypselobarbus pulchellus, H. kolus*and *Amblypharyngodon mola*, which are important in aquaculture. Among catfishes, magur*(Clariasbatrachus)* is the only species that is widely cultured, while the catfish, ‘Singhi’ *(Heteropneustes fossilis)* is cultured to some extent in the eastern states.

Attempts have also been made to culture the other catfishes like *Pangasius pangasius, Wallago attu, Sperata seenghala, S. aor*and*Ompokpabda*. The finfish species of importance include climbing perch *(Anabastestudineus),* murrels *(Channa striata*and*C. marulius)* and tilapia *(Oreochromismossambicus*and*Oreochromis niloticus).*

**PREPARATION OF PONDS**

Pond preparation involves making the ponds weed and predator-free and generating adequate natural food for the survival and growth of fishes. Control of aquatic weeds, removal of undesirable flora and fauna and improvement of soil and water quality are important aspects of fish management.Weeds have to be removed from the ponds first, after which the tank is fertilized with both organic and inorganic fertilizers, such as Oil Cake and raw Cow Dung @ 5,000 kg/acre.

The PH of pond water should be 7.5 – 8.00, for which lime is added in the tanks @ 200 kg/acre per annum. The lime increases pH and also helps in eradicating fish parasites.The organic fertilizer in the form of raw cow dung is added in the tank @ 500 kg/acre per annum. This is followed by the application of inorganic fertilizers like Super Phosphate @ 120 kg/acre and Ammonium Sulphate @ 200 kg/acre, in spaced intervals.

**SPAWNING**

Because of constant temperature and favourable weather conditions, carps spawn all the year round in India. Spawning takes place early in the morning when the water surface cools down to about18 degrees. The female carp swims near the water surface followed by the male carp in nuptial swimming and rubbing each other’s bodies. Female lays egg and male releases its milt and eggs are fertilised.

Three days after fertilization, the eggs begin to hatch. The newly hatched larva is about 5.5 mm long, delicate and transparent, with a yolk sac attached to the belly. It rarely swims but settles on the bottom or on some floating object. On the second day, the larva starts swimming and on the third day swims actively from surface to bottom. During these stages, the larva or fry gets its nourishment from the yolk sac, which disappears on the third day and the fry now must search for food and eat. Supplementary fry-feed in the form of hard-boiled egg yolk or powdered milk can be applied on the water surface at this time.

Carpscan feed on almost anything like insects, shells and worms and can also eat aquatic plants, bread crumbs, rice bran and fish meal made from corn, copra and soybean.

**CHOOSING BROODERS**

Both female and male brood fish should be carefully tended for 2-3 months before induced spawning operations are carried out and males and females should be segregated and kept in separate ponds.

 To be good brooders the fish must be more than one year old and 150 gm in weight. Sex can be determined by the shape of the genital papilla which is pointed in male and oval in female.  When the female is ready for induced spawning operations, It should have a bulging abdomen that is soft to touch. The cloaca is reddish and prominent, and the contour of the enlarged ovaries can be seen on both sides of the abdomen. The head should be small and the snout pointed.

**Nursery ponds** are constructed to rear carp fry or larvae. A normal sized nursery pond measures 5 x 10 m, with a depth of 0.5 m. Before filling up water the pond should be cleaned thoroughly to get rid of predators and parasites that may be destructive the larvae.About 1,500 to 3,000 fries can be stocked in the nursery pond andfed with milk, wheat flour or boiled egg yolk by spreading it on the water surface. This feed can be supplemented with rice bran, bread crumbs or fish meal, which can be given twice a day, in the morning and in the afternoon.

**Rearing ponds**, where adult carpsare cultured until they reach marketable size, are needed, which have dimensions of 15 x 50 m and depth of 1.5 to 2 m. Rearing ponds should also be thoroughly cleaned before filling them with water. This is done by exposing the bottom and letting it dry thoroughly.Next step involves application of fertilizers, which encourages growth of aquatic plants, moss and algae, which are important natural food and also lead to growth of micro fauna. Manure in the form of chicken dropping is the most commonly used being cheaper and more readily available in large quantities. When carp fry reaches the length of about 5 to 7 cm, they are transferred from the nursery pond to the rearing pond and allowed to grow to adult stage.

**STOCKING OF PONDS**

Ponds are stocked with fish fries of appropriate size. Fingerlings of over 10 cm in size are recommended for stocking in culture ponds. Stocking of smaller fishes may result in higher mortalities and slow growth during the initial months. In fish polyculture a fingerling size of 50-100 g is preferred for stocking to ensure higher survival and better growth. Generally, a density of 5,000 fingerlings is kept as a standard stocking rate per ha for carp polyculture, which will give a yield of 3-5 ton/ha/yr.

Prior to stocking, the fish fries should be dippedin 3-5% potassium permanganate solution for 15 seconds to kill parasites. In composite fish farming, a combination of six species are cultured, namely, Catla, Rohu, Mrigal and exotic Carps like Silver Carps, bass and common Carp.Supplementary feeds like Groundnut Oil cake and Rice Bran are fed to fishes during culture. At the end of the culture period of say 12 months, the fish will reach marketable size and fetch attractive prices.

**POST-STOCKING POND MANAGEMENT**

While fertilizing the carp ponds, 20-25% of the total amount of organic manures is applied a fortnight before stocking and the remaining amount is applied in equal instalments on a bimonthly basis. Other commonly used organic manures include poultry manure, pig dung, duck droppings, cow dung, domestic sewage, etc. *Azolla*, a nitrogen-fixing fern is used as a bio-fertilizer for aquaculture at the rate of 40 tonnes/ha/yr, which supplements nutrients required for intensive carp culture. The bio-processed organic manure, biogas slurry has also been used as manure in carp culture.

The supplementary feed in carp polyculture is usually restricted to a mixture of groundnut/mustard oil-cake and rice bran. Grass carps are fed with aquatic vegetation such as*Hydrilla, Najas, Ceratophylum,* duck weeds, etc. which can be kept in special enclosures in corners of the pond. Feeding preferably twice-a-day is advocated @ 5% of the initial biomass of stocking material for first month and then gradually reducing it.

Aeration may be done mechanically to increase the concentration of dissolved oxygen in ponds, by paddle wheel aerators, aspirator aerators and submersible pond aerators. It is also necessary to replace certain amount of water at regular intervals.

**HARVESTING**

Harvesting of fishes is usually done after a culture period of 10 months to one year. However, fishes attaining the marketable size can be harvested periodically depending on several factors, which also reduces the pressure of density in the ponds and thereby providing sufficient space for the growth of fishes.

**INDUCED REPRODUCTION IN FISH**

 Inducedfish farming has allowed farmers to breed and raise species that do not naturally reproduce in captivity, to manipulate the timing of reproduction to suit production cycles, getting fish to spawn on a predetermined date and fertilise and incubate eggs under hatchery conditions. There are two main strategies used to induce reproduction. The first is to provide an environment similar to that in which spawning occurs in nature. Catfish, for example, likes to spawn in enclosed spaces and goldfish in vegetation and at high temperatures.

The second strategy is to inject the fish with one or more naturally occurring reproductive hormones or their synthetic analogs to manipulate maturation of gonads and ovulation. The hormones injected include, **Gonadotropin Releasing Hormone (GnRH)** analogs, dopamine antagonists and gonadotropins. **Leutinizing Hormone Releasing Hormone (LHRH)** is a mammalian hormone that has been employed successfully to induce reproduction in fishes. **Dopamine** inhibits the action of LHRH and hence a dopamine antagonists are given for induced breeding.

Two types of **gonadotropin** extracts have been used to induce ovulation in fishes, namely, **Human Chorionic Gonadotropin (HCG)** and **fish pituitary extract**. Pituitary extracts are made by removing the pituitary from a fish and extracting the hormones, which may then be injected into another fish. HCG offers three major advantages over the pituitary extract, namely,**1)** it is much less expensive, **2)** it is more stable and **3)**it comes in a purified form. An intraperitonial injection is given through the ventral part of the fish behind the pelvic or pectoral fin. Intramuscular injections are commonly given on the dorsal part of the fish above the lateral line and below the dorsal fin.Two doses with a time gap of 12 to 24 hours are given.

**OBTAINING PITUITARY EXTRACT (HYPOPHYSATION)**

Pituitary gland contains **gonadotropin** hormone, which stimulates the production of sex steroids in the gonads and induce maturation of gametes. Gonadotropin is composed of **follicle stimulating hormone (FSH)** and **luteinizing hormone (LH),** which are responsible for the egg development and egg ovulation.The skullof the fish from which,**hypophysis** is to be collected, is cut open with a knife to remove the bone and meninges. The pituitary gland can be seen after the mid-brain has been folded back by using forceps.The gland is taken out and ground in the homogenizer and then distilled water is added and the gland is again ground. The solution is taken in a syringe for injection. The female can be injected with two doses with the time interval of 6 hours. The intramuscular injection is given in the area between the base of the dorsal fin and lateral line.

**OVULATION & FERTILISATION**

**Stripping** the fish is done by holding the female around the caudal fin with one hand, while applying slight pressure to the abdomen with the other hand. A stream of eggs will eject through the genital opening. The abdomen should be messaged from front to back to strip out all the eggs.Ovulation occurs about ten hours after the second injection of hormone.  The eggs are collected in a dry plastic container. At the same time, the milt from a male fish is made to drip on the eggs by pressing the testes with fingers and pouring the water through the fine mesh cloth. Eggs and sperm are mixed and stirred gently. After about two minutes, water is added two or three times to cleanse the fertilized eggs and then they are transferred to the hatching **happas**, where most of the fertilized eggs hatch out within 24 hours. The

The yolk sac is absorbed in the body of fry in about 2 days, and then the larvae are transferred from happas to the nursery tanks, where the fries feed and grow. Food has to be given during the first 3 weeks and when the fries reach the size of 2–3 cm, they are distributed in the rearing tanks where they are cultured further to adult marketable stage.

**PEARL CULTURE**

The technical requirements for establishment of Pearl farm and its successful operation are briefly described below:

**PROCESS OF PEARL CULTURE**

The process of pearl culture includes the follwing steps which are very crucial for obtaining high grade of pearls with good commercial value.

**Step 1: Construction of pearl farm**

Construction of a pearl farm includes three steps. They are,

* Selection of farm site
* Construction of farm
* Well-planned work schedule

**Selection of farm site:** This step determines the type of pearls produced, and the oyster survival rate. Some of the points to be noted while selecting the site are:

**\*** Natural features like mountains and reefs are needed to protect the farm from winds, currents, storms, etc.

**\*** Constant regularity of temperature

**\*** Type of sea bed, such as rocky or sandy.

**\*** Gentle currents are essential for the survival of the oysters as they bring food and oxygen.

**Construction of pearl farm:** The whole pearl farm system is based on series of floating wooden rafts. Ten units of wooden rafts are used. Each raft consists of two to five pieces of wood making the total length to 20 ft. The raft is covered with wire mesh baskets, each of which house 10 oysters.

**Well-planned work schedule:** A typical work schedule plays a very critical role in pearl culture. The timing for collecting and seeding the oysters must be scheduled and followed strictly.

**Step 2: Collecting oysters**

After the construction of pearl farm, the divers set out to the bottom of the sea, to collect the oysters. Divers are pulled by large lugger boats in the direction of the tidal flow. Oysters are generally located on a flat rock bottom and are usually covered with marine animals and a thin layer of silt. Therefore, it is often very difficult for divers to recognise them. The shells collected, are cleaned, sized, and placed into baskets for storage until they are transferred to the pearl farm.

**Step 3: Seeding**

Two-three year old healthy oysters are considered for surgical implantation known as seeding. This is a very delicate operation and involves three stages:

**Preparation of the graft:** A donor oyster is sacrificed to obtain mantle. Mantle is needed by the host oyster to accept the nucleus. The mantle is located on the outer section of the oyster and Mantle produces the nacre which forms pearl. Before a graft is taken from the mantle, the oysters are starved for several days to slow down the metabolism of the oyster. This helps to decrease the risk of core rejection and open the oyster easily.

**Attaching the graft:**The oyster is opened with special wedges and pliers, then a scalpel slit is made in the soft tissue near the reproductive organ and a graft of living mantle is inserted into the slit.

**Inserting the core:** A nucleus is placed in the scalpel slit and the oyster is then returned back to the water. The inserted core irritates the oyster, provoking it to gradually coat the core with thin layers of mother of pearl nacre. After some time, the oysters are collected, and x-rayed to see whether the implants have been accepted. Oysters which have rejected the implant are returned to the water and are once again operated. The oysters which have accepted the implant are transferred to the pearl farm.

The person who is seeding must be extremely careful not to harm the tiny pea-crab which lives unharmed within every healthy oyster. It is presumed that the crab assists the oyster by keeping it clean and by sharing the debris which the oyster sucks in.

**Step 4: Caring the oyster**

The shells which have been collected and transferred to the pearl farm are placed in baskets or panels which are attached to long lines connected to the floating rafts. The rafts are dropped down into the ocean with the oyster securely inside the basket, where they remain until they become operated on for further seeding.

The oyster can produce more than one pearl in its lifetime. Regular cleaning of the shells to remove seaweed results in better pearls plus makes them easier to handle. The cleaning is done by a machine which uses water jets and brushes to clean off any seaweed. The oysters need very tender loving care so as to be productive when harvested.



**METHODS IN PEARL CULTURE**

**Harvesting**

After 2-3 years, the oysters are harvested. It is necessary to make a trial harvest to determine whether the pearls have a sufficient coating. If it is not sufficient then an additional six months to a year of culturing is necessary. The oysters are split open and pearl bags are cut by the scalpel to remove the pearls. Collected pearls should be thoroughly dried after the harvest to prevent loss of luster.

**Sorting pearls**

There are many different steps involved with the sorting of pearls. Firstly, the pearls are sorted according to whether they can be used for the cultured pearl industry or not. These are categorised into three sections:

* Unmarked pearls
* Pearls with one major blemish
* Pearls with more than one major blemish

**PRAWN CULTURE**

The prawn production in India accounts for about 15% of the total world production of prawn and shrimps. For marine prawns, the percentage of Indian production to the world production is about 20%. The major commercial prawn species reared in India are *Macrobrachium. rosenbergii*and*M. malcolmsonii.*

**BIOLOGY**

***Macrobrachiumrosen bergii****,* also known as the giant river prawn or the **giant freshwater prawn,** is native to the Indo-Pacific and northern Australian Regions. The adult is found in freshwater, while its larval stages live in brackish water after the juvenile stage. During mating, the male attaches its spermatophore on the ventral side of the abdomen of female’s body and the eggs coming out of female genital opening are fertilised by the sperms derived from spermatophores.

The fertilised eggs are held in the brood chamber or egg basket, which is made by the interlocking ***appendix interna*** of the pleopods and are aerated by vigorous movements of the swimmerets for 2-3 weeks. This is in contrast to shrimps, whose fertilized eggs are released into the sea. Females can lay 80,000-100,000 eggs during one spawning and eggs take an average of 20 days at 28°C to hatch into larvae.

After hatching, larvae are dispersed by the rapid movements of the abdominal appendages of the female. Larvae are planktonic and swim upside down actively with tail first posture and feed on small planktons. Larvae complete development in 15-20 days and metamorphose into post larvae, which resemble miniature adults and generally feed near bottom and then begin to migrate upstream into freshwater rivers within one or two weeks after metamorphosis and are soon able to swim against the rapidly flowing currents (contranatant behaviour) and can also crawl over the stones in shallow waters.

**HATCHERIES AND NURSERIES**

Freshwater prawn hatcheries need supplies for both freshwater and sea water; the latter can be drawn from areas where the salinity is 30 to 35 ppt. The brackish water derived from the mixture of seawater, brine or artificial sea salts mixed with freshwater should have salinity of 12-16 ppt, pH of 7.0 to 8.5 and dissolved oxygen level of 5 ppm.

 The prawn farm site should also have the following facilities:

* A secure power supply to ensure that the components of hatchery, e.g. aeration, water flow etc. can continue to function uninterrupted.
* An uninterrupted access for incoming and outgoing materials by road.
* Access to the uninterrupted seawater and freshwater supplies.
* Farm should not be close to cities, mines and industrial centres or to other activities that may pollute the water supply.
* Farm should be situated in a climate where the temperature range of 28-31°C can be easily maintained.
* Food supplies for larvae should be easily procured when required.
* Should have access to biological and veterinary assistance whenever required.
* Should be close to other nursery facilities feed sites.
* Should be close to the market for quick selling after harvesting.

**OBTAINING BERRIED FEMALE PRAWNS**

Berried females are those that carry fertilised eggs in their egg basket. They can be obtained from rivers, canals, lakes and estuaries, where they are most abundant in the beginning of rainy season. In the tropics, berried females can be obtained all the year round from farm ponds containing adult animals.   Selecting fast-growing, berried females from ponds has a positive effect on the weight of prawns at harvest.

In the tropics, where berried females are readily available, special brood holding facilities are not required but in temperate areas, indoor brood stocking facilities are essential. Brood stock is disinfected by placing into freshwater containing 0.2-0.5 ppm of copper sulphate or 15-20 ppm of formalin for about 30 minutes.  Prawns should be fed daily at the rate of 1-3% of total biomass.

Berried females can be collected from the holding system and placed in tanks where the eggs will hatch into first instar larvae, which are collected by netting. The hatching tanks should be covered to prevent bright sunlight to reach larvae for which the inner side of the tanks should also be painted with black epoxy-resin paint.

**LARVAL REARING TANKS**

Different designs of containers can be used to grow freshwater prawn larvae, which may be circular flat-bottom tanks, circular conical-bottomed plastic tanks, plastic-lined wooden tanks, rectangular concrete tanks, concrete-faced brick tanks and earthen water jars. Good drainage system is essential as water has to be removed from tanks at harvesting time. Mixing tanks are also required for preparing the brackish water to be used in the hatchery as well as storage tanks.  Aeration of water is also essential which can be done through PVC pipes, with holes cut at one foot intervals.

Larvae should not be exposed to direct sunlight, for which 90% of the tan area should be covered and shady. Some natural light is essential for good larval survival, which can be provided through transparent roofs over the hatcheries. Physical filters that include sand filters, drum screen filters, and medium filters should be easy to clean and designed to minimize water loss.

Water needs to be chemically treated before it can be used in rearing tanks and also should be physically filtered by passing through the sand bed before transferring it to another tank for treatment. Mix the seawater or brine with freshwater to form 12 ppt of brackish water. The optimum temperature range for *M. rosenbergii* is 28-31°C. Below 24-26°C the larvae will not grow well and the time taken for them to reach metamorphosis will be longer.

**LARVAL FEEDING**

A wide range of feeding material is used by different hatcheries, which includes nauplius larvae of shrimps, freshwater cladocerans, fish eggs, squid flesh, frozen adult *Artemia*, rotifers, fish flesh, egg custard, worms and commercial feeds available in the market. The quantity of food to be given depends on the utilization of feed by larvae that vary from place to place. The quantity of feed consumed will increase as the larvae grow.

**HARVESTING POST LARVAE**

When post larvae are about 7-8 mm long, they can withstand transfer from 12 ppt water into freshwater. However, they should not be harvested from the larval tanks and transferred directly into holding tanks containing freshwater but should be acclimatized to fresh water in the larval tanks itself. When majority of larvae have metamorphosed, water level in tanks should be reduced to about 35 cm. Then gradually the tank should be flushed with freshwater over a period of 12 hours. The post larvae can then be collected and transferred or the larval tanks can be refilled to 70 cm with fresh water and the animals temporarily held in them. The best way to harvest post larvae from the larval tanks is to reduce the water level and then remove them by nets.

**HOLDING POST LARVAE BEFORE SALE**

 Post larvae cannot be held in holding tanks for more than a week or two prior to stocking in nurseries. When the post larvae are in the holding tanks, the rearing water should be changed every 2-3 days) to provide aeration. Post larvae can be stocked at densities of about 5,000/m2 for one week, although survival increases by reducing the density.

**REARING PONDS**

Pond size should be such that can be managed easily. Generally most farms have ponds of around 0.2-0.6 ha size. Large ponds are normally wider than 30 m and often drained for harvesting. The average depth of water in freshwater prawn ponds in tropical areas should be about one meter; with a minimum of 0.75 m and a maximum of 1.2 m. Deeper ponds are used in colder areas to maintain more stable water temperatures. The banks of the ponds or embankments or bunds must be high enough for the highest water level expected in the pond, which generally should be 1-2 feet higher than water level. The flow of water into each pond must be controlled by valves, stop-logs or plugs. Paddle wheels are the most efficient method of increasing dissolved oxygen levels in the pond water.

**STOCKING**

Stocking the ponds quickly reduces competitors and predators, which have less time to become established. Often post larvae that are a week or two old after metamorphosis are used to stock ponds, where they remain until harvesting.  A stocking density of about 40,000/ha is recommended for the monoculture of *Macrobrachiumrosenbergii*.  Using larger juveniles for stocking increases the survival rate as well as the average weight of the animals by as much as about 30%.

**FEED TYPE**

 Natural productivity of the ponds generally gives small production from the ponds. Therefore, intensive farming must involve supplementary feeding to increase productivity.  Some farms claim to rely on fertilizers, rather than feeding at the beginning of the rearing period, which stimulates algal bloom and lot of micro flora and fauna in the ponds. Others find that providing feed from the beginning of the rearing period improves performance and is cost-effective. Commercial feeds are the most productive and reliable to use but they are expensive and unaffordable to small farmers.

**HARVESTING MARKETABLE PRAWNS**

Basically there are two methods of harvesting: **culling** and **draining**. The time of harvesting depends partly on the growth rate and partly on the size of animals for market requirements. Culling is used to harvest market-sized animals from the ponds to remove faster growing prawns which increase density quickly. In tropics culling usually starts 5-7 months after post larvae have been stocked to take out the market-sized animals for selling and keeping the smaller ones and soft-shelled animals in the pond for further growth. After about 8-11 months ponds are drained and all animals are sold. In cull harvesting, a seine net is pulled through the pond to remove market-sized animals, while in drain harvesting, a harvesting sump is installed in front of the gate or outside the pond, in which prawns will accumulate while water is being drained.

**DISEASE CONTROL**

Several diseases affect freshwater prawn larvae as well as adults. Some hatcheries use formalin at the rate of 200 ppm daily as an effective remedy for protozoan and hydrozoan parasites and fungal diseases. Formalin can also be used at a lower level of about 30 ppm for longer periods, followed by water change after 24 hours.  Larvae can also be transferred to disinfecting tanks every 5-10 days to get rid of diseases and parasites. Daily dip of larvae in Malachite green (0.2 ppm) for 30 min has also been used for treatment. Also, dipping in copper sulphate 0.4 ppm solution for 6 hours is recommended. Antibiotics and sulfa drugs are sometimes used to control filamentous bacteria and some hatcheries use lime (CaO) as a prophylactic measure.

**VERMICULTURE**

Vermiculture means worm farming or culturing worms for selling them either to fishermen or to compost manufacturers. When earthworms are used for the production of compost it is called **vermicomposting.**Earthworms burrow through the soil and feed on decaying organic matter, excreting castings that are rich in nutrients and beneficial micro-organisms, which are about 20 times more in worm castings than in normal soil. These beneficial organisms not only make available nutrients to the plants but also suppress the growth of pathogens leading to healthy plants.

The most common worms used in vermiculture are, red worms *(Eisenia foetida, Eisenia andrei,* and *Lumbricus rubellus).* These worms thrive at temperatures between 20-30°C and can be cultured indoor in boxes. Other worms like *Perionyx excavatus* and *Eudrillius eugiene*are are suitable for warmer climates.

**Vermiculture Medium**

Crop residues, dry leaves, cattle dung are the basic materials for culturing earthworms, along with saw dust, coir waste, paddy husk, slurry from biogas plant, poultry waste and vegetable wastes. Earth worm culturing should be done under shelter to avoid direct sunlight and flooding by heavy rain.

**Containers for Vermiculture**

Brick lined pits, plastic tubs, wooden boxes, earthen pots or any other suitable containers can be used for culturing earthworms. The ideal size is 1 m x 1 m x 0.3 m but dimensions can be changed to suit the amount of waste material and convenience but the depth of pit should not be more than 45 cms. Sometimes a heap of organic matter over plain ground in shady area can also be used for culturing.

**METHOD OF CULTURING EARTHWORMS**

**1)** Select a container or dig a pit of suitable dimensions in shady areas.

**2)** At the bottom of the pit or container, make a **wormibed** of 10 cm height using coir waste, paddy husk, sugar cane trash, old papers etc. and spread a layer of soil over it. Wet the bed by sprinkling sufficient water over it to obtain a relative humidity of 40-45%.

**3)** Mix the organic waste, cattle dung and slurry from biogas plant or any other organic material and spread it over the bed. Keep this mixture for two weeks for **half digestion,** during which heating of substrate will take place and temperature will rise to 50-55°C. Add 5-10 % of neem cake in this material. Neem cake has beneficial effect on the growth of worms and kills harmful microorganisms.

**4)** Once the organic feeding material has cooled down to about 30°C, introduce worms by spreading them over the bed at the rate of 500 worms for every 100 kg of organic material.

**5)** Cover the bed with jute cloth, straw or similar material to provide shade and protection to the worms. Water has to be sprinkled over this cover to maintain the moisture content at 45-50% and temperature between 20-30°C. The pH of the raw material should not exceed 6.5-7.

The worms feed actively on organic matter and excrete mounds of castings near the surface. In about 60 days the compost will be ready.

**6)** To separate the worms from compost, take out the vermicompost and spread it in a heap in sunlight on a plastic sheet. In about two hours all the worms will move to the bottom of the heap. The compost can be removed from the top and used in fields, and the worms from the bottom can be carefully collected and used for further vermicomposting.

**VERMICOMPOSTING TECHNIQUE FOR FARMERS**

* The vermicomposting is done by digging pits 3.0 m long, 1.0 m wide and 1.0 m deep
* At the bottom of the pits, broken pieces of earthen pots or bricks are spread to provide adequate drainage.
* Over the layer of bricks, a bed of paddy husk or dry leaves is spread and then a layer of 2.5 cm thick soil is spread over it.
* Cattle dung and other organic wastes are then spread over the bed in about three inches thick layer.
* This organic material is allowed half digestion for about two weeks when temperature will increase to about 50°C.
* Worms can be introduced after this incubation period is over and when the temperature has come down to about 30°C. About 500 earthworms are then introduced into the pit, and a layer of paddy straw is placed over them. Water should be sprinkled and the pit is covered with coconut fibres or paddy straw or dry leaves to protect the worms from sunlight and predators.
* Fresh layers of organic waste can be added over this material every 3 or 4 days and covered with a layer of soil and paddy husk.
* The earthworms will move to the upper layer after finishing food material in the lower layers.
* The pit can be charged with all kinds of organic wastes in layers of about 5 cm, covered with a layer of soil till the material reaches the top of the pit.
* When the pit is full, it should be covered with husk and a layer of soil, and left for 30-60 days, during which compost will be fully formed.
* To procure the compost, top layer should be exposed to sunlight to force the earthworms to move to the deeper layers, so that compost could be removed from the top.
* The worms collected at the base can be used for inoculating new vermicomposting pits.
* The quality of vermicompost is far superior to other composts in terms of nutrients and other plant growth promoting substances.

Vermicompost production using worms such as *Eisenia foetida*, *Lumbricus rubellus*and *Eudrilus eugeniae*can be enhanced by using cattle urine for moistening organic wastes during the preliminary composting stage before the addition of worms. This simple technique can yield vermicompost of a higher Nitrogen content. Moreover, worms have been found to become more active and vermicompost can be harvested at least 10 days earlier if cattle urine is used.

**AIDS**

AIDS stands for Acquired Immunodeficiency Syndrome. It is the most advanced stage of infection with the Human Immunodeficiency Virus (HIV) which kills or damages cells of the body’s immune system. HIV most often spreads through unprotected sex with an infected person, by sharing drug needles or through contact with the blood of an infected person. Women can give it to their babies during pregnancy or childbirth.

The first signs of HIV infection may appear as swollen glands and flu-like symptoms which may come and go a month or two after infection. Severe symptoms may not appear until months or years later. The CD4 count indicates how far the HIV disease has advanced. CD4 counts in adults range from 500 to 1,500 cells per cubic millimeter of blood. In general, the CD4 count goes down as HIV disease progresses, to below 200, regardless of whether the persons are sick or not.

**MODE OF INFECTION**

Once HIV enters the human body, it attaches itself to a White Blood Cell (WBC) called CD4, also called T4 cell, which are the main disease fighters of the body. Whenever there is an infection, CD4 cells lead the infection-fighting army of the body to protect it from falling sick. Hence damage of these cells can affect a person’s disease-fighting capability and general health. After making a foothold on the CD4 cell, the virus injects its RNA into the cell. The RNA then produces its DNA by using enzyme reverse transcriptase. The viral DNA then gets attached to the DNA of the host cell and thus becomes part of the cell’s genetic material.

It is a virtual takeover of the cell. Using the cell’s division mechanism, the virus now replicates and churns out hundreds of thousands of its own copies. These cells then enter the blood stream, get attached to other CD4 cells and continue to replicate. As a result the number of virus in the blood rises and CD4 cell count declines.

There are several common ways that HIV can be passed from person to person that include:

* Having unprotected sex with someone who is infected
* Using needles or syringes that have been used by people who are infected
* Receiving infected blood products or transplanted organs.
* Transmission from mother to child – An infected mother may pass the virus to her developing fetus during pregnancy, birth or through breastfeeding.

**SYMPTOMS**

Many people do not develop any symptoms when they first become infected with HIV. Some people, however, get flu-like illness within three to six weeks after exposure to the virus. This illness, called Acute HIV Syndrome may include fever, headache, tiredness, nausea, diarrhea and enlarged lymph nodes. These symptoms usually disappear within a week to a month and are often mistaken for another viral infection. During this period, virus in the body abounds and spreads to different parts, particularly to lymphoid tissue. At this stage, the infected person is more likely to pass the infection to others.

More severe symptoms may not surface for several years, even a decade or more after the first entry of the virus or within two years in children born with the virus. Some people may begin to have symptoms as soon as a few months while others may be symptom-free for more than 10 years. During the “asymptomatic” period, the virus will be actively multiplying, infecting, and killing cells of the immune system. The following symptoms may appear in the infected person:

* Lack of energy.
* Weight loss.
* Frequent fevers and sweats.
* A thick, whitish coating on the tongue or mouth that is caused by a yeast infection and sometimes accompanied by a sore throat.
* Severe or recurring vaginal yeast infections.
* Chronic pelvic inflammatory disease or severe and frequent infections like *Herpes zoster.*
* Periods of extreme and unexplained fatigue that may be combined with headaches, lightheadedness or dizziness.
* Rapid loss of weight that is not due to increased physical exercise or dieting.
* Bruising more easily than normal.
* Long-lasting bouts of diarrhea.
* Swelling or hardening of glands located in the throat, armpit or groin.
* Periods of continued, deep and dry coughing.
* Increasing shortness of breath.
* The appearance of discolored or purplish growths on the skin or inside the mouth.
* Unexplained bleeding from skin mucous membranes or from any opening in the body.
* Recurring and unusual skin rashes.
* Severe numbness or pain in the hands or feet, loss of muscle control and reflex and paralysis or loss of muscular strength.
* An altered state of consciousness, personality change or mental deterioration.
* Children’s growth may be slow or they may fall sick frequently. HIV positive persons are also found to be more vulnerable to cancers.

**SYMPTOMS IN FEMALES**

Although most of the symptoms of HIV infection are similar in men and women, some are more typical of females. For example: Vaginal yeast infections may be chronic, more severe and difficult to treat in women with HIV infection than in healthy women.

Pelvic inflammatory disease, an infection of the female reproductive organs, may also be more frequent and severe in women with HIV infection. Human papillomavirus (HPV) infection, which causes genital warts may occur more frequently in HIV-infected women and can lead to pre-cancerous lesions of the cervix or cancer of the cervix.

**OPPORTUNISTIC INFECTIONS**

The number of CD4 cells per ml of blood which ranges from 500 to 1,500 in a healthy individual falls below 200 in AIDS infected people. The Viral Load will be very high at this stage. Opportunistic infections are caused by bacteria, virus, fungi and parasites. Some of the common opportunistic infections that affect HIV positive persons are: *Mycobacterium avium*, Tuberculosis, Salmonellosis, Bacillary Angiomatosis, Cytomegalovirus, Viral hepatitis, Herpes, Human papillomavirus, Progressive multifocal leukoencephalopathy; Candidiasis, Cryptococcal meningitis and Pneumocystis Carinii pneumonia, Toxoplasmosis, Cryptosporidiosis. HIV positive persons are also prone to cancers like Kaposi’s sarcoma and lymphoma.

**DIAGNOSIS**

In the early stages of infection, HIV often produces no symptoms and the infection can be diagnosed only by testing a person’s blood. Two tests are available to diagnose HIV infection — one that looks for the presence of antibodies produced by the body in response to HIV and the other that looks for the virus itself. If antibodies are present, the test gives a positive result.

A positive test has to be confirmed by another test called **Western Blot** or **Immunoflouroscent Assay**(IFA). All positive tests by **ELISA** need not be accurate and hence Western Blot and other tests are necessary to confirm a person’s HIV status. ELISA requires specialized equipment and blood samples need to be sent to a laboratory. To cut short this waiting period, **Rapid Tests** that give results in 5 to 30 minutes are increasingly being used the world over.

The HIV antibodies generally do not reach detectable levels in the blood till about three months after infection. This period, from the time of infection till the blood is tested positive for antibodies is called the **Window Period.** Sometimes, the antibodies might take even six months to show up. Even if the tests are negative during the Window Period, the amount of virus may be very high in an infected person.

**PREVENTION**

Because there is no effective vaccine and no cure for HIV, the only way to protect one is by taking preventive measures.

People should either abstain from having sex or use latex condoms during sex. People who are allergic to latex can use polyurethane condoms.

Although some laboratory evidence shows that spermicidal creams can kill HIV, there is no conclusive evidence if it can prevent transmission.

The risk of HIV transmission from a pregnant woman to her baby is significantly reduced if she takes **AZT**during pregnancy, labour and delivery and her baby takes it for the first six weeks of life. **Nevirapine** is also found to be useful.

Having a sexually transmitted disease (STD) can increase a person’s chances of getting HIV through sexual contact. Hence it is necessary to treat STD as soon as possible.

All donated blood must be screened for HIV as well as for Hepatitis B and Syphilis.

**TREATMENT**

Three classes of drugs are available for treatment of AIDS.

**1. Nucleoside analogueReverse Transcriptase Inhibitors (NRTIs)**. These were first antiretroviral drugs that were developed for inhibiting the replication of HIV in the early stage by inhibiting an enzyme called Reverse Transcriptase. The drugs include **Zidovudine** (Retrovir, AZT), **Lamivudine** (Epivir, 3TC), **Didanosine** (Videx, ddI), **Zalcitabine** (Hivid, ddC), **Stavudine** (Zerit, d4T) and **Abacavir** (Ziagen).

The major reported side effect of **Zidovudine** is bone marrow suppression, which causes a decrease in the number of red and white blood cells. The drugs **ddI, ddC** and **d4T** can damage peripheral nerves, leading to tingling and burning sensation in hands and feet. Treatment with **ddI** can also cause pancreatitis, and **ddC**may cause mouth ulcers. Approximately 5 percent of people treated with **Abacavir** experience hypersensitivity with rash along with fever, fatigue, nausea, vomiting, diarrhea and abdominal pain. Symptoms usually appear within the first 6 weeks of treatment and generally disappear when the drug is discontinued.

**2. Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs).** These drugs bind directly to the enzyme, Reverse Transcriptase. There are three NNRTIs currently approved for clinical use: **Nevirapine** (Viramune), **Delavirdine** (Rescriptor) and **Efavirenz** (Sustiva). A major side effect of all NNRTIS is appearance of rash. In addition, people taking Efavirenz may also have side effects such as abnormal dreams, sleeplessness, dizziness and difficulty concentrating.

**3. Protease Inhibitors (PIs).** They interrupt HIV replication at a later stage in its life cycle by interfering with an enzyme known as HIV protease. This causes HIV particles in the body to become structurally disorganized and noninfectious. Among these drugs are **Saquinavir** (Fortovase), **Ritonavir** (Norvir), **Indinavir** (Crixivan), **Nelfinavir** (Viracept), **Amprenavir** (Agenerase) and **Lopinavir** (Kaletra).

The triple cocktail treatment, also known as **Highly Active Antiretroviral Therapy(HAART),** is the closest thing that medical science has to an effective therapy, which has the ability to disrupt HIV at different stages of replication. Reverse transcriptase inhibitors, which usually make up two drugs in the HAART regimen, restrain an enzyme crucial in the early stage of HIV replication. Protease inhibitors hold back another enzyme that functions near the end of the HIV replication process.

**As of now, there is no vaccine to prevent HIV infection.**

**MALARIA**

Malaria is one of the most common infectious diseases and an enormous public health problem. The disease is caused by a protozoan parasites of the genus *Plasmodium,* which is usually referred to as malaria parasites.

The term malaria originated from the medieval Italian term, *mala aria* meaning “bad air” and the disease was formerly called marsh fever due to its association with swamps.

In 1880, a French army doctor working at the military hospital in Algeria named **Charles Louis Alphonse Laveran** observed malarial parasites for the first time inside the red blood cells of people suffering from malaria. For this and later discoveries, he was awarded the 1907 Nobel Prize for Physiology or Medicine. The protozoan was named *Plasmodium* by the Italian scientists **Ettore Marchiafava** and **Angelo Celli**.

A year later, **CarlosFinlay**, a Cuban doctor treating patients with yellow fever in Havana, first suggested that mosquitoes were transmitting disease to humans. However, it was **SirRonaldRoss** working in India who finally proved in **1898** that malaria was transmitted by mosquitoes to birds. He isolated malarial parasites from the salivary glands of mosquitoes that had fed on infected birds. For this work Ross received the **1902**Nobel Prize in Medicine. The findings of Finlay and Ross were confirmed by a medical board headed by Walter Reed in 1900.

 **MALARIA PARASITES**

Malaria is caused by protozoan parasites of the genus *Plasmodium* (Phylum Apicomplexa). In humans malaria is caused by ***P. falciparum, P. malariae, P. ovale,*** and ***P. vivax,*** the last one is the most common one responsible for about 80 % of all malaria cases. However, *P. falciparum* is the most deadly one, responsible for about 15% of infections but 90% of deaths. Parasitic *Plasmodium* species also infect birds, reptiles, monkeys, chimpanzees and rodents. There have been documented human infections with several **simian species** of malaria, namely ***P. knowlesi, P. inui, P. cynomolgi*, *P.simiovale, P. brazilianum, P. schwetzi* and *P. simium.***

 **LIFE CYCLE**

The parasite’s primary (definitive) hosts and vectors are female mosquitoes of the *Anopheles* genus. A mosquito becomes infected when it takes a blood meal from an infected human. Once ingested, the parasite’s **gametocytes,** taken up along with the blood differentiate into male or female **gametes**, which fuse to form **zygote**in the mosquito gut. The zygote is also called **ookinete** that penetrates the gut lining and produces an **oocyst** outside the stomach wall.

The diploid zygote first undergoes reduction division and then divides by multiple fission to produce haploid sporozoites inside the oocyst. When the oocyst ruptures, **sporozoites**are released that migrate through the mosquito’s body to reach salivary glands, where they are ready to infect a new human host when the mosquito bites a healthy man. This type of transmission is occasionally referred to as anterior station transfer. Only female mosquitoes feed on blood, thus males do not transmit the disease..

Malaria in humans develops via two phases: an **exoerythrocytic** (hepatic) and an **erythrocytic** phase. When an infected mosquito pierces a person’s skin to take a blood meal, sporozoites in the mosquito’s saliva enter the bloodstream and migrate to the liver. Within 30 minutes of being introduced into the human host, they infect hepatocytes, multiplying asexually to form **schizont** for a period of 6–15 days.

Once in the liver they produce thousands of **cryptozoites** and secondary **metacryptozoites,**which, following rupture of their host cells escape into the blood and infect red blood cells, thus beginning the erythrocytic stage of the life cycle. The parasites escape from the liver undetected by wrapping themselves in the cell membrane of the host liver cell. Within red blood cells the parasites multiply further asexually producing **schizont** that burst to release about two dozens of **merozoites** that invade fresh red blood cells. Such cycles continue to occur every 48 hours causing chill and fever at the release of merozoites from RBCs.

Some *P. vivax* and *P. ovale* sporozoites do not immediately develop into exoerythrocytic merozoites but instead produce **hypnozoites** that remain dormant for periods ranging 6–12 months to as long as three years. After a period of dormancy, they reactivate and produce merozoites. **Hypnozoites** are responsible for long incubation and late relapses in these two species of malaria.

The parasite is protected from attack by the body’s immune system because for most of its life it resides within the liver and blood cells and is hidden from immune surveillance. However, circulating infected blood cells are destroyed in the spleen. To avoid this, *P. falciparum* produces adhesive proteins on the surface of the infected blood cells, causing the blood cells to stick to the walls of smaller blood vessels, thereby sequestering the parasite from the passage through the general circulation and spleen.

This stickiness of RBCs is the main factor that gives rise to hemorrhagic complications associated with *falciparum* malaria. The smallest branches of the circulatory system can be blocked by the attachment of masses of these infected red blood cells. In cerebral malaria the sequestrated red blood cells can breach the blood brain barrier, leading to coma.

Although the red blood cell surface adhesive proteins (called **PfEMP1** for *Plasmodiumfalciparum* erythrocyte membrane protein 1) are exposed to the immune system, they do not serve as good immune targets because of their extreme diversity. There are at least 60 types of these proteins within a single parasite and perhaps limitless types in general parasite populations. Also, the parasite switches between a broad repertoire of **PfEMP1** surface proteins thus staying one step ahead of the pursuing immune system.

**TREATMENT**

The first effective treatment for malaria was the bark of **cinchona tree**, which contains **quinine**. This tree grows on the slopes of the Andes, mainly in Peru.

**Treatment with Chloroquine**

|  |  |
| --- | --- |
| Day 1 | 4 tablets (600mg base) or 10 mg/kg first dose.2 tablets (300mg base) or 5 mg/kg 6-8 hours later. |
| Day 2 | 2 tablets (300mg base) or 5 mg/kg. |
| Day 3 | 2 tablets (300mg base) or 5 mg/kg |
| Next 14 days | **Primaquine,** 2 tablets (each tablet contains 7.5 mg base daily with food). |

Most drugs used in treatment of malaria are active against the parasite stages in blood and include the following:

**Chloroquine**

**Sulfadoxine-pyrimethamine combination**

**Mefloquine**

**Atovaquone-proguanil combination**

**Quinine**

**Doxycycline**

**Artemisinin derivatives**

In addition, **primaquine** is active against the dormant parasite in liver called **hypnozoites** and hence prevents relapses. **Primaquine** should not be taken by pregnant women or by people who are deficient in G6PD (glucose-6-phosphate dehydrogenase).

**Mefloquine** is an antimalarial agent that acts as a blood schizonticide. It is effective against all species of malaria *(P. falciparum, P. vivax, P. malariae and P. ovale).* Its exact mechanism of action is not known. Mefloquine is active against the erythrocytic stages of *Plasmodium* species. However, the drug has no effect against the exoerythrocytic (hepatic) stages of the parasite and mature gametocytes. **Mefloquine** is effective against malaria parasites resistant to chloroquine and other 4-aminoquinoline derivatives, proguanil, pyrimethamine and pyrimethamine-sulphonamide combinations.

**Malarone** (**Atovaquone** 250 mg plus **Proguanil** 100 mg), 4 tablets daily for three consecutive days. This combination therapy is relatively new and appears to be very effective but it is also very expensive.

For over 1,500 years Chinese have used leaves from *Artemisia annua*shrub (sweet wormwood) to treat malaria. However, it is only in the late 1960s that its anti-malarial ingredient, **artemisinin** was identified and extracted. Today, **artemisinin** is considered the treatment of choice for uncomplicated *falciparum* malaria, as prescribed by the World Health Organization in 2001.

**BENEFICIAL EFFECTS OF MALARIA**

 **Sickle-cell disease**

Distribution of malaria.The best-studied influence of the malaria parasite upon the human genome is the blood disease, sickle-cell disease. In sickle-cell disease, there is a mutation in the HBB gene, which encodes the beta globin subunit of haemoglobin. The normal allele encodes a glutamate at position six of the beta globin protein, while the sickle-cell allele encodes a valine. This change from a hydrophilic to a hydrophobic amino acid encourages binding between haemoglobin molecules, with polymerization of haemoglobin deforming red blood cells into a “sickle” shape. Such deformed cells are cleared rapidly from the blood, mainly in the spleen, for destruction and recycling.

In the merozoite stage of its life cycle the malaria parasite lives inside red blood cells, and its metabolism changes the internal chemistry of the red blood cell. Infected cells normally survive until the parasite reproduces, but if the red cell contains a mixture of sickle and normal haemoglobin, it is likely to become deformed and be destroyed before the daughter parasites emerge. Thus, individuals heterozygous for the mutated allele, known as sickle-cell trait, may have a low and usually unimportant level of anaemia, but also have a greatly reduced chance of serious malaria infection. This is a classic example of heterozygote advantage.

Individuals homozygous for the mutation have full sickle-cell disease and in traditional societies rarely live beyond adolescence. However, in populations where malaria is endemic, the frequency of sickle-cell genes is around 10%. The existence of four haplotypes of sickle-type hemoglobin suggests that this mutation has emerged independently at least four times in malaria-endemic areas, further demonstrating its evolutionary advantage in such affected regions. There are also other mutations of the HBB gene that produce haemoglobin molecules capable of conferring similar resistance to malaria infection. These mutations produce haemoglobin types HbE and HbC which are common in Southeast Asia and Western Africa, respectively.

**Thalassaemias**

Another set of mutations found in the human genome associated with malaria are those causing blood disorders known as thalassaemias. Studies in Sardinia and Papua New Guinea have revealed that the gene frequency of **?-thalassaemias** is related to the level of malarial endemicity in a populations. A study on more than 500 children in Liberia revealed that those suffering with ?-thalassaemia had a 50% decreased chance of getting clinical malaria. Similar studies have found links between gene frequency and malaria endemicity in the **?+** form of **?-thalassaemia.** Presumably these genes have also been selected in the course of human evolution with malaria epidemic.

**Duffy antigens**

The **Duffy antigens** are antigens expressed on red blood cells and other cells in the body acting as a chemokine receptors. The expression of Duffy antigens on blood cells is encoded by **Fy genes** (Fya, Fyb, Fyc etc.). *Plasmodium vivax* malaria uses the Duffy antigen to enter blood cells. However, it is possible to express no Duffy antigen on red blood cells owing to the absence of Fy genes (Fy-/Fy-). This genotype confers complete resistance to *P. vivax* infection. The genotype is very rare in European, Asian and American populations, but is found in almost all indigenous population of West and Central Africa. This is thought to be due to high exposure of populations to *P. vivax* in Africa in the last few thousand years.

**G6PD**

Glucose-6-phosphate dehydrogenase **(G6PD)** is an enzyme which normally protects from the effects of oxidative stress in red blood cells. However, a genetic deficiency in this enzyme results in increased protection against severe malaria.

**HLA and interleukin-4**

**HLA-B53** is associated with low risk of severe malaria. This MHC class I molecule presents liver stage and sporozoite antigens to **T-Cells**. **Interleukin-4** is produced by activated **T-cells** and promotes proliferation and differentiation of antibody-producing **B-cells**. A study of the Fulani of Burkina Faso found that the **IL4-524T** allele was associated with elevated antibody levels against malaria antigens, which raises the possibility that this might be a factor in increased resistance to malaria.

**FILARIA**

Filariasis is an infectious tropical disease caused by three thread-like parasitic filarial worms, *Wuchereria bancrofti, Brugia malayi,*and*Brugia timori*, all transmitted by mosquitoes. Lymphatic Filariasis, known as **Elephantiasis** puts at risk more than a billion people in more than 80 countries. Over 120 million are already affected by and over 40 million of them are seriously incapacitated and disfigured by the disease. One-third of the infected people live in India, one third in Africa and the rest are in South Asia, the Pacific and the Americas.

**PATHOGENS**

Pathogenic filarial parasites affect the lives of millions of people, especially those living in tropical countries. The filarial parasites that pose the most serious public health threats are *Wuchereria bancrofti, Brugia malayi, Brugia timori, Onchocerca volvulus*, and *Loa loa.* All of these cause cutaneous manifestations. One filarial nematode, *Mansonella streptocerca*, also causes cutaneous changes but is not a significant public health threat.

**Human Filarial Parasites and Their Vectors**

|  |  |  |
| --- | --- | --- |
| **Disease** | **Parasite** | **Vector** |
| Onchocerciasis | *O. volvulus* | Blackflies: *Simulium* species |
| Bancroftian filariasis | *W. bancrofti* | Mosquitoes: *Anopheles, Aedes, Culex,* and *Mansonia*species |
| Malayan filariasis | *B. malayi* and *B. timori* | Mosquitoes: *Anopheles, Aedes, Culex,* and *Mansonia*species |
| Loiasis | *Loa loa* | Red flies: *Chrysops* species |
| Mansonelliasis | *M. streptocerca* | Midges: *Culicoides* species |
| Dirofilariasis | *Dirofilaria* species | Mosquitoes: *Culex* species |

**LIFE CYCLE**

The thread-like, parasitic filarial worms *Wuchereria bancrofti* and *Brugia malayi* that cause lymphatic filariasis live almost exclusively in humans. These worms are lodged in the lymphatic system, the network of nodes and vessels that maintain the delicate fluid balance between the tissues and blood and are an essential component for the body’s immune system. They live for 4-6 years, producing millions of immature microfilariae (minute larvae) that circulate in the blood.

The disease is transmitted by mosquitoes that bite infected humans and pick up the microfilariae that develop, inside the mosquito, into the infective stage in a process that usually takes 7-21 days. The larvae then migrate to the mosquitoes’ mouth-parts, ready to enter the punctured skin following the mosquito bite and completing the cycle.

**SYMPTOMS**

Patients suffer from hydrocoel (fluid-filled balloon-like enlargement of scrotal sacs) and elephantiasis of the legs and penis. Elephantiasis of the entire leg, the entire arm, the vulva or the breast (swelling up to several times normal size) can take place.

Elephantiasis affects mainly the lower extremities and is caused when the parasites are lodged in the lymphatic systemand block lymph flow. *W. bancrofti* can affect the legs, arms, vulva, breasts, while *Brugia timori* rarely affects the genitals. Infection by *Onchocerca volvulus* and the migration of its microfilariae through the cornea of eye is a major cause of blindness.

**DIAGNOSIS**

Until very recently, diagnosing lymphatic filariasis had been extremely difficult, since parasites had to be detected microscopically in the blood, and in most parts of the world, the parasites have a “nocturnal periodicity” that restricts their appearance in the blood to only the hours around midnight. The diagnosis is made by identifying microfilariae on a stained blood film. Blood must be drawn at night, since the microfilariae circulate at night when their vector, the mosquito, is most likely to bite.

The new development of a very sensitive, very specific simple “card test” to detect circulating parasite antigens without the need for laboratory facilities and using only finger-prick blood droplets taken anytime of the day has completely transformed the approach to diagnosis.

**TREATMENT**

Vector control, use of mosquito nets, and improved living conditions are still vital for the control of these infections.

The drugs of choice for killing adult worms are **Albendazole** and **Ivermectin**.

**Ivermectin** (dihydroavermectin) is the drug of choice for the treatment of onchocerciasis. It is a macrocyclic lactone derived from the actinomycete, *Streptomyces avermitilis* found in soil. It functions as a single dose and is effective microfilaricide for *O.volvulus*.

Unlike **diethylcarbamazine** (DEC), ivermectin does not produce reaction in onchocerciasis because it acts by paralyzing the microfilariae in the skin tissue spaces and lymphatics. They are then swept away into the local lymph nodes, which may swell up and only cause some local limb edema. On the other hand, DEC “unmasks” the microfilariae in the tissue spaces where they are attacked by the various protective cells which cause reaction in the skin.

The addition of oral **doxycycline** (100 mg/d) given for 6 weeks from the start of ivermectin to kill off *Wolbachia* organisms enhanced the effects of ivermectin.

**Diethylcarbamazine**or **DEC** (Hetrazan) is a microfilaricide with no effect on the adult worm. It produces Mazzotti reactions that become severe in heavily infected persons. A low dose of dexamethasone (3 mg/d) after onset of Mazzotti reaction controls the progression of reaction without interfering with the macrofilaricidal efficacy of DEC.

**Suramin**is a microfilaricide given intravenously, starting with a test dose of 100 mg of fresh 10% solution over 2 minutes. If no hypersensitivity develops, weekly dosages of 0.2 g, 0.4 g, 0.6 g, 0.8 g, and 1 g are given to adult patients. Rarely, patients experience eye lesions, dermatitis, kidney damage, a Mazzotti-like reaction and/or death. Thus, the use of suramin requires great caution and hence is generally not recommended.

**Amocarzine** is a new oral macrofilaricidal compound that has promising effects on onchocerciasis in Latin America.

**Doramectin** (Dectomax, Pfizer) is a new drug related to ivermectin. Its efficacy and safety in onchocerciasis are untested.

**NODULECTOMY**

A useful adjunct to chemotherapy popular in South America is removal of the palpable nodules. In Africa, nodulectomy has never been practiced widely because the nodules tend to be deeper and located near delicate joint spaces. Alternatively, **chloroquine** can be injected into young nodules that kill the worms.

*Wolbachia* organisms appear to play a critical role in the biology and metabolism of filarial worms. The use of tetracycline to kill *Wolbachia* appears to be lethal to the adult *O. ochengi* and recent evidences suggest that it is also effective against *O.volvulus* and perhaps other filarial worms.

**CHOLERA**

Cholera is an acute intestinal infection caused by ingestion of food or water contaminated with the bacterium *Vibrio cholerae*. It has a short incubation period of one to five days and produces a toxin that causes painless, watery diarrhoea and vomiting that can quickly lead to severe dehydration and death.

The genus **Vibrio** consists of Gram-negative straight or curved rod-like bacteria, with a single polar flagellum. Vibrios are capable of both respiratory and fermentative metabolism. Most species are oxidase-positive. *V. cholerae* and *V. parahaemolyticus* are pathogens of humans. *V.parahaemolyticus* is an invasive organism affecting primarily the colon, while *V.* Another species, *Vibrio vulnificus* is another emerging pathogen of humans that causes *cholerae* is noninvasive affecting the small intestine by producing an enterotoxin. wound infections, gastroenteritis or a syndrome known as “primary septicaemia.”

**HISTORY**

During the 19th century cholera spread repeatedly from the Ganges delta in India to the rest of the world before receding to South Asia. Six epidemics were recorded that killed millions of people across Europe, Africa and the Americas. Cholera is mainly transmitted through contaminated water and food and is closely related to unhygienic conditions of surrounding environment.

The absence or shortage of safe drinking water and insufficient sanitation, combined with an unhygienic environmental status are the main causes of spread of the disease. Cholera still remains a global threat to public health and one of the key indicators of social development. While the disease is no longer an issue in countries where minimum hygiene standards are met, it remains a threat in almost every developing country where populations are large.

The number of cholera cases reported to WHO during 2006 rose dramatically, reaching the level of the late 1990s. A total of 236 896 cases were notified from 52 countries, including 6311 deaths, an overall increase of 79% compared with the number of cases reported in 2005.

**CHOLERA TOXIN**

Cholera toxin activates the adenylate cyclase enzyme in cells of the intestinal mucosa leading to increased levels of intracellular cAMP, and the secretion of H20, Na+, K+, Cl–, and HCO3– into the lumen of the small intestine. The effect is dependent on a specific receptor, monosialosyl ganglioside (GM1 ganglioside) present on the surface of intestinal mucosal cells.

The bacterium produces invasin, neuraminidase, during the colonization stage which has the interesting property of degrading gangliosides to the monosialosyl form, which is the specific receptor for the toxin. Once it has entered the cell, the A1 subunit enzymatically transfers ADP ribose from NAD to a protein (called Gs or Ns), that regulates the adenylate cyclase system which is located on the inside of the plasma membrane of mammalian cells. Enzymatically, fragment A1 catalyzes the transfer of the ADP-ribosyl moiety of NAD to a component of the adenylate cyclase system. Adenylate cyclase (AC) is activated normally by a regulatory protein (GS) and GTP.

**TRANSMISSION**

The highly liquid diarrhea during cholera infection is loaded with bacteria that can spread under unsanitary conditions to infect water used by other people. Cholera is transmitted from person to person through ingestion of faeces-contaminated water.

The sources of contamination are typically other cholera patients whose diarrhoeal discharge is allowed to get into waterways or into groundwater or drinking water supply. Any infected water or food washed in such water and fish and shellfish living in the affected waterways can cause infection. Cholera is rarely spread directly from person to person. *V. cholerae* occurs naturally in the planktons of fresh, brackish, and salt water, attached primarily to copepods. Both toxic and non-toxic strains exist. Coastal cholera outbreaks typically follow zooplankton blooms.

**COLONIZATION OF INTESTINE**

There are several characteristics of pathogenic *V. cholerae* that help it in the colonization process, namely, **adhesins**, **neuraminidase**, intestinal motility, chemotaxis and toxin production. *V. cholerae* is resistant to bile salts and can penetrate the mucus layer of small intestine, possibly aided by secretion of neuraminidase and proteases (mucinases). They also withstand the propulsive gut motility by their own swimming ability and chemotaxis directed against the gut mucosa.

Two other possible adhesins in *V. cholerae* are a surface protein that agglutinates red blood cells **(hemagglutinin)** and a group of outer membrane proteins which are products of the **acf** (accessory colonization factor) genes. **acf** mutants have been shown to have reduced ability to colonize the intestinal tract. It has been suggested that *V. cholerae* might use these nonfimbrial adhesins to mediate a tighter binding to host cells than is attainable with fimbriae alone

**VACCINES**

The oral vaccines are made from a live attenuated strain of *V. cholerae*. The ideal properties of such a vaccine of the bacterium would be to possess all the pathogenicity factors required for colonization of the small intestine but not to produce toxin molecules. Ideally it should produce only the **B** subunit of the toxin which would stimulate formation of antibodies that could neutralize the binding of the native toxin molecule to epithelial cells.

A new vaccine has been developed to combat the Bengal strain of *Vibrio cholerae* that has started spreading in epidemic fashion in the Indian subcontinent and Southeast Asia. The Bengal strain differs from previously isolated epidemic strains in that it is sero group is 0139 rather than 01, and it expresses a distinct polysaccharide capsule. Since previous exposure to 01 *Vibrio cholerae* does not provide immunity against 0139, populations suffer from the Bengal form of cholera.

The noncellular vaccine is relatively nontoxic and contains little or no LPS and other impurities. The vaccine will be used for active immunization against *Vibriocholerae* O139 and other bacterial species expressing similar surface polysaccharides. In addition, human or other antibodies induced by this vaccine could be used to identify *Vibriocholerae* Bengal for the diagnosis of the infection and for environmental monitoring of the bacterium.

**TREATMENT**

Cholera can be simply and successfully treated by immediate replacement of body fluids and salts lost through diarrhoea and vomiting. Patients can be treated with **Oral Rehydration Solution,** a mixture of sugar and salts to be mixed with water and taken in large amounts but patients who become severely dehydrated must be given intravenous fluids. With prompt rehydration, less than 1% of cholera patients die.

In severe cases, an effective antibiotic can reduce the volume and duration of diarrhoea and the period of *Vibrio* excretion. **Tetracycline** is the usual antibiotic of choice, but resistance to it is increasing. Other antibiotics that are effective include, **cotrimoxazole, erythromycin, doxycycline, chloramphenicol and furazolidone.**

**Antibiotics used to treat cholera**

**Doxycycline,**a single dose of 300 mg tablet

**Tetracycline,**12.5 mg/kg or 500 mg tablet, 4 times per day

for 3 days.

**Trimethoprim/sulfamethoxazole (TMP/SMX),**TMP 5 mg/kg and

SMX 25 mg/kgc TMP 160 mg and SMX 800 mg twice a day for 3 days.

**Furazolidone,**1.25 mg/kg or 100 mg tablet, 4 times per day for 3 days.

**Erythromycin** or **chloramphenicol** may be used when the antibiotics recommended above are not available, or where *Vibrio cholerae* O1 is resistant to them.

**Doxycycline** is the antibiotic of choice for adults but not for pregnant women.

**TMP-SMX** is the antibiotic of choice for children.

**Tetracycline** is equally effective in all age groups.

**Furazolidone** is the antibiotic of choice for pregnant women.

**TUBERCULOSIS**

Tuberculosis (TB) is an infectious disease caused by the bacterium, *Mycobacteriumtuberculosi*. TB most commonly affects the lungs but can involve almost any organ of the body. There is also a group of organisms referred to as atypical tuberculosis. These involve other types of bacteria of *Mycobacterium*family. At times, these bacteria can cause an infection that sometimes appears as typical tuberculosis. These “atypical” mycobacteria are: *M. kansasii* that may produce similar clinical and pathologic symptoms of disease. *M. avium-intracellulare*(MAI) seen in persons with AIDS and is not primarily a pulmonary pathogen but occurs mostly in organs of the mononuclear phagocyte system.

Tuberculosis outside the lungs can appear in the following kinds:

**SkeletalTuberculosis**: Tuberculous osteomyelitis, known as **Pott’s disease,** involves mainly the thoracic and lumbar vertebrae followed by knee and hip. There is extensive necrosis and bony destruction with compressed fractures with kyphosis and extension to soft tissues.

**GenitalTractTuberculosis**: *Tuberculous salpingitis* and *endometritis* result from infection of the fallopian tube that leads to *granulomatous salpingitis*, which can drain into the endometrial cavity and cause a *granulomatous endometritis* with irregular menstrual bleeding and infertility. In the male, tuberculosis involves prostate and epididymis leading to infertility.

**Urinary Tract Tuberculosis**: WBC’s appear in urine but a negative routine bacterial culture may suggest the diagnosis of renal tuberculosis. Progressive destruction of renal parenchyma occurs if not treated. Drainage to the ureters can lead to inflammation and ureteral stricture.

**CNS Tuberculosis**: A meningeal spread can occur and the cerebrospinal fluid typically shows a high protein, low glucose, and lymphocytosis. The base of the brain is often involved, so that various cranial nerves may be affected. Rarely, a solitary granuloma, or “tuberculoma”, may form and manifest with seizures.

**Gastrointestinal Tuberculosis**: This is uncommon today because routine pasteurization of milk has eliminated *Mycobacterium bovis* infections. However, *M. tuberculosi* coughed up in sputum may be swallowed through contamination. The classic lesions are circumferential ulcerations with stricture of the small intestine, with ileo-caecal involvement.

**Adrenal Tuberculosis**: Spread of tuberculosis to adrenals is usually bilateral, so that both adrenals are markedly enlarged. Destruction of cortex leads to Addison’s disease.

**Scrofula**: *Tuberculous lymphadenitis* of the cervical nodes is caused by *Mycobacteriumscrofulaceum*and may produce a mass of firm, matted nodes just under the mandible.

There can be chronic draining fistulous tracts to overlying skin. This complication may appear in children.

**Cardiac Tuberculosis**: The pericardium is the usual site for tubercular infection of heart. The result is a *granulomatous pericarditis* that can be hemorrhagic. There can be fibrosis with calcification, leading to a constrictive pericarditis.

**HISTORY**

**Robert Koch** isolated the tubercular bacillus in 1882 and established TB as an infectious disease. In the 19th century, due to the absence of antibiotics, patients were isolated in sanatoria and given treatment. Attempts were made to remove the infectious tissue by surgery called thoracoplasty. Till the first half of 20th century, no effective treatment was available. **Streptomycin**, the first antibiotic to fight TB, was introduced in 1946, and **isoniazid** (Laniazid, Nydrazid) became available in 1952.

*M*. *tuberculosis* is a rod-shaped, slow-growing bacterium. Its cell wall has high acidic content, which makes it hydrophobic, resistant to oral fluids. The cell wall absorbs a certain dye and maintains a red color, hence the name acid-fast bacilli.

**MODE OF INFECTION**

A person can become infected with tuberculosis bacteria through inhalation of droplets containing bacillus from the air. The bacteria get into the air when someone with tuberculosis lung infection coughs, sneezes or spits. TB is not transmitted by just touching the clothes or shaking the hands of someone who is infected. Tuberculosis is spread primarily from person to person by breathing infected air especially in closed rooms. TB caused by *Mycobacterium bovis,* however, is transmitted by drinking unpasteurized milk. Earlier this bacterium was a major cause of TB in children, but rarely causes TB now since most milk is pasteurized.

**PATHOLOGY**

When the inhaled tuberculosis bacteria enter the lungs, they can multiply and cause pneumonia. The local lymph nodes associated with the lungs may also become involved with the infection and usually become enlarged. The infection can also spread to other parts of the body. The body’s immune system in healthy people can fight the infection and stop the bacteria from spreading.

If the body is able to form scar tissue (fibrosis) around the TB bacteria, then the infection is contained in an inactive state. Such an individual typically has no symptoms and cannot spread TB to other people. The scar tissue and lymph nodes may eventually harden due to the process of calcification of the scars. However, if the body’s immune system is weakened, the TB bacteria can break through the scar tissue.

The breakthrough of bacteria can result in recurrence of the pneumonia and a spread of TB to other parts of the body. It may take many months from the time the infection initially gets into the lungs until symptoms develop. The usual symptoms that occur with an active TB infection are a generalized tiredness or weakness, weight loss, fever, and night sweats. If the infection in the lung worsens, then further symptoms can include coughing, chest pain, coughing up of sputum or blood, and shortness of breath. If the infection spreads beyond the lungs, the symptoms will depend upon the organs involved.

**DIAGNOSIS**

TB can be diagnosed in several different ways, including chest X-rays, analysis of sputum, and skin tests. The chest x-rays can reveal evidence of active tuberculosis pneumonia or scarring (fibrosis) or hardening (calcification) in the lungs. Examination of the sputum on a slide (smear) under the microscope can show the presence of the tuberculosis bacteria. A sample of the sputum can also be cultured in special incubators so that the tuberculosis bacteria can subsequently be identified.

Several types of skin tests are used to screen for TB, e.g. tuberculin skin tests that include the **Mantouxtest**, the **Tine test,**and the **PPD** (Purified Protein Derivative) test. In each of these tests, a small amount of purified extract from dead tuberculosis bacteria is injected under the skin. If a person is not infected with TB, then no reaction will occur at the site of the injection. If a person is infected with tuberculosis, however, a raised and reddened area will appear around the site of the test injection within 48 to 72 hours after the injection.

If the infection with tuberculosis has occurred recently, the skin test may be negative, because usually it takes 2 to 10 weeks after infection for the skin to test positive. The skin test can also be falsely negative if a person’s immune system is weakened due to another illness such as AIDS or cancer or he is on medication that can suppress the immune response such as cortisone or anti-cancer drugs.

**TREATMENT**

Treatment with antibiotics is recommended to treat as well as to prevent the TB from turning into an active infection in those where it is dormant. The antibiotic used for this purpose is called **isoniazid** (INH). If taken for 6 to 12 months, it will prevent the TB from becoming active in the future. In fact, if a person with a positive skin test does not take INH, there is a 5 to 10% lifelong risk that the TB will become active.

Taking **isoniazid** is not advisable (contraindicated) during pregnancy or for those suffering from alcoholism or liver disease. Also, isoniazid can have side effects such as rashes, tiredness or irritableness. Liver damage from isoniazid is rare and typically reverses once the drug is stopped. Very rarely, however, in older people, the liver damage (INH hepatitis) can even be fatal. It is important therefore, for the doctor to monitor a patient’s liver by periodically carrying out liver function tests during the course of INH therapy.

Active TB is treated with a combination of medications with **isoniazid**, **Rifampicin** (**Rifadin**), **ethambutol**(Myambutol) and **pyrazinamide**. Drugs are often taken for the first two months of therapy to help kill any potentially resistant strains of bacteria. Then the number is usually reduced to two drugs for the remainder of the treatment based on drug sensitivity testing. **Streptomycin**, a drug that is given by injection, may be used as well, particularly when the disease is extensive. Treatment usually lasts for many months and sometimes for years. Successful treatment of TB is dependent largely on the compliance of the patient.

Drug-resistant TB has become a very serious problem in recent years in certain populations. For example, INH-resistant TB is seen among patients in Southeast Asia.  Even more serious problem is the multi-drug resistant TB that has been seen in prison populations. Poor compliance by the inmates is thought to be the main reason for the development of multi-drug resistance.

Surgery on the lungs may be indicated to help cure TB when medication has failed, but in most cases is not required. Treatment with appropriate antibiotics will usually cure the disease. Without treatment, however, tuberculosis can be lethal and hence early diagnosis is important.

**SUMMARY**

Tuberculosis (TB) is an infection primarily of lungs (a pneumonia), caused by bacteria called *Mycobacteriumtuberculosis*. It is spread usually from person to person by breathing infected air during close contacts.

TB can remain in an inactive (dormant) state for years without causing symptoms or spreading to other people.

When the immune system of a patient with dormant TB is weakened, the TB can become active and cause infections in the lungs or other parts of the body.

The risk factors for acquiring TB include close-contact situations, alcohol and IV drug abuse, and certain diseases (e.g., diabetes, cancer, and HIV) and occupations (e.g., health care workers).

The most common symptoms of TB are fatigue, fever, weight loss, coughing, and night sweats.

The diagnosis of TB involves skin tests, chest x-rays, sputum analysis (smear and culture), and PCR tests to detect the genetic material of the causative bacteria.

Inactive tuberculosis may be treated with an antibiotic, isoniazid (INH), to prevent the TB infection from becoming active.

Active TB is treated, usually successfully, with INH in combination with one or more of several drugs, including **rifampicin, ethambutol, pyrazinamide**, and streptomycin.

Drug-resistant TB is a serious, as yet unsolved, public health problem, especially in Southeast Asia and in prison populations.

The occurrence of HIV has been responsible for an increased frequency of tuberculosis. Control of HIV in the future, however, should substantially decrease the frequency of TB.

**PEST OF SUGRACANE (PYRILLA PERPUSIELLA)**

**Host:** This insect is a serious pest of sugarcane in northern India where it also occasionally feeds on maize, millets, rice, barley, oats, sorghum, bajra and wild grasses.

**Damage:** The pest is found gregariously on the under surface of the leaves where they suck up plant sap that causes yellowing and eventually drying of leaves. Under low infestation yellow patches appear on the leaves. Photosynthesis is reduced resulting in the reduction of sucrose content of the juice by up to 30%. Hoppers secrete a sweet substance called honey dew that coats the leaves and attracts a blackish fungus, which reduces photosynthesis resulting in yield loss.

**Life cycle:** Adult hoppers are straw coloured to brownish, 7-8 mm long, with a pointed snout bearing piercing and sucking mouth parts. They are found gregariously and jump off readily when disturbed. Adults are active fliers, migrating from one crop to another and breed throughout the year. Eggs are light yellowish in colour, oval, one mm long and laid on the lower surface of the leaf, near the midrib in groups of about 20 eggs, which hatch in 6-15 days depending on temperature.

Nymphs are initially greenish, later turn pale brownish, wingless and with a pair of anal filaments covered with whitish fluffy waxy material. There are 5 nymphal instars which take 40-60 days to complete development. Multiplication of the pest is favoured by high humidity and luxuriant plant growth as in heavily manured and irrigated field or in rainy season.

**Distribution:** The pest is found throughout the Indian subcontinent from Afghanistan to Burma and Thailand.

**Control:** The pest can be controlled by spraying 0.05% of parathion, malathion, thiodon, fenitrothion or rogor. Dusting the plants with 10% Aldrin or dieldrin also helps.

Conservation of the following natural enemies helps in containing the pest:

Egg parasitoids:*Tetrastichus pyrillae, Cheiloneurus pyrillae, Ooencyrtus pyrillae, O. pipilionus, Agoniaspis pyrillae.*

Nymphal parasitoid: *Lestodryinus pyrillae, Pyrilloxenos ompactus, Chlorodryinus pallidus.*

Predators:*Coccinella septempunctata, C. undecimpunctata, Chilomenes sexmaculata, Brumus suturalis.*

Egg-predators:*Nimboa basipunctata, Goniopteryx pusana.*

**PEST OF OILSEED (ACHAEAJANATA)**

**Host:**This is a pest of castor, pomegranate, rose, *Zizyphus, Euphorbia, Tridax, Cardiospermum, Ficus, Bauhinia,*Citrus, mango etc.

**Damage:**Larvae defoliate plants quickly by feeding gregariously and voraciously. Midribs and veins are left intact and other parts of the leaves eaten up. Being larger in size, their capacity to cause damage is enormous. Young plants cannot sustain damage and die. Adults are fruit-sucking moths that prefer to suck juice from mango and citrus and fruits.

**Life cycle:**Adult moths are grayish-brown in colour with wavy lines on the fore wings. Hind wings are black in colour and have one large median and three marginal white spots. They are medium sized robust moths.

Eggs are round, bluish green in color, ridged and are laid singly on tender shoots, usually on the undersurface of the leaves. Fecundity of a female is 450 eggs. Incubation period varies between 2-5 days after which a tiny larva hatches out which is slender and yellowish-green in colour.

Young larvae are usually gregarious but as they grown they get scattered on leaves. A full grown larva is a typical semilooper, has a bluish-black body with a black head and reddish spots on the back and a reddish anal tubercle. Legs are missing on the median segments which makes it walk with looping action. Sometimes there are faint reddish-brown or whitish stripes on the body.

Full grown larva measures about 7 cms. There are 5-6 instar and the whole larval period is about 15-20 days. Pupation takes place in soil or among fallen leaves. Pupal period is 10-15 days but may be prolonged to few months under winter conditions.

**Distribution:**The pest is distributed in the whole of Indian subcontinent. Thailand, Malaysia, Philippines and Indonesia.

**Control:**As the larvae are large and prominent on the leaves, destroying them by handpicking is quite easy. They are also eaten by birds in large numbers.

Chemical control can be achieved by spraying endrin 0.02%, parathion 0.025% or by spraying 0.02% of diazinon, toxaphen, carbaryl, endosulfan and methyl parathion.

Biological control involves conservation of the following parasitoids:

Egg parasites: *Trichogramma evanescens.*Larval parasite: *Apanteles sundanus, A. ruidus, Microplitis maculipennis, M. ensirus, M. similes, Euplectus leucostomus, Paniscus ocellaris, Zamesochorus orientalis, Tetrastichus ophiusae, Rogas percurrens*and*Enicospilus*sp.

**PEST OF RICE (SITOPHILUS ORYZAE)**

**Distribution**

Sitophilus Oryzae is also called as Rice weevils. These insects are cosmopolitan in distribution and have originated in Far East region. They can exist anywhere where physical conditions for growth are favourable and the grain is left undisturbed for some time.

**Habit and Habitat**

Sitophilus Oryzae is usually found in grain storages and processing plants. They infest wheat, oats, rye, barley, rice, and corn. Sometimes, they are also found infesting beans, sunflower seeds and dried corn. These insects do not bite nor damage wood.

**Identification**

Weevils have chewing type of mouthparts. The most significant identification feature of weevils is their snout, which is pretty long. The adult rice weevil is reddish-brown in color. It has irregularly shaped pits on its thorax, also four light spots are found on wing covers.

Rice weevils can fly. During larval stage they are legless, white to creamy white in color, with a small head. Weevils in the pupa stage have snouts just like the adults.

**Life Cycle of Rice weevil**

A single generation can be completed in 28 days. Rice weevils pretend death by drawing their legs close to body and remaining silent even when disturbed.

**Egg:** Rice weevil lays its eggs in crevices of kernels or dust. Female rice weevil lays about 4 eggs/day. During its life span of 5 months, it lays a total of about 250-400 eggs. The eggs hatch in 3 days.

**Larva:** They feed inside the grain kernel for 18 days. The larva is the only stage during which the insect grows. Inside the seed, its cuticle hardens and matures. It consumes several times its own weight and periodically moults to increase in size.

**Pupa:** The pupa stage lasts for 6 days. The pupa does not feed. In some species, the pupa is enclosed in a cocoon constructed by the larva. The pupa goes through great changes both internally and externally. Finally the insect emerges out as a developed adult.

**Adult:** Adults are between 0.1 and 1.7 cm long. They have three pairs of legs and their bodies are divided into head, thorax and abdomen. Adults move and penetrate deeply into bulk of grains and get widely distributed.

**\*** Head includes the mouth parts and sense organs

**\*** Thorax bears the legs and wings

**\*** Abdomen contains the reproductive organs

**Feeding habit and development of rice weevil**

Females drill a tiny hole in the grain kernel through whic it deposits its egg and then plugs the hole with a gelatinous substance. Inside the grain kernel, the egg hatches into a young larva which migrates toward the centre of the grain kernel. Now inside the grain the larva feeds, grows and develops into a pupa. The pupa undergoes major internal and external changes inside the grain to develop into an adult. The adult emerges out through the hole of emergence. The emerged adults are ready to mate and begin their generation.  .

**Damage caused by rice weevil**

Rice weevils are the most destructive pests of stored grain and they completely destroy the grain. Attack is evidenced by,

* Surface heating of grain
* Dampness which may even cause germination
* Presence of numerous adults

**Control measures to avoid rice weevil**

* Removal of infested food products
* Discarding heavily infested material
* Repackaging material into new infection-free containers
* Placing the products in the freezer for several weeks may help to kill adults and larvae

**BIOTECHNOLOGY**

Biotechnology deals with techniques of using live organisms or enzymes from organisms to produce products and processes useful to humans.

Making curd, bread or wine, which are all microbe-mediated processes, could also be thought as a form of biotechnology.

However, it is used in a restricted sense today, to refer to such of those processes which use **genetically modified organisms** to achieve the same on a larger scale.

Modern biotechnology using genetically modified organisms was made possible only when man learnt to alter the chemistry of DNA and construct recombinant DNA. This key process is called **recombinant DNA technology**or**genetic engineering**.

This process involves the use of **restriction endonucleases, DNA ligase, appropriate plasmid or viral vectors** to isolate and ferry the foreign DNA into host organisms, expression of the foreign gene, purification of the gene product, i.e., the functional protein and finally making a suitable formulation for marketing. Large scale production involves use of bioreactors.

**Genetic Engineering**

* Genetic engineering involves the techniques to alter the chemistry of genetic material (DNA and RNA) and thus **change the phenotype** of the host organism.
* Asexual reproduction preserves the genetic information, while sexual reproduction permits variation.
* Traditional hybridisation procedures used in plant and animal breeding, very often lead to inclusion and multiplication of undesirable genes along with the desired genes.
* The techniques of genetic engineering which include **creation of** **recombinant DNA,**use of**gene cloning** and **gene transfer**, overcome this limitation and allows us to isolate and introduce only one or a set of desirable genes without introducing undesirable genes into the target organism.
* There are three basic steps in genetically modifying an organism —
1. identification of DNA with desirable genes;
2. introduction of the identified DNA into the host;
3. maintenance of introduced DNA in the host and transfer of the DNA to its progeny.

**Cloning**

* DNA which is somehow transferred into an alien organism would not be able to multiply itself in the progeny cells of the organism.
* But, when it gets integrated into the genome of the recipient, it may multiply and be inherited along with the host DNA. This is because the alien piece of DNA has become part of a chromosome, which has the ability to replicate.
* In a chromosome there is a specific DNA sequence called the **origin of replication**, which is responsible for initiating replication.
* Therefore, for the multiplication of any alien piece of DNA in an organism it needs to be a part of a chromosome(s) which has a specific sequence known as ‘origin of replication’.
* Thus, an alien DNA is linked with the origin of replication, so that, this alien piece of DNA can replicate and multiply itself in the host organism. This can also be called as **cloning or making multiple identical copies of any template DNA**.

**Recombinant DNA (rDNA)**

* Recombinant DNA (rDNA) molecules are DNA molecules formed by laboratory methods of genetic recombination (such as molecular cloning) to bring together genetic material from **multiple** sources, creating sequences that would not otherwise be found in the genome.
* Recombinant DNA is possible because DNA molecules from all organisms share the **same** chemical structure. They **differ** only in the **nucleotide sequence** within that identical overall structure.
* In most cases, organisms containing recombinant DNA have apparently normal phenotypes. That is, their appearance, behavior and metabolism are usually unchanged.



* The cutting of DNA at specific locations became possible with the discovery of the so-called **‘molecular scissors’- restriction enzymes**.
* Restriction enzymes belong to a larger class of enzymes called **nucleases**. These are of two kinds; exonucleases and endonucleases.
* Exonucleases remove nucleotides from the ends of the DNA whereas, endonucleases make cuts at specific positions within the DNA.
* The cut piece of DNA was then linked with the plasmid DNA. These plasmid DNA act as vectors to transfer the piece of DNA attached to it.
* You probably know that mosquito acts as an insect vector to transfer the malarial parasite Into human body.
* In the same way, a plasmid can be used as vector to deliver an alien piece of DNA into the host organism.
* The linking of antibiotic resistance gene with the plasmid vector became possible with the enzyme **DNA ligase**, which acts on cut DNA molecules and joins their ends. This makes a new combination of circular autonomously replicating DNA created in vitro and is known as **recombinant DNA**.
* When this DNA is transferred into Escherichia coli, a bacterium closely related to Salmonella, it could replicate using the new host’s DNA polymerase enzyme and make multiple copies. The ability to multiply copies of antibiotic resistance gene in E. coli was called cloning of antibiotic resistance gene in E. coli.

**Applications of Recombinant DNA Technology**

* Recombinant DNA is widely used in biotechnology, medicine and research.
* Recombinant DNA is used to identify, map and sequence genes, and to determine their function.

**Recombinant DNA is used to produce**

* Recombinant human insulin,
* Recombinant human growth hormone,
* Recombinant blood clotting factor VIII,
* Recombinant hepatitis B vaccine,
* Insect-resistant crops etc.

**Cloning Vectors**

* You may be surprised to know that we have learnt the lesson of transferring genes into plants and animals from bacteria and viruses which have known this for ages – how to deliver genes to transform eukaryotic cells and force them to do what the bacteria or viruses want.
* For example, Agrobacterioum tumifaciens, a pathogen of several dicot plants is able to deliver a piece of DNA known as ‘T-DNA’ to transform normal plant cells into a tumor and direct these tumor cells to produce the chemicals required by the pathogen.
* Similarly, retroviruses in animals have the ability to transform normal cells into cancerous cells.
* A better understanding of the art of delivering genes by pathogens in their eukaryotic hosts has generated knowledge to transform these tools of pathogens into useful vectors for delivering genes of interest to humans.
* The tumor inducing (Ti) plasmid of AgrobcLCterium tumifaciens has now been modified into a cloning vector which is no more pathogenic to the plants but is still able to use the mechanisms to deliver genes of our interest into a variety of plants.
* Similarly, retroviruses have also been disarmed and are now used to deliver desirable genes into animal cells.
* So, once a gene or a DNA fragment has been ligated into a suitable vector it is transferred into a bacterial, plant or animal host (where it multiplies).
* Plasmids and bacteriophages [vectors] have the ability to replicate within bacterial cells independent of the control of chromosomal DNA.

**Competent Host – Methods to Induce Alien DNA into Host Cells**

* Since DNA is a **hydrophilic molecule**, **it cannot pass through cell membranes**. In order to force bacteria to take up the plasmid, the bacterial cells must first be made ‘competent’ to take up DNA. Recombinant DNA can then be forced into such cells by incubating the cells with recombinant DNA on ice, followed by placing them briefly at 420C (heat shock), and then putting them back on ice. This enables the bacteria to take up the recombinant DNA. This is not the only way to introduce alien DNA into host cells.
* In a method known as micro-injection, recombinant DNA is directly injected into the nucleus of an animal cell.
* In another method, suitable for plants, cells are bombarded with high velocity micro-particles of gold or tungsten coated with DNA in a method known as **biolistics or gene gun**.
* And the last method uses ‘disarmed pathogen’ vectors, which when allowed to infect the cell, transfer the recombinant DNA into the host.

**Biotechnology And Its Applications**

* Biotechnology essentially deals with industrial scale production of biopharmaceuticals and biologicals using genetically modified microbes, fungi, plants and animals.
* The applications of biotechnology include therapeutics, diagnostics, genetically modified crops for agriculture, processed food, bioremediation, waste treatment, and energy production.

**Cloning**

* Cloning is the production of an exact copy of a cell, any other living part, or a complete organism.
* Cloning of an animal was successfully performed for the first time by Ian Wilmut and his colleagues at the Roslin Institute in Edinburgh, Scotland.
* They cloned successfully a sheep named Dolly. Dolly was born in 1996 and was the first mammal to be cloned.
* During the process of cloning Dolly, a cell was collected from the mammary gland of a female Finn Dorsett sheep. Simultaneously, an egg was obtained from a Scottish blackface ewe. The nucleus was removed from the egg. Then, the nucleus of the mammary gland cell from the Finn Dorsett sheep was inserted into the egg of the Scottish blackface ewe whose nucleus had been removed. The egg thus produced was implanted into the Scottish blackface ewe. Development of this egg followed normally and finally Dolly was born. Though Dolly was given birth by the Scottish blackface ewe, it was found to be absolutely identical to the Finn Dorsett sheep from which the nucleus was taken. Since the nucleus from the egg of the Scottish blackface ewe was removed, Dolly did not show any character of the Scottish blackface ewe.



* Dolly was a healthy clone of the Finn Dorsett sheep and produced several offspring of her own through normal sexual means.
* Since Dolly, several attempts have been made to produce cloned mammals. However, many die before birth or die soon after birth. The cloned animals are many-a-time found to be born with severe abnormalities.

**Biotechnological applications in agriculture**

* Let us take a look at the three options that can be thought for increasing food production
1. agro-chemical based agriculture;
2. organic agriculture; and
3. genetically engineered crop-based agriculture.
* Plants, bacteria, fungi and animals whose genes have been altered by manipulation are called Genetically Modified Organisms (GMO). GM plants have been useful in many ways. Genetic modification has:
1. made crops more tolerant to abiotic stresses (cold, drought, salt, heat).
2. reduced reliance on chemical pesticides (pest-resistant crops).
3. helped to reduce post harvest losses.
4. increased efficiency of mineral usage by plants (this prevents early exhaustion of fertility of soil).
5. enhanced nutritional value of food, e.g., Vitamin ‘A’ enriched rice.
* In addition to these uses, GM has been used to create tailor-made plants to supply alternative resources to industries, in the form of starches, fuels and pharmaceuticals.
* **Bt toxin** is produced by a bacterium called **Bacillus thuringiensis** (Bt for short).
* Some strains of Bacillus thuringiensis produce proteins that kill certain insects such as tobacco budworm, armyworm, beetles and dipterans flies, mosquitoes.
* Why does this toxin not kill the Bacillus? Actually, the Bt toxin protein exist as inactive protoxins but once an insect ingest the inactive toxin, it is converted into an active form of toxin due to the alkaline pH of the gut which solubilise the crystals.
* Bt toxin gene has been cloned from the bacteria and been expressed in plants to provide resistance to insects without the need for insecticides; in effect created a bio-pesticide.
* Examples are Bt cotton, Bt corn, rice, tomato, potato and soyabean etc.

**Biotechnological applications in medicine**

* The recombinant DNA technological processes have made immense impact in the area of healthcare by enabling **mass production of safe and more effective therapeutic drugs**.
* Further, the recombinant therapeutics **do not induce unwanted immunological responses** as is common in case of similar products isolated from non-human sources.
* At present, about 30 recombinant therapeutics have been approved for human-use the world over. In India, 12 of these are presently being marketed.

**Genetically Engineered Insulin**

* Management of adult-onset diabetes is possible by taking insulin at regular time intervals. What would a diabetic patient do if enough human-insulin was not available?
* If you discuss this, you would soon realise that one would have to isolate and use insulin from other animals. Would the insulin isolated from other animals be just as effective as that secreted by the human body itself and would it not elicit an immune response in the human body?
* Now, imagine if bacterium were available that could make human insulin. Suddenly the whole process becomes so simple. You can easily grow a large quantity of the bacteria and make as much insulin as you need.
* Think about whether insulin can be orally administered to diabetic people or not. Why?
* Insulin used for diabetes was earlier extracted from pancreas of slaughtered cattle and pigs.
* Insulin from an animal source, though caused some patients to develop allergy or other types of reactions to the foreign protein.
* Insulin consists of two short polypeptide chains: chain A and chain B, that are linked together by **disulphide**
* In mammals, including humans, insulin is synthesised as a **pro-hormone** (like a pro-enzyme, the pro-hormone also needs to be processed before it becomes a fully mature and functional hormone) which contains an extra stretch called the C peptide. This C peptide is not present in the mature insulin and is removed during maturation into insulin.The main challenge for production of insulin using rDNA techniques was getting insulin assembled into a mature form.
* In 1983, Eli Lilly an American company prepared two DNA sequences corresponding to A and B, chains of human insulin and introduced them in plasmids of E. coli to produce insulin chains. Chains A and B were produced separately, extracted and combined by creating disulfide bonds to form human insulin.

**Gene Therapy**

* If a person is born with a hereditary disease, can a corrective therapy be taken for such a disease? Gene therapy is an attempt to do this.
* Gene therapy is a collection of methods that allows correction of a gene defect that has been diagnosed in a child/embryo.
* Here genes are inserted into a person’s cells and tissues to treat a disease. Correction of a genetic defect involves delivery of a normal gene into the individual or embryo to take over the function of and compensate for the non-functional gene.
* The first clinical gene therapy was given in 1990 to a 4-year old girl with **adenosine deaminase (ADA)** This enzyme is crucial for the immune system to function.
* The disorder is caused due to the deletion of the gene for adenosine deaminase.
* In some children ADA deficiency can be cured by bone marrow transplantation; in others it can be treated by enzyme replacement therapy, in which functional ADA is given to the patient by injection.
* But the problem with both of these approaches that they are not completely curative.
* As a first step towards gene therapy, lymphocytes from the blood of the patient are grown in a culture outside the body. A functional ADA cDNA (using a retroviral vector) is then introduced into these lymphocytes, which are subsequently returned to the patient.
* However, as these cells are not immortal, the patient requires periodic infusion of such genetically engineered lymphocytes.
* However, if the gene isolate from marrow cells producing ADA is introduced into cells at early embryonic stages, it could be a permanent cure.

**Molecular Diagnosis**

* You know that for effective treatment of a disease, early diagnosis and understanding its pathophysiology is very important.
* Using conventional methods of diagnosis (serum and urine analysis, etc.) early detection is not possible.
* **Recombinant DNA technology**, **Polymerase Chain Reaction (PCR)** and **Enzyme Linked Immuno-sorbent Assay (ELISA)** are some of the techniques that serve the purpose of early diagnosis.
* Presence of a pathogen (bacteria, viruses, etc.) is normally suspected only when the pathogen has produced a disease symptom. By this time the concentration of pathogen is already very high in the body.
* However, very low concentration of a bacteria or virus (at a time when the symptoms of the disease are not yet visible) can be detected by amplification of their nucleic acid by PCR.
* PCR is now routinely used to **detect HIV** in suspected AIDS patients. It is being used to detect mutations in genes in suspected **cancer** patients too. It is a powerful techqnique to identify many other genetic disorders.
* ELISA is based on the principle of **antigen-antibody interaction**. Infection by pathogen can be detected by the presence of antigens (proteins, glycoproteins, etc.) or by detecting the antibodies synthesised against the pathogen.

**Transgenic animals**

* Animals that have had their DNA manipulated to possess and express an extra (foreign) gene are known as transgenic animals.
* Transgenic rats, rabbits, pigs, sheep, cows and fish have been produced, although over 95 per cent of all existing transgenic animals are mice.
* Why are these animals being produced? How can man benefit from such modifications? Let us try and explore some of the common reasons.
* **Normal physiology and development:** Transgenic animals can be specifically designed to allow the study of how genes are regulated, and how they affect the normal functions of the body and its development, e.g., study of complex factors involved in growth such as insulin-like growth factor. By introducing genes from other species that alter the formation of this factor and studying the biological effects that result, information is obtained about the biological role of the factor in the body.
* **Study of disease:** Many transgenic animals are designed to increase our understanding of how genes contribute to the development of disease. These are specially made to serve as models for human diseases so that investigation of new treatments for diseases is made possible. Today transgenic models exist for many human diseases such as cancer, cystic fibrosis, rheumatoid arthritis and Alzheimer’s.
* **Biological products:** Medicines required to treat certain human diseases can contain biological products, but such products are often expensive to make. Transgenic animals that produce useful biological products can be created by the introduction of the portion of DNA (or genes) which codes for a particular product such as human protein. Similar attempts are being made for treatment of phenylketonuria (PKU) and cystic fibrosis. In 1997, the first transgenic cow, Rosie, produced human protein-enriched milk (2.4 grams per litre). The milk contained the human alpha-lactalbumin and was nutritionally a more balanced product for human babies than natural cow-milk.
* **Vaccine safety:** Transgenic mice are being developed for use in testing the safety of vaccines before they are used on humans. Transgenic mice are being used to test the safety of the polio vaccine. If successful and found to be reliable, they could replace the use of monkeys to test the safety of batches of the vaccine.
* **Chemical safety testing:** This is known as toxicity/safety testing. The procedure is the same as that used for testing toxicity of drugs. Transgenic animals are made that carry genes which make them more sensitive to toxic substances than non-transgenic animals. They are then exposed to the toxic substances and the effects studied. Toxicity testing in such animals will allow us to obtain results in less time.

**Biotechnology: Ethical Issues**

* The manipulation of living organisms by the human race cannot go on any further, without regulation. Some ethical standards are required to evaluate the morality of all human activities that might help or harm living organisms.
* Going beyond the morality of such issues, the biological significance of such things is also important. Genetic modification of organisms can have unpredicatable results when such organisms are introduced into the ecosystem.
* Therefore, the Indian Government has set up organisations such as **GEAC (Genetic Engineering Approval Committee)**, which will make decisions regarding the validity of GM research and the safety of introducing GM-organisms for public services.
* The modification/usage of living organisms for public services (as food and medicine sources, for example) has also created problems with patents granted for the same.
* There is growing public anger that certain companies are being granted patents for products and technologies that make use of the genetic materials, plants and other biological resources that have long been identified, developed and used by farmers and indigenous people of a specific region/country.
* Rice is an important food grain, the presence of which goes back thousands of years in Asia’s agricultural history. There are an estimated 200,000 varieties of rice in India alone. The diversity of rice in India is one of the richest in the world.
* Basmati rice is distinct for its unique aroma and flavour and 27 documented varieties of Basmati are grown in India. There is reference to Basmati in ancient texts, folklore and poetry, as it has been grown for centuries.
* In 1997, an American company got patent rights on Basmati rice through the US Patent and Trademark Office. This allowed the company to sell a ‘new’ variety of Basmati, in the US and abroad. This ‘new’ variety of Basmati had actually been derived from Indian farmer’s varieties.
* Indian Basmati was crossed with semi-dwarf varieties and claimed as an invention or a novelty. The patent extends to functional equivalents, implying that other people selling Basmati rice could be restricted by the patent.
* Several attempts have also been made to patent uses, products and processes based on Indian traditional herbal medicines, e.g., turmeric neem. If we are not vigilant and we do not immediately counter these patent applications, other countries/individuals may encash on our rich legacy and we may not be able to do anything about it.
* **Biopiracy** is the term used to refer to the use of bio-resources by multinational companies and other organisations without proper authorisation from the countries and people concerned without compensatory payment.
* Most of the industrialised nations are rich financially but poor in biodiversity and traditional knowledge. In contrast the developing and the underdeveloped world is rich in biodiversity and traditional knowledge related to bio-resources.
* Traditional knowledge related to bio-resources can be exploited to develop modern applications and can also be used to save time, effort and expenditure during their commercialisation.
* There has been growing realisation of the injustice, inadequate compensation and benefit sharing between developed and developing countries. Therefore, some nations are developing laws to prevent such unauthorised exploitation of their bio-resources and traditional knowledge.
* The Indian Parliament has recently cleared the second amendment of the Indian Patents Bill, that takes such issues into consideration, including patent terms emergency provisions and research and development initiative.

**Summary**

* Biotechnology has given to humans several useful products by using microbes, plant, animals and their metabolic machinery.
* Recombinant DNA technology has made it possible to engineer microbes, plants and animals such that they have novel capabilities.
* Genetically Modified Organisms have been created by using methods other than natural methods to transfer one or more genes from one organism to another, generally using techniques such as recombinant DNA technology.
* GM plants have been useful in increasing crop yields, reduce post-harvest losses and make crops more tolerant of stresses.
* There are several GM crop plants with improved nutritional value of foods and reduced the reliance on chemical pesticides (pest-resistant crops).
* Recombinant DNA technological processes have made immense impact in the area of healthcare by enabling mass production of safe and more effective therapeutics.
* Since the recombinant therapeutics are identical to human proteins, they do not induce unwanted immunological responses and are free from risk of infection as was observed in case of similar products isolated from non-human sources. Human insulin is made in bacteria yet its structure is absolutely identical to that of the natural molecule.
* Transgenic animals are also used to understand how genes contribute to the development of a disease by serving as models for human diseases, such as cancer, cystic fibrosis, rheumatoid arthritis and Alzheimer’s.
* Gene therapy is the insertion of genes into an individual’s cells and tissues to treat diseases especially hereditary diseases. It does so by replacing a defective mutant allele with a functional one or gene targeting which involves gene amplification.
* Viruses that attack their hosts and introduce their genetic material into the host cell as part of their replication cycle are used as vectors to transfer healthy genes or more recently portions of genes.