CE 789 : Sanitary microbiology

Introduction

1. Epidemiological terminology

2. Infection disease cycle

3. Air borne diseases

4. Water borne diseases

5. Detection techniques

1. Epidemiological terminology

Term	**Definition**
Epidemiology	Science that evaluates the occurrence, determinants, distribution and control of health and disease in a defined human population
Endemic disease	Disease that is maintained at low level frequency and at regular interval ; ex : common cold
Epidemic	Sudden increase in the occurrence of a disease above the expected level ; ex : AIDS : see figure 1
Pandemic	Increase in disease occurrence within a large population over a very wide region, for example among continents ; ex : influenza
Zoonose	Animal disease that can be transmitted to humans
Incidence	Number of new cases of a disease during a specific period in a specific population Ex : 700 new cases of influenza occurred this year in City of 100,000 habitants
Morbidity rate	(number of new cases of a disease during a specific period = incidence) / (number of individuals in the population) ex : in our case, the morbidity rate is 0.7% in City (700 / 100,000 * 100)
Prevalence	Total number of individuals infected in a population at any one time no matter when the disease began ex : the 1st of January 2002, 350 persons were infected by influenza virus ; the prevalence of influenza this year is 350 + 700 = 1,050 in City
Mortality rate	(number of deaths due to a given disease) / (size of the population with the same disease) ex : if there were 15,000 deaths due to AIDS in a year, and the total number of people infected was 30,000, the mortality rate is 50%.

Table 1 : Terminology in epidemiology

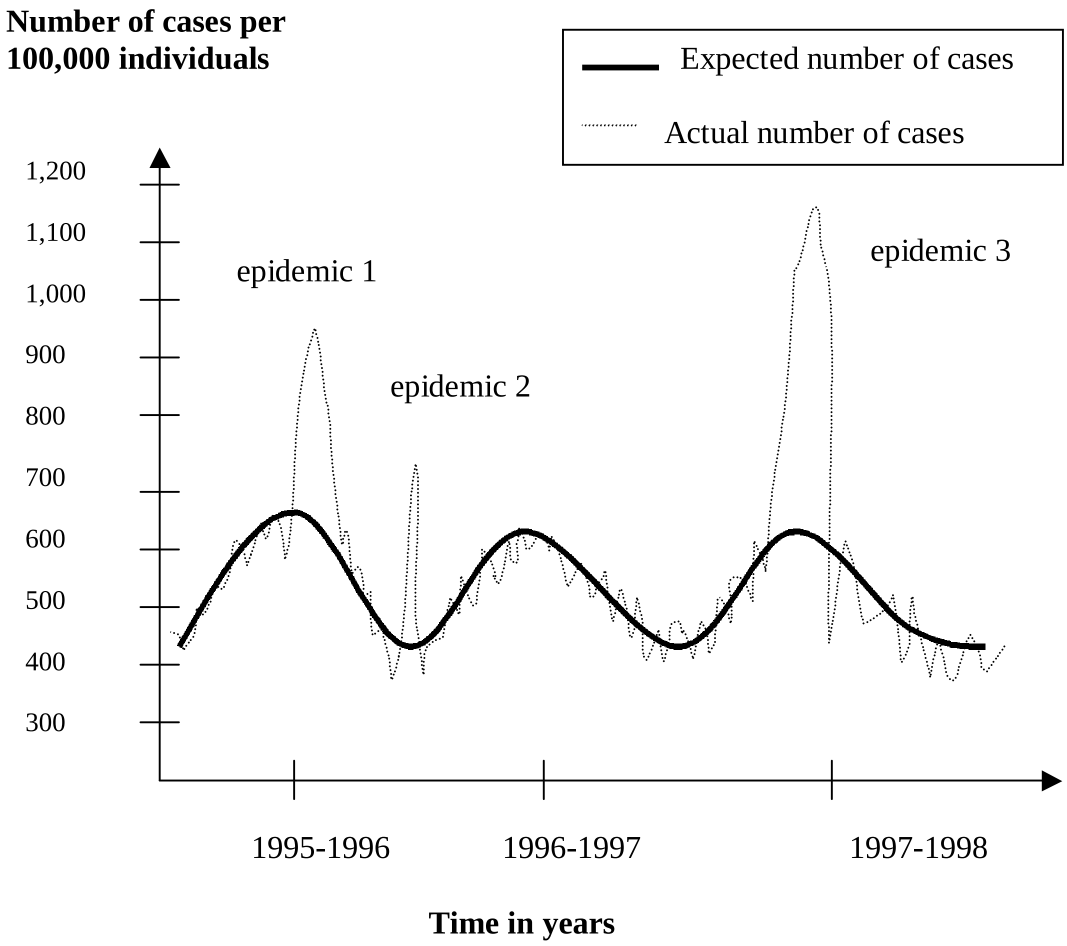


Figure 1 : A graph illustrating three epidemics

An infectious disease epidemic is usually a short – term increase in the occurrence of the disease in a particular population ; two major types of epidemic are recognised : common source and propagated :

	Common source epidemic	**Propagated epidemic**
Description	sharp rise to a peak and then rapid, but not as pronounced, decline in the number of individuals infected	slow and prolonged rise and then a gradual decline in the number of individuals infected
Duration of the cases	one incubation period	several incubation periods
Origin	single common contaminated source as food or water	introduction of a single infected individual into a susceptible population
Example	food poisoning or legionnaires' disease	mumps, chickenpox

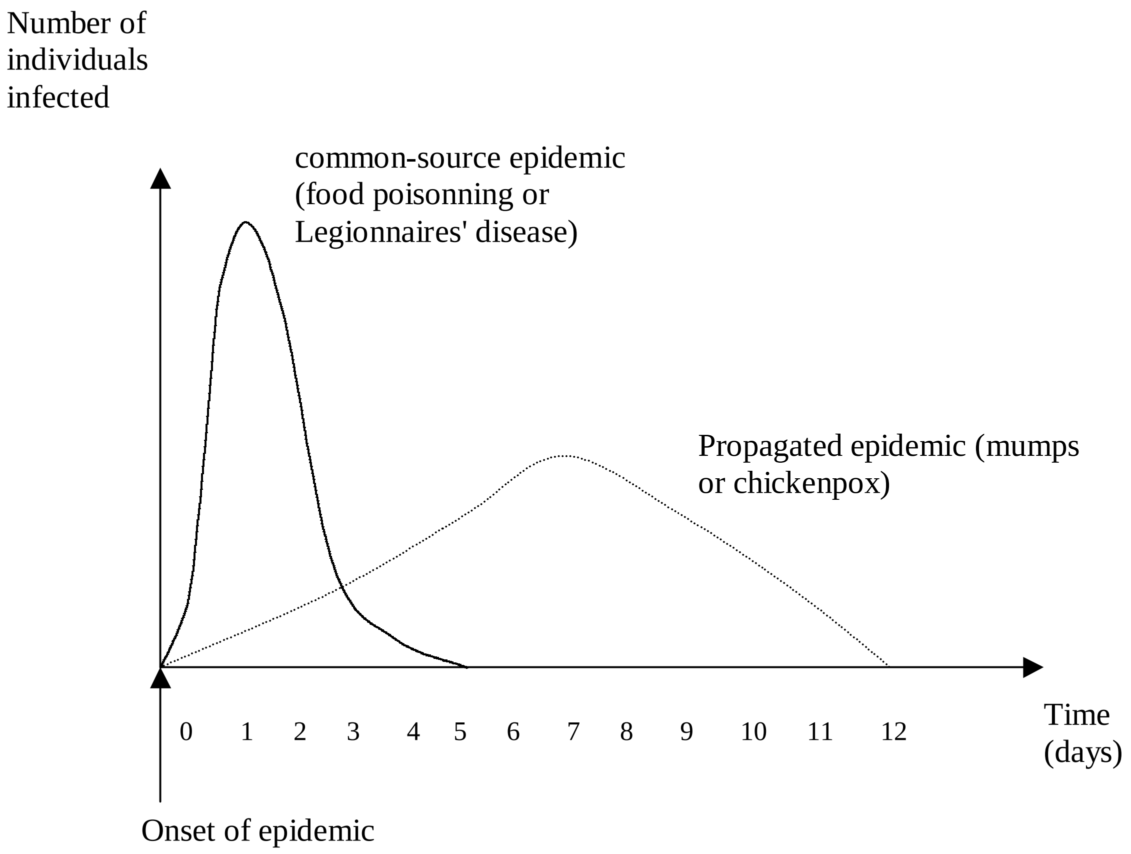


Figure 2 : epidemic curves

To understand how epidemics are propagated, see figure 3 :

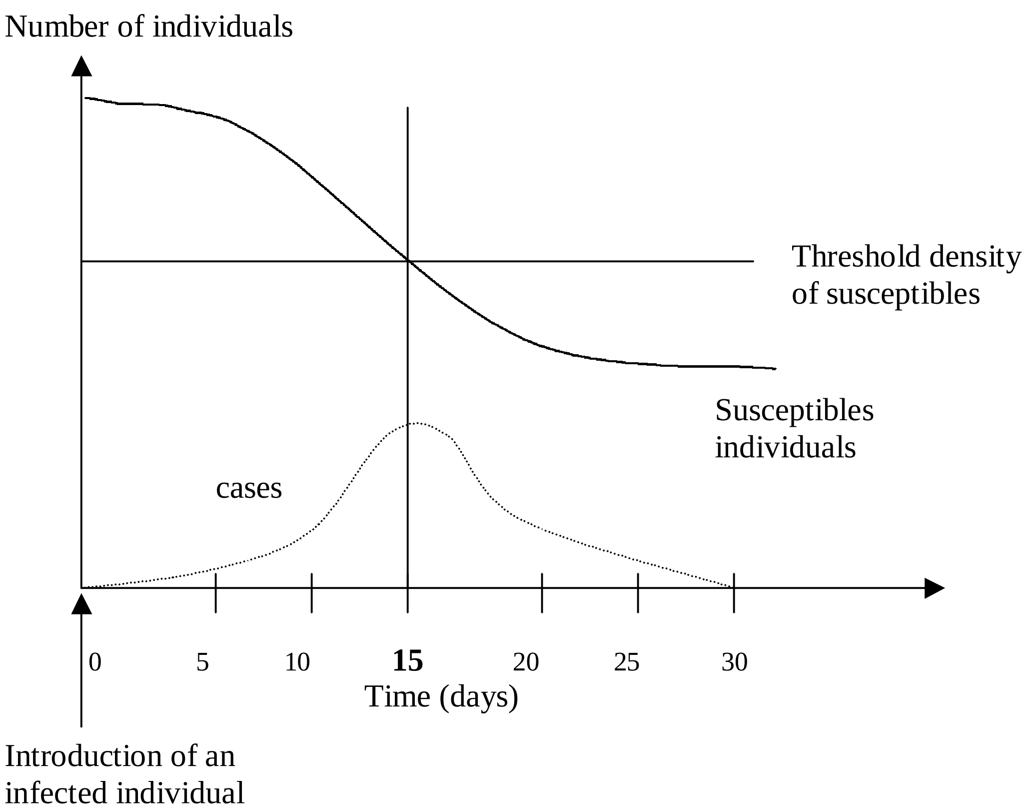


Figure 3 : Diagrammatic representation of the spread of an imaginary propagated epidemic

At time 0, all individuals in this population are susceptible to a hypothetical pathogen ; the introduction of an infected individual initiates the epidemic outbreak, which spreads of reach a peak (day 15).
As individuals recover from disease, they become immune and no longer transmit the pathogen ; the number of susceptible individuals decreases and to a threshold density : it is the minimum number of individuals necessary to continue propagating the disease ; this threshold coincides with the peak of the epidemic wave, and the incidence of new cases declines because the pathogen cannot propagate itself.

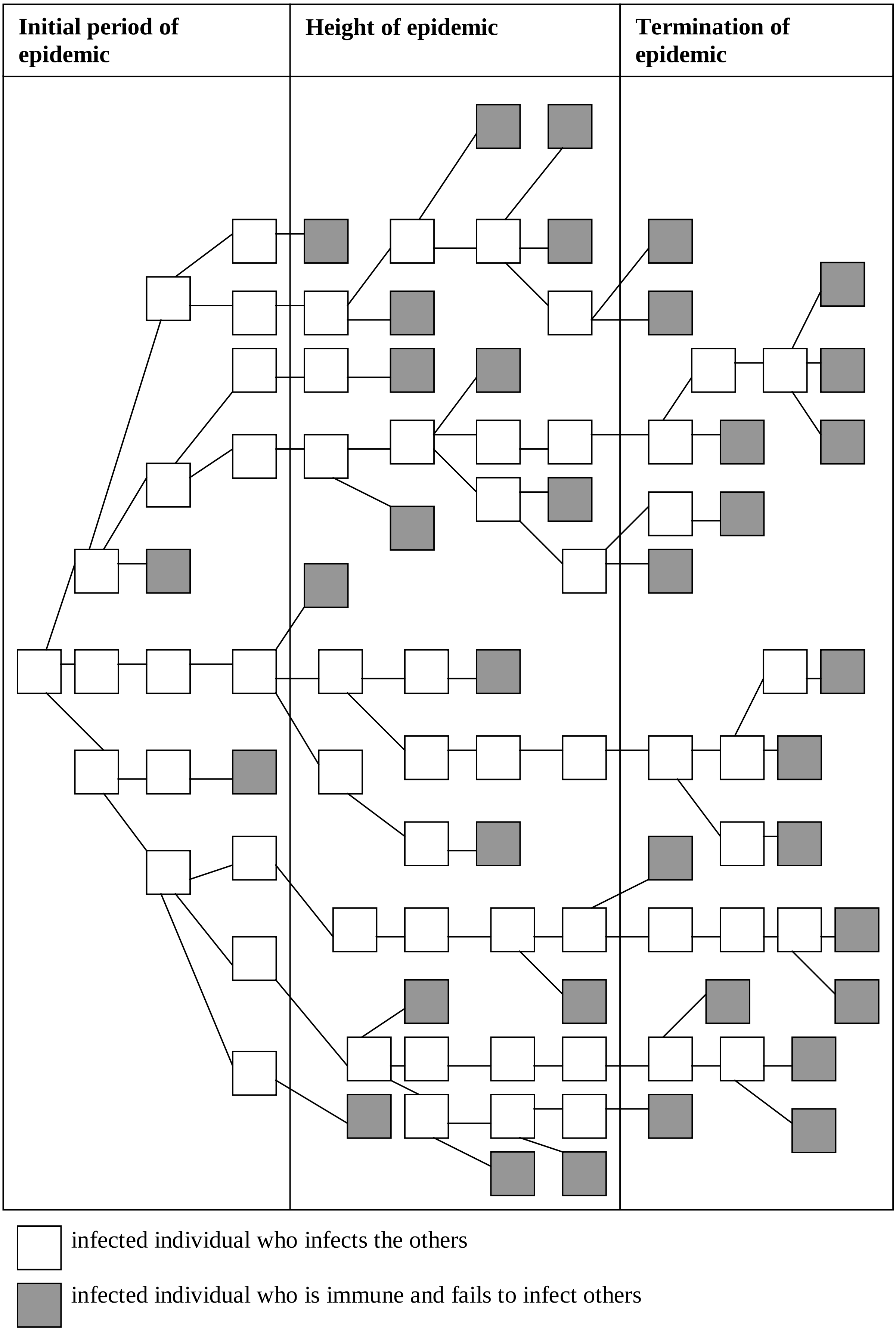


Figure 4 : Herd immunity

Herd immunity : resistance of a population to infection and pathogen spread because of the immunity of a large percentage of the population : figure 4 illustrates the kinetics of the spread of an infectious disease and the effect of increasing the number of immune individuals in the population in limiting the disease.
The larger the proportion of those immune, the smaller the probability of effective contact between infective and susceptible individuals – that is, many contacts will be with immunes, and thus the population will exhibit a group resistance.
A susceptible member of such an immune population enjoys an immunity that is not of his or her own making, but instead arises because of membership in the group.

<u>2. Infection disease cycle</u>

<u>2.1. Definitions</u>

Organisms can live **without any relationship : <u>saprophytes</u>**

or they can establish a relationship with another organism : **<u>symbiosis</u>**
We will define three kind of relationship :
- **Mutualism** in which some reciprocal benefit accrues to both partner ; <u>syntrophism</u>, association in which the growth of one organism is improved by substances provided by another one, is an example of mutualism (cross-feeding) ;
ex : the rumen symbiosis
microorganisms degrade cellulose (ruminants can't synthesise cellulase) in glucose, which is fermented in organic acids, true energy source for the ruminant and methane which is eructed.

- **Commensalism** benefits to only one partner ; the other is neither harmed or helped
ex : the non-pathogenic strains of *E.coli* live in the human colon

- **Parasitism** if a symbiont harms the other organism (<u>the host</u>)
When a parasite is **growing** within a host, the host is said to have in **infection** ; an infection may or may not result in overt disease
Parasitism is defined by **pathogenicity**.
Pathogen can be **opportunistic** : it is generally harmless but becomes pathogenic in a compromised host (debilitated and lower resistance to infection) due to alcoholism, malnutrition, trauma from injury, altered microbiota from the prolonged use of antibiotics, viruses (HIV)…ex : mycosis, infection due *Pseudomonas aeruginosa*…

All symbiotic relationship are dynamic, and shifts among them can occur :
- a saprophytic organism can become commensal
- a commensal can become pathogen

Pathogenicity has two components :
- **virulence** which is the ability of the pathogen to invade (attachment and colonization) the host
- **toxigenicity** which is its ability to produce toxin ; there are two kinds of toxins :
  - proteins that are **exotoxins**, secreted by the pathogen *: Shigella dysenteriae (C),* ETEC (E), *Clostridium tetani* and *botulinum(N), Vibrio cholerae* (E) : **Neurotoxins, Enterotoxins and Cytotoxins** (general tissues)
  - endotoxins which are lipopolysaccharides (LPS) localised in the outer membrane of the bacteria cell wall ; this LPS is released when the bactreia lyses ; LPS induces inflammation, hypotension, internal haemorrhaging : *Salmonella typhi*

The lethal dose 50 (LD_{50}) and the infectious dose 50 (ID_{50}) refer respectively to the number of pathogens that kill or infect 50% of an experimental group of hosts within a specified period.

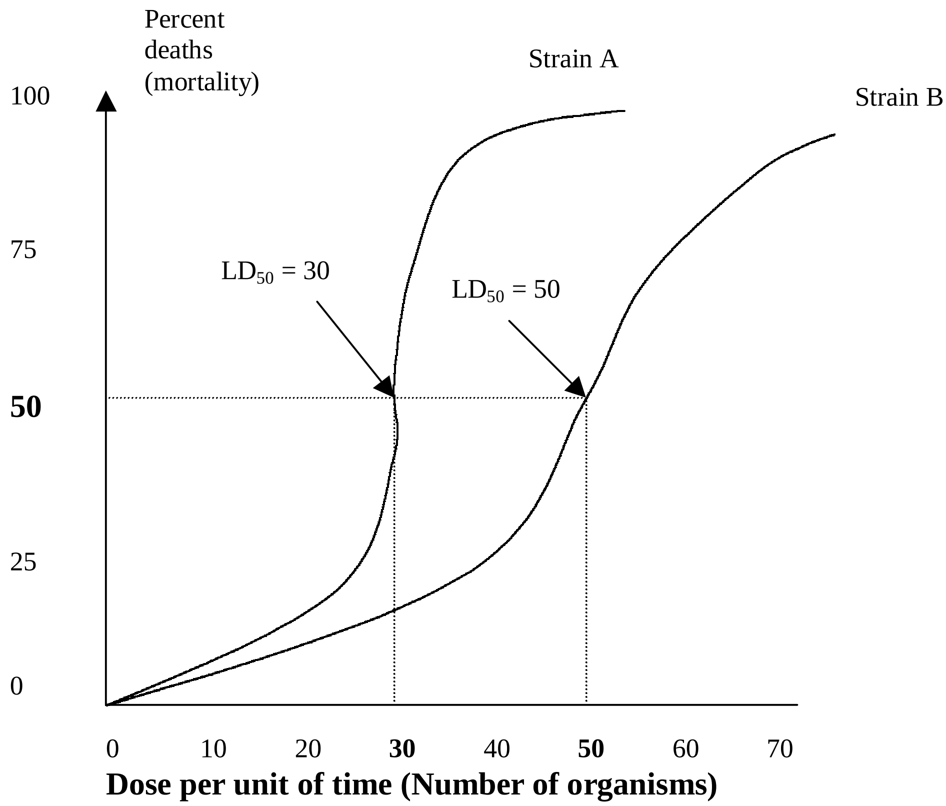


Figure 5 : **Determination of the LD_{50} of a pathogenic microorganism**
Various doses of a specific pathogen (strains A and B) are injected into host animals ; strain A is more pathogenic than strain B

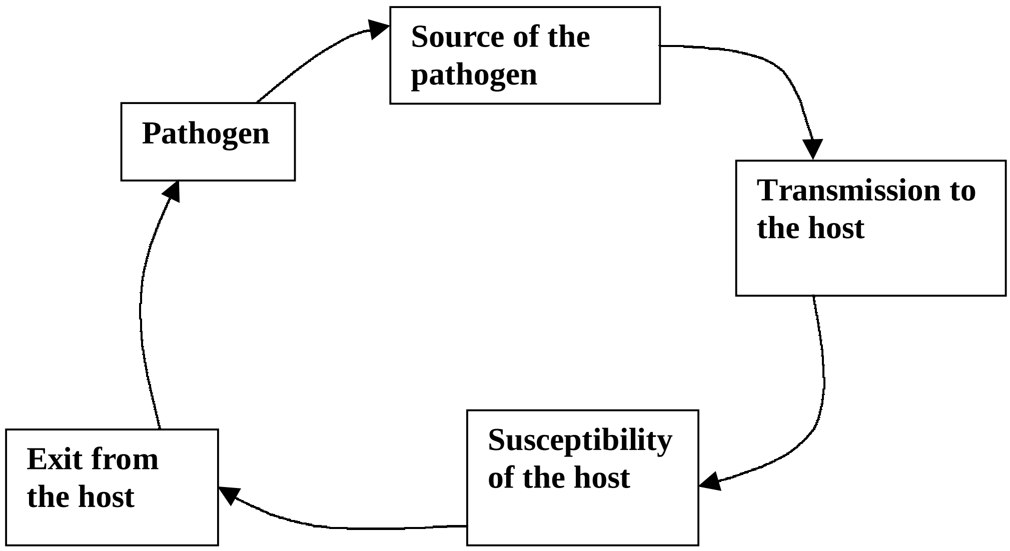


Figure 6 : Infection disease cycle

A communicable disease is caused by a pathogen which is transmissible from one individual to another.

Source / reservoir of the pathogen

Source : location from which the pathogen is immediately transmitted to the host, either directly through the environment, or indirectly through an intermediate agent.
Source can be animate : humans, animals
or inanimate : water, food…

Reservoir : natural environmental location in which the pathogen is normally found living, and from which infection of the host occurs ; reservoir can be animate or inanimate.
Human hosts who are source of the pathogens are called carriers : 4 types :
- active carrier : has an overt clinical case of the disease
- convalescent carrier : has recovered from the infection but continues to harbor large number of pathogens
- healthy carrier : harbor pathogens but is not ill
- incubatory carrier : is incubating pathogen but is not yet ill.

2. 2. Pathogen transmission to the host

Transmission of the disease occurs by 4 main routes : airborne, contact, vehicle and vector borne.

2.2.1. Air borne transmission : pathogen is suspended in the air, contained within droplet nuclei or dust.
Droplet nuclei : small particles, 1 to 4 µm in diameter, usually propelled from respiratory tract into the air by individual's coughing, sneezing…

Microorganism	**Disease**
Viruses	
Varicella	Chickenpox
Influenza	Flu
Rubeola	Measles (*rougeol*e)
Rubella	German measles (*rubéole*)
Mumps	Mumps
Poliomyelitis	Polio
Bacteria	
Actinomyces	Lung infection
Bordetella pertussis	Whooping cough
Chlamydia	Psittacosis
Corynebacterium diphteriae	Diphtheria
Mycoplasma pneumoniae	Pneumonia
Mycobacterium tuberculosis	Tuberculosis
Neisseria	Meningitis
Streptococcus	Pneumonia, ,sore throat
Fungi	
Blastomyces	Lung infection
Candida	Disseminated infections
Coccidioides	Coccidioidomycosis
Histoplasma capsulatum	Histoplasmosis

Table 2 : Some airborne pathogens and the diseases they cause in humans

2.2.2. Contact transmission

It implies the touching between the source or reservoir of the pathogen and the host.
Direct contact : person – to – person contact : sexually transmitted disease (AIDS, syphilis), by contact with oral secretion or body lesions (herpes, boils), or transmitted by contact with animals (Salmonella, Campylobacter)
Indirect contact : transmission of the pathogen through an inanimate objects (Pseudomonas is transmitted by eating utensils…)

2.2.3.Vehicle transmission
Inanimate material involved in pathogen transmission are called vehicles ; food and water are important common vehicles : table 3

2.2.4. Vector-borne transmission

Living transmitters of a pathogen are called vectors : arthropods (insects, ticks, mites, fleas) or vertebrates (dogs…) ; this kind of transmission can be :
- external : the pathogen is carried on the body surface of the vector without any modification (flies carrying *Shigella)*
- internal : the pathogen is carried inside the body of the vector, without any modification (*Yersinia pestis* in the rat flea from rat to human), or with a physiological modification (development of the malaria parasite inside the mosquito vector).

Summary regarding the pathogen transmissions : figure 6

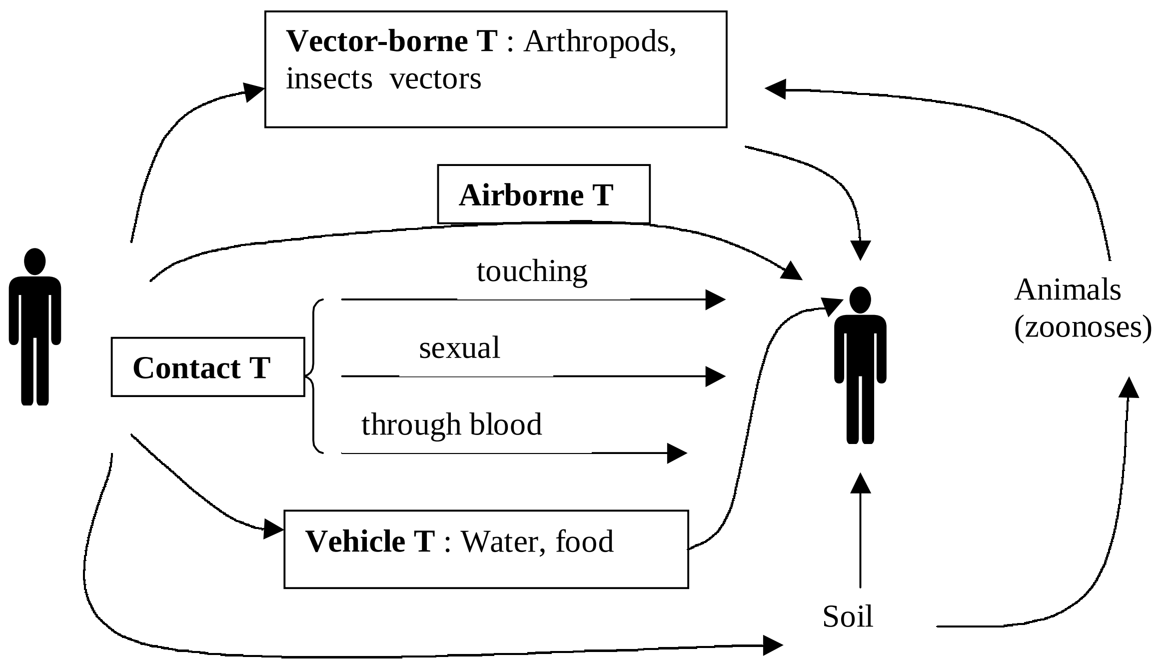


Figure 7 : Sources and transmission routes for transmissible diseases

2.2.5. Susceptibility of the host

I depends on both
- pathogenicity of the organism : virulence (attachment, colonisation, invasion, growth and multiplication of the pathogen) and toxigenicity (secretion of toxins)
- defence mechanisms of the host : non specific (skin, enzymes in respiratory systems, chemicals in saliva…)and specific (specific immunity : immune response in which lymphocytes recognise the presence of particular foreign agent ; it allows the formation of antibodies and prevent ulterior infection)

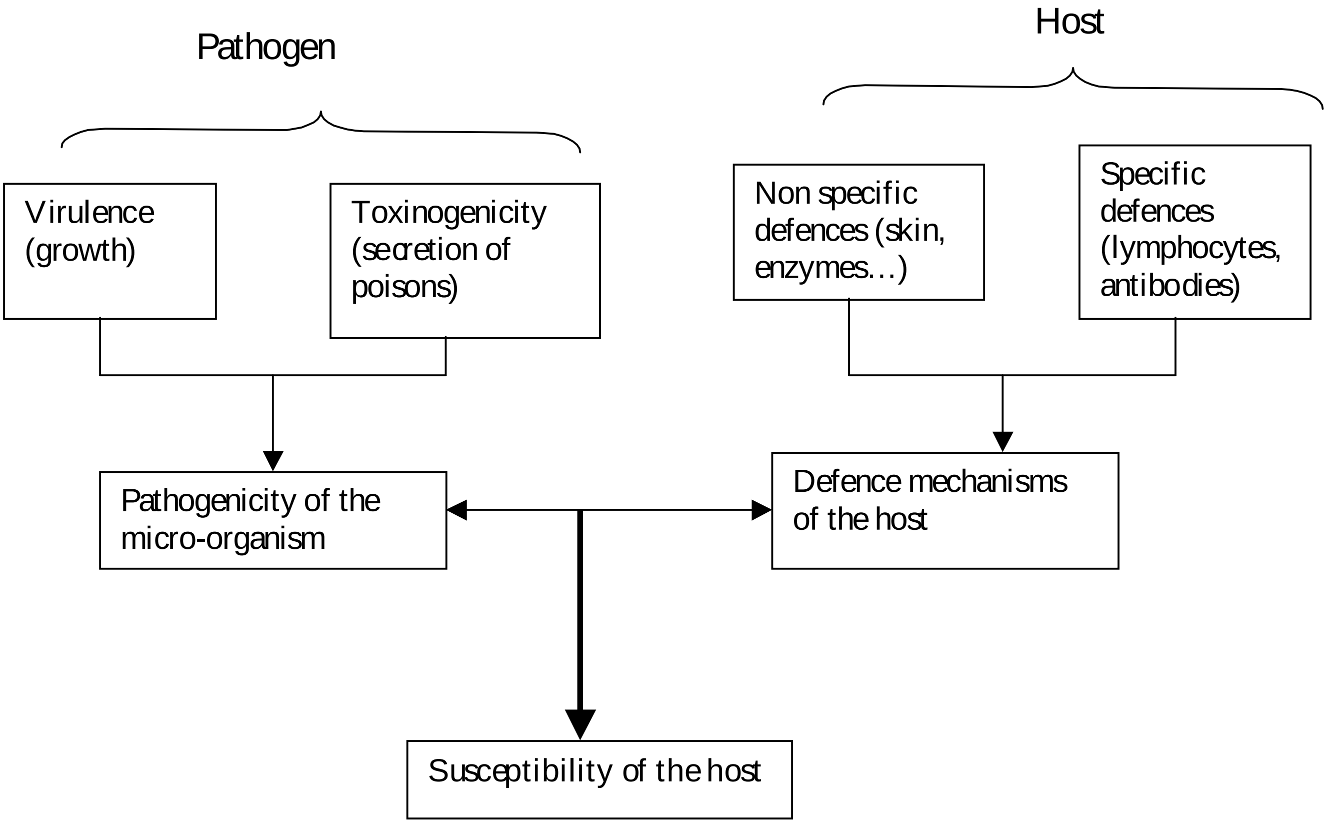


Figure 8 : Determinants of the susceptibility of the host

2.2.6. Exit of the pathogen from the host

Active escape : the pathogen actively moves to a portal of exit and leaves the host ; ex : parasitic helminths migrate through the body of their host, reach the surface and exit
Passive escape : the pathogen leaves the host in faeces, urine, droplets, saliva, desquamated cells.

2.3. Control of epidemic

- ☞ quarantine and isolation of cases and carrier
- ☞ destruction of an animal reservoir of infection
- ☞ destruction of the link between the source of the infection and susceptible individuals : chlorination of water supplies, pasteurisation of milk, inspection of food and people who handle food, destruction of vectors by spraying insecticides
- ☞ treatment of sewage to reduce water contamination
- ☞ therapy that reduces or eliminates infectivity of the individuals : immunisation

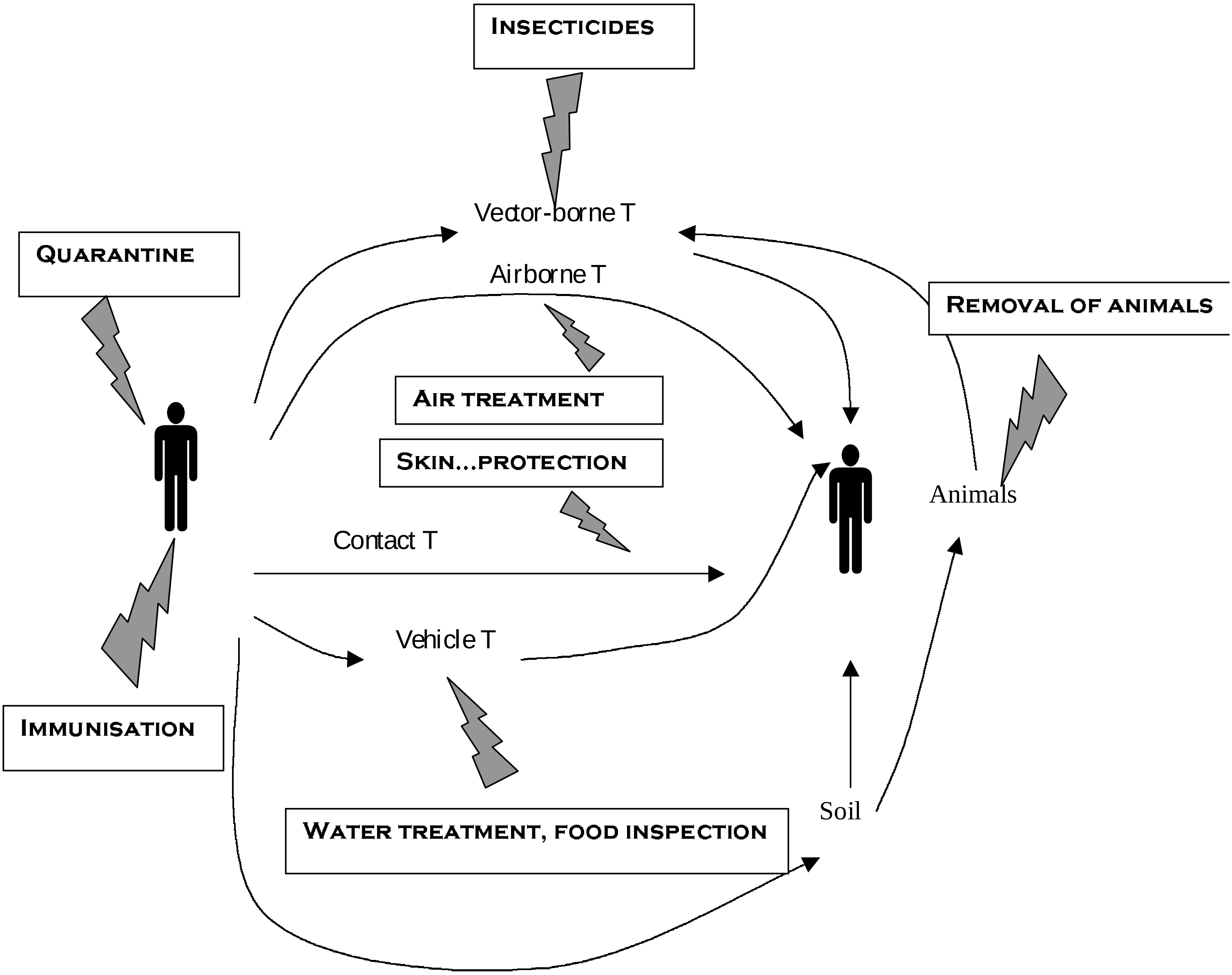


Figure 9 : Control of epidemics

2.4. Nosocomial infections

Nosocomial infection are produced by infectious pathogens that develop within hospital and are acquired by patients.

In USA : 5% of the patients admitted for 4-13 days ; cost : 4.5 billion dollars / year ; 60,000 deaths per year.

Sources :
endogenous sources : patients' own microbiota
exogenous source : animate (staff…) or inanimate (food, urinary catheters, water systems…).

Control :
- aseptic techniques
- proper handling of equipment, supplies, food, excreta
- surgical wound care
- dressings

Bacteriema due to E.coli and Staphylococcus aureus
Burn wounds due to Pseudomonas aeruginosa and S. aureus
Respiratory tract infections due to S.aureus and P. aeruginosa, Legionnella pneumophila
Surgical site infection due to S.aureus and Enterococcus
Urinary tract infections due to E.coli, Enterococcus , P.aeruginosa

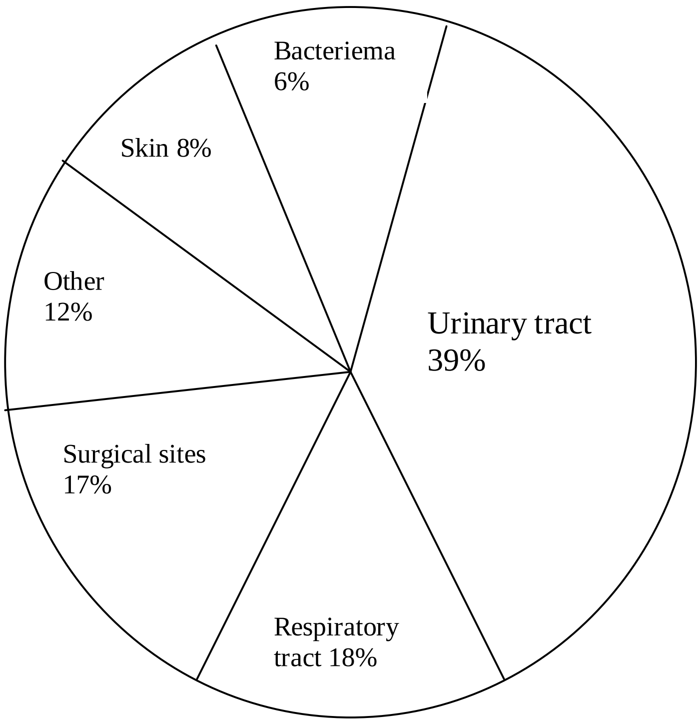


Figure 10 : Nosocomial infections

3. Airborne diseases

3.1. Human diseases caused by viruses

These virus are propelled by respiratory tract by an individual coughing, sneezing or vocalising : table 4

Health statistics : www.medindia.net

Table 3 : Reported cases of measles in India

Year	Cases
1986	155072
1987	247519
1988	157800
1989	162560
1990	87446
1991	79655
1992	29297
1993	65077
1994	56858
1995	26986

Disease	Virus	Transmission	Symptoms	Incubation	Concerned population	Treatment	Vacccine	Statistics
Chikenpox	Family of HerpesV	droplet inhalation	skin disease	10 – 23 days	children 2 – 7 years	acyclovir	attenuated virus	4 milli on cases / year in US
Influenza	3 types of OrthomyxoV ; problem of antigenic shift	droplet inhalation ;	chills, fever, headach, muscular pain ; death if followed by S.aureus infection	1-2 days	everybody	aspirin, amatidine	attenuated virus	21 billion of deaths in 1918
Measles (Rubeola)	ParamyxoV	inhalation	cough, fever, skin eruption : pink lesions ; then permanent immunity	10-21 days	everybody	-	attenuated virus	3,000 / year in US ; 1.5 million people non vaccinated are killed in the world / year
Mumps	ParamyxoV	saliva, respiratory droplets	swelling and tenderness of parotid glands	16-18 days	primarly in schood age children	-	attenuated virus	3,000/year in US
Rubella (German measles)	TogaV	respiratory droplets	eruption of small red spots	12-23 d	5-9 years old children ; very serious for first trimester of pregnancy	-	attenuated virus	1,000/year in US
Variola (smallpox)	PoxV	respiratory droplets	vesicles on the skin ; mortality = 50%	1 month	everybody	-	attenuated virus	eradicated ; virus kept in some labs ; biological weapon ?

Table 4 : Airborne disease due to virus

3.2. Human air borne disease due to bacteria

Most of these bacteria involve respiratory system ; others cause skin diseases : Table 5

Table 5 : Reported cases of Diphtheria in India

Year	Cases
1986	9426
1987	12952
1988	17146
1989	9790
1990	8425
1991	12550
1992	6811
1993	7131
1994	3040
1995	1257

Table 6 : Reported prevalence of tuberculosis in India

Age group (YRS)	Infected (%)
0-4	1
5-9	6.4
10-14	15.4
15-24	31.9
25-34	47.3
35/44	54.8
45/54	60.7
above 55	62.1
total	30.4

Table 7 : Reported incidence of whooping cough in India

Year	Cases
1986	167225
1987	163786
1988	145469
1989	137374
1990	113016
1991	73520
1992	61648
1993	47612
1994	36279
1995	16210

Disease	Bacteria	Transmission	Symptoms	Concerned population	Treatment	Vacccine	Statistics
Diphteria	Corynebcterium diphteriae	droplet inhalation	Fever, cough, mucopurulent nasal discharge, pseudomembrane formation on pharynx, destruction of cardiac, kidney and nervous tissues	Crowded conditions	Penicillin, erythromycin	Diphteria-Pertussis-Tetanus Vaccine	eradication is possible ; 100 cases/year in US
Legionnaires' disease	Legionella pneumophila	droplet inhalation from air conditioning systems and showers	fever, dry cough, headache, neurological manifestation and bronchopneumonia	males over 50 smoking, alcoholism, chronic illness	Erythromicin, rifampicin Water / air treatment	no	30,000/year in US
Meningitis	Streptococcus pneumoniae, Neisseria meningitidis , Haemophilus inflenzae	droplet inhalation from respiratory secretion	initial respiratory illness, sore throat, vomiting, headache, stiffness in the neck	everybody	penicillin…	Against Meningitis due to S.penumoniae and N.meningitidis	20,000/year in US
Mycobacterium pneumonia	M.avium and M. intracellulare	respiratory and gastrointestinal tracts	pulmonary infection	humans, insects, birds ; AIDS patients	clarithromycin coupled with a second drug, as tuberculosis	no	40% of AODS patients in US
Pertussis (whooping cough)	Bordetella pertussis	droplet inhalation	incubation : 7-14 days ; common cold, prolonged cough, final recovery take several months	children	erythromycin…	DPT	95% of the population ; 500,000 deaths/year in the world
Streptococcal disease	S. pyogenes	droplet inhalation	sore throat, impetigo, fever, erysipelas, rheumatic fever, scarlet fever…	everybody	penicillin	no	
Tuberculosis	Mycobacterium tuberculosis	droplet inhalation	incubation 4 to 12 weeks ; fever, fatigue, weight loss, cough, expectoration of bloody sputum	homeless, elderly, malnourished people, AIDS patients	isoniazid + rifampicin, ethambutol, and pyrazinamide for 9 months	no	20% of the population is infected, 10 million new cases par year, 3 million deaths per year

Table 8 : Air-borne diseases due to bacteria

4. Water – borne diseases

Diarrheal diseases are the leading cause of childhood deaths in the world : 10 million per year

4.1. Water – borne diseases due to viruses

4.1.1. Gastroenteritis

4 major categories of viruses : seen in infants 1 to 11 months of age
- rotaviruses
- Norwalk viruses
- caliciviruses
- astroviruses

Transmission : fecal-oral route ; infection occur in winter in contrast to bacterial-caused diarrheal diseases which occur generally in summer.

Physiopathology : virus attacks the upper intestinal epithelial cells, cause malabsotprion, impairment of sodium transport and diarrhea ; generally self limited ; treatment through use oral fluid replacement with isotonic liquid

Virus	Epidemiological characteristics	Clinical characteristics
Rotavirus	Endemic diarrhea in infants worldwide ; 3.5 million and 150 deaths of cases per year in US ;	Dehydrating diarrhea for 5-7 days ; fever ; abdominal cramps, nausea and vomiting common
Norwalk viruses	Epidemic of vomiting and diarrhea in older children and adults ; often associated with infected food	Acute vomiting, headache lasting 1-2 days
Caliciviruses	Pediatric diarrhea associated with infected food	Rotavirus-like in children ; Norwal-like in adults
Astroviruses	Pediatric diarrhea ; reported in nursing homes	Water diarrhea for 1-3 days

Table 9 : Medically important gastroenteritis viruses

4.1.2. Hepatitis A and E

Fecal-oral contamination
Generally , the HAV multiply in the intestine ; occasionally viremia occurs and it spread to the liver ;
Symptoms during 20 days : anorexia, fever, diarrhea, chills and jaundice if the liver is infected
- 30,000 cases / year in US
- Low mortality (<1%)
- Vaccine

Hepatitis E is implicated in many epidemics in Asia… : usually like HAV, self limited

But 10 % of women infected in their 3 last months of pregnancy die of fulminant hepatic failure

4.1.3. Poliomyelitis
Caused by poliovirus, very stable in food and water ; fecal-oral transmission ; multiply in the throat and the intestine ; generally, no symptoms or a brief illness (fever, headache, vomiting)

Table 10 : Reported cases of Poliomyelitis in India

Year	Cases
1986	20169
1987	28264
1988	24257
1989	13866
1990	10408
1991	6028
1992	9390
1993	7576
1994	5881
1995	3406

4.2.2. Water – borne diseases due to bacteria

Symptoms : gastroenteritis ; they can occur in two ways :

- infection : colonisation of tissues and/or secretion of exotoxin
- intoxication when the toxin is ingested by the host : the presence of living bacteria is not required ; these toxins are enterotoxins and they cause nausea, vomiting an diarrhea.

13,600 children per day die from diarrahal disease (500 / year in US)

Organism	Incubation period (hours)	Vomiting	Diarrhea	Fever	Epidemiology	Major food involved
Staphylococcus aureus	1-8	+++	+	-	grow in food, produces enterotoxins	meat, diary, bakery
Bacillus cereus	2-16	+++	++	-	causes vomiting or diarrhoea	reheated fried rice
Clostridium perfringens	8-16	+	+++	-	grow in food ; huge numbers ingested	rewarded meat dishes
Clostridium botulinum	18-24	+	-	-	grow in anaerobic foods and produce toxin	anaerobic food in tins
Escherichia coli (enterotoxigenic strain)	24-72	+	++	-	grow in gut and major cause of traveller's diarrhea	undercooked ground beef, raw milk, water
Vibrio parahaemolyticus	6-96	+	++	-	grow in food and in gut and produce toxin, or invade	seafood, shellfish
Vibrio cholerae serogoupO1 biotype El Tor and 0139in India (endemic)	24-72	+	+++	-	grow in gut and produce toxins ; mortality rate > 50%	water
Shigella spp.	24-72	+	++	+	grow in superficial gut epithelium ; S. dysenteriae produces toxins	eggs products, pudding
Salmonella spp.	8-48	+	++	+	grow in gut	meats, poultry, fish, eggs, dairy products, water
Salmonella typhi	10-14 days	+	+	++	invade gut, lymph nodes, liver, spleen and gallbladder	
Clostridium difficile	7days	-	+++	+	Antobiotic-associated colitis	water
Campylobacter jejuni	2-10 days	-	+++	++	infection by pral route from food, pets ; grow in small intestine	water
Yersinia enterocolitica	4-7days	+	++	+	fecal-oral transmission ; food-borne ; infected animals	milk, meat products

Table 11 : Bacteria that cause acute diarrhoeas and food poisonings

4.2.2.1. Cholera

2 serogroups can cause epidemics :
- Serogroup O1 divided in two biotypes : classic and El Tor ; El Tor caused epidemics since 1961
- and Serogroup O139 emerged in India in 1992

Cholera is endemic in India
Mortality rate > 50%
The bacteria adhere to the small intestine where they are not invasive but secrete cholera toxin ; it stimulates hypersecretion of water ; the diarrhea can be so profuse than a person can lose 10 to 15L of fluid during the infection ; death may result from the elevated concentration of blood proteins, which leads to circulatory shock and collapse.

Table 12 : Notified cholera cases and deaths in India (www.medindia.net)

Year	Cases	Deaths
1950	176307	86997
1960	14167	5250
1970	17268	3801
1980	8717	309
1990	3704	87
1994	4958	32

4.2.2.2. Botulism

Clostridium botulinum is found in soil and aquatic sediment ; the source of infection is home-canned food that have not been heated sufficiently to kill the bacteria endospores ; the endospores germinate and a toxin is produced during vegetative growth.
The endospore are naturally present in honey and can germinate in infant intestine.
Symptom : flaccid paralysis
Mortality rate = 33% from respiratory failure
Treatment : antitoxin
100 cases per year in US
Prevention :
- safe food-processing practices
- educating the public on safe home-canning methods for food
- not feeding honey to infants younger than one year old.

4.2.2.3. Campylobacter jejuni gastroenteritis

50% of chicken secrete this bacteria ; it is also present in surface water.
Transmission : fecal-oral, ingestion
2 million cases of GE per year in US
Invade small intestine, secrete exotoxin : diarrhoea, fever, severe inflammation of the intestine with ulceration and bloody stools (feces)
Self limited disease, antibiotic in severe cases
Prevention : good personal hygiene and food handling precaution

4.2.2.4. Salmonella gastroenteritis
More than 2,000 subspecies

Most reported is Salmonella typhimurium , acquired from infected food (water, eggs, poultry…)
in US : 2 to 3 millions of cases per year
The bacteria invade the intestinal mucosa and secrete enterotoxin and cytotoxin that destroy epithelial cells : cramps, diarrhea, vomiting, fever for 2 to 5 days
Most adult patient recover.
Tretament by fluid replacement
Prevention by good food-processing…

4.2.2.5. Shigellosis or bacillary dysentery
Shigella sonnei and flexneri
Most prevalent among children 1 – 4 year olds
30,000 cases / year in US
500,000 deaths a year in the world
Bacteria multiply in the colon epitehlium, produce both endotoxin and exotoxins
The watery stools contain blood, mucus, pus
Disease self limited for adults ; maybe fatal for children
Prevention in good food-processing, water treatment

4.2.2.6. Traveller's diarrhea and E.coli infections

4 subspecies :
ETEC enterotoxigenic
EIEC enteroinvasive
EHEC enterohemorrhagic
EPEC enteropathogenic

ETEC : secretion of two toxins, same mechanism than cholera toxin ; responsible for many diarrhea among infants in developing countries

EIEC is responsible for a dysenteric syndrome, like Shigella

EHEC : hemmoragic colitis, abdominal pain, cramps, bloody diarrhea ; caused by E.coli O157 : H7 : 20,000 cases and 250 deaths a year in US

EPEC : important cause of diarrhea in children residing in developing countries.

Diagnosis of traveller's diarrhea caused by E.coli is based on past travel history and symptoms ; laboratory diagnosis is isolation of the specific type of E.coli from feces and identification using DNA probes. Treatment is with fluid and electrolytes plus doxycycline and trimethroprimsulfamethoxazole. Recovery is usually without complications ; prevention and control involve avoiding contaminated food and water.

4.2.2.7. Typhoid fever
Caused by Salmonella typhi ; fecal – oral transmission.
Once in the small intestine, the incubation period is about 14 days ; the bacteria colonize the small intestine, spread in the blood, liver and gallbladder
Symptoms include fever, abdominal pain, headache, anorexia, malaise, several weeks.
After 3 months, most individuals stop shedding bacteria in their feces.
However, few individuals continue to shed the bacteria but show no symptoms (carriers)

Treatment with antibiotics
Prophylactic measures : water treatment, prevention of food handling by carriers, complete isolation of patients
Vaccine for high-risk individuals
in US : 500 cases a year

4.3. Parasitosis

This chapter is regarding parasitosis linked to water quality : either the parasites or the vector live in or near water

4.3.1. Human diseases caused by protozoa
A survey in rural population of a hill district in North India revealed the presence of parasitic infection in 66 percent of the population
150 millions cases of malaria in the world / year
In Africa, 1 million of children / year die under the age of 14
8 million cases of trypanosomiasis
12 million of leishmaniasis
55 million of amebiasis yearly

4.3.1. 1.Amebiasis
Due to Entameba histolytica responsible for amebic dysentery
100,000 people die per year
This very common parasite is endemic in warm climates where adequate sanitation and effective personal hygiene is lacking
Prevalence : 10% in developing countries

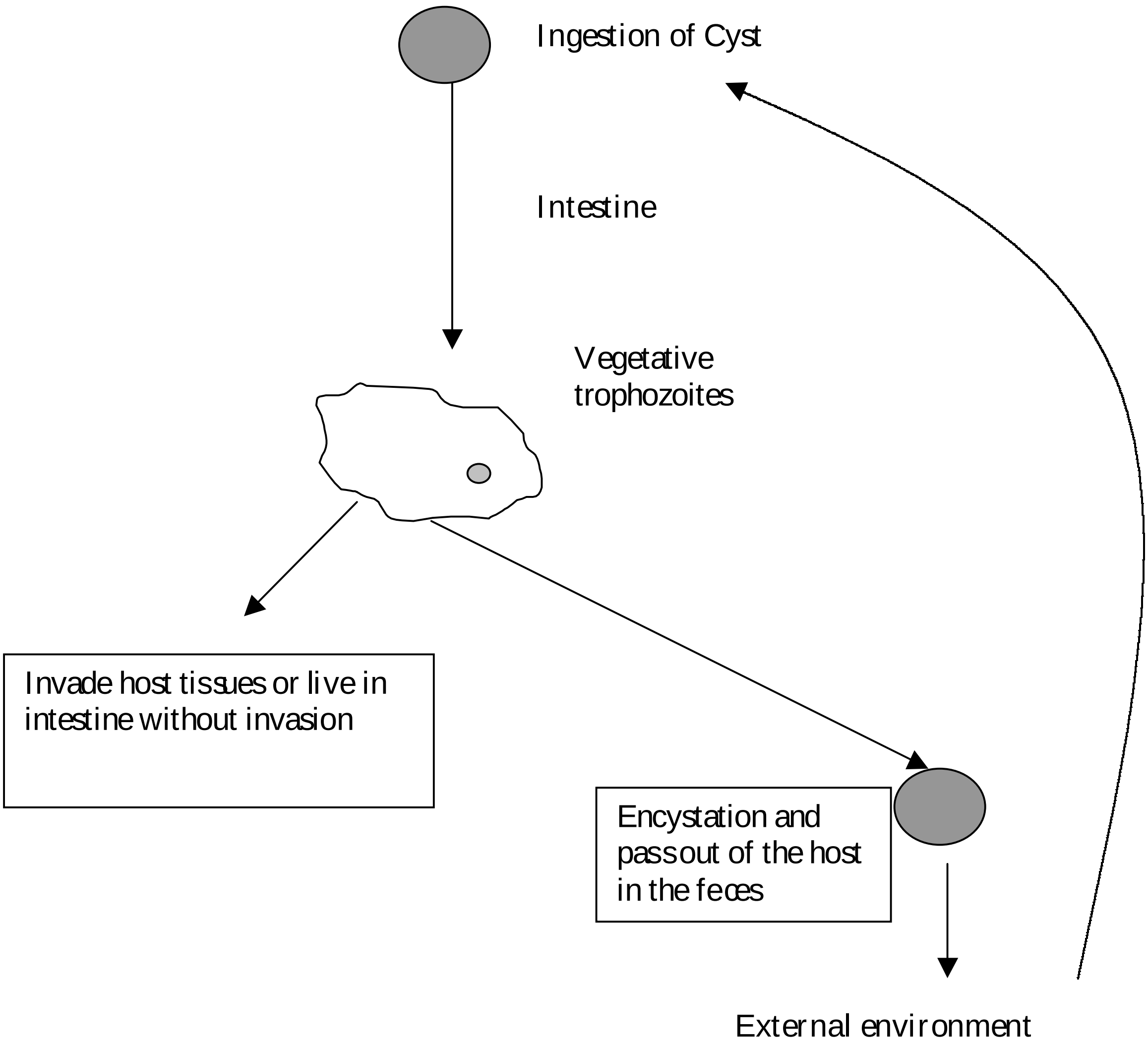


Figure 11 : Cycle of ***Entamoeba histolytica***

Symptoms :
- diarrhea with blood and mucus
- abcesses in the liver, lungs or brain

Therapy : iodoquinol or amebaquin for carriers and Flagyl for symptomatic intestinal amebiasis

Prevention : water treatment ; hyperchlorination or iodination

4.3.1.2. Cryptosporidiosis

Cryptosporidium parvum
Found in 90% of sewage samples, 75% of river waters and 28% of **drinking waters** ; its cycle seems amibiase cycle (the vegetative form invade enterocyts, and the cysts are excreted in feces (5 µm in diameter, can't be removed by classical sand filters, resistant to chlorine)
- low infection dose : 10 to 100 cysts
- remains viable 6 months in a moist environment

Symptoms : diarrhea cholera-like, fever, abdominal cramps

No therapy : rehydratation ; severe for AIDS

4.3.1.3. Giardiasis

Giardia lamblia

in US, 7% of the population is healthy carrier
Prevalence : 11%in India
Transmission by cyst-contaminated water supplies
Amoebia – like cycle
Acute : diarrhea, cramps, anorexia
Diagnosis : identification of trophozoïtes or cysts in stools
Treatment : Flagyl, Atabrine for adults furazolidone for children
Prevention : use of slow and filtration in drinking water production

4.3.1.4. Malaria
Sporozoa *Plasmodium falciparum*
Endemic around the equator
In Africa : 100 million people infected and one million die annually
Vector - borne transmission
Cycle :
1. the parasite infects the blood of a mosquito : Anopheles **: Vector**
2. the Mosquito injects *Plasmodium* in human blood, which migrate to the liver and then to the erythrocytes
3. In erythrocytes, the Plasmodium multiplies, every 48 and 72 hours, infecting other erythrocytes and releasing toxins generates chills and fever characteristic of malaria.
4. Occasionally, another mosquito injects blood and Plasmodium , and when it bites another human **host**, the cycle continues

Anaemia, , hypertrophy of spleen and liver.
Diagnosis : made by demonstrating the presence of parasites within stained erythrocytes.

Treatment : chloroquine…

Table 13 : Malaria situation in India

Year	Annual parasite Incidence per thousand population	Positive cases in Million
1976	11.25	6.47
1985	8.52	1.86
1990	2.57	2.02
1995	3.19	2.8

4.3.2. Human diseases caused by metazoan pathogens

Most of metazoan parasites cycles transit to water and concern worms (helminthiasis)

This chapter is regarding the most frequent parasitosis linked to water quality, and occurring in India : nematodiasis

Disease	Parasite	Vector	Transmission	Symptoms	Prevalence (www.midcoast.com)	Diagnostic / Treatment	Prevention
Ascaridiosis	Ascaris lumbricoidis	-	water (fecal – oral)	Cough and then abdominal cramps, diarrhea, vomiting, surgical complications	around 30% in India	Observation of eggs in stools / Solaskil, Zentel, Fluvermal…	good food-processing installation of sewers, water treatment…
Hookworm disease	Ancylostoma duodenale and Necator amireicanus	-		Alternance diarrhea/ constipation and then anaemia, development perturbation for children	around 40% in India		
Dracunculosis	Guineaworm : Dracunculosis medinensis	Soft –water shellfish : Cyclops (3 mm)	Vector and Vehicle- Borne (water)	Hypodermic infestation	153,000 / year in the world 2185 cases in India in 1991	Manual extraction of the worm	Water filtration

Table 14 : Most frequent parasitosis occuring in India and due to nematodes

5. Detection techniques of micro-organisms in water

5.1. Total micro-organisms count in drinking waters

5.1.1. Principle

Field of study : drinking water
Guide level in drinking water : 10 / mL at 37°C and 100/ mL at 22°C
Indicates a global microbiologic quality of the drinking water ; all type of water contains a lot of micro-organisms ; their determination indicates the quality of water supply system. ; an increase of the micro-organism concentration indicates a contamination of the water supply network, or of the raw water.

The concentration of micro-organisms in water is determined by counting colonies after development on a growth media, in aerobiosis, and incubation at 36°C during 22 h and at 22°C during 72h . These micro-organisms are bacteria, yeast and mould.

5.1.2. Procedure

Samples of tested water must be taken in sterile recipients, with all kind of necessary conditions of asepsis ; they must be then conserved at low temperature (between 2 and 10°C) and water must be analysed before 12 hours after their conditioning.
If the concentration of micro-organisms is too high, carry on dilution (serial dilutions of one mL of water in 9mL of dilution black)
Pour-plate technique :

Placenutrient agar deeps into the boiling water bath for melting.
Remove this tube and cool to 45°C .
Introduce, with a sterile transfer pipette, 1 mL of the sample of tested water in the bottom of a sterile Petri dish

Pour the liquid agar medium (40 to 50 °C) in this Petri dish and rotate gently in order to obtain a regular repartition of the colonies ; harden the medium on a horizontal and cold surface
Label the side of the Petri dish (name, date, temperature of incubation).
Turn over the Petri dish and incubate your pour-plate at 22°C during 72h or at 36°C during 24h.

Numeration of the colonies:
Consider the Petri dish which present less than 300 colonies.

Each colony has been formed from the development of a unique cell.

If N is the number of colonies counted in a Petri dish containing the dilution 10^n :

C (microorganisms / mL) = $N / 10^n$

Ex :
a) 102 colonies counted in the Petri dish seeded with non diluted sample of tested water :
$C = 102 / 10^0 = 102$ microorganisms / mL
b) 68 colonies counted in 10^{-4} dilution plate (1 : 10,000) :

$C = 68 / 10^{-4} = 680{,}000$ microorganisms / mL

☞ one colony can be formed from the development of two cells or more: sometimes, the viable number is expressed in terms of colony forming units (CFUs) : 102 or 680,000 CFUs / mL.

☞ if you seed two agar plates per dilution, you can add the number of colonies and divide this number by <u>the sum</u> of the poured volumes:

Dilution / poured volume equivalent (mL) for one plate	Number of colonies in plate 1	Number of colonies in plate 2	conclusion
10^0 / 1	Too numerous to count	Too numerous to count	Non valid (>300)
10^{-1} / 0.1	332	303	Non valid
10^{-2} / 0.01	45	51	96
10^{-3} / 0.001	6	4	10
10^{-4} / 0.0001	1	1	2

C = [96+10+2] / (0.02 + 0.002 + 0.0002) = 4865 CFUs / mL

<u>5.2. Numeration of Fecal contamination indicators : thermoduric and total coliform and streptococci in drinking water by Filtration technique</u>

<u>5.2.1. Generalities</u>

The intestinal flora includes several germs, indicated following this decreasing predominance :
- rod shaped Gram negative bacteria, anaerobic and called bacteroids
- the genus Bifidobacterium (rod shaped, Gram positive, anaerobe)
- genus Clostridium (cocci, gram positive and sporulating) and lactic bacteria

These three kind of bacteria are difficult to grow in laboratory.
- the specie E.coli
- the genus Enterococcus

These two types of bacteria are easy to grow
And finally,
- enterobacteria (like Citrobacter), genus Staphylococcus, genus Bacillus, yeasts…

FCI are commensally of the intestine ; when they are present in water, there is a risk of contamination due to pathogenic bacteria like Salmonella, Shigella, Vibrio cholerae…
Fecal contamination is due to the discharge of waste water in surface water ; MWW contain 10^6 to $10^è$ bacteria / mL.

There are three categories of FCI :
- total coliforms, thermoduric coliforms and E.coli :they are commensally of the intestines and can't survive for a long time in water ; E.coli is the main representing of thermoduric coliforms
- fecal streptococci (Enterococcus faecalis) , also commensally of the intestine, but can survive longer in water : they are the indicator of an older contamination
- sulphite reducing Clostridium, which are the less reliable FCI because they can also live like saprophytes in water.
The most reliable FCI in water are thermoduric coliforms.

5.2.2. Principle and definitions

Coliforms :
Micro-organism growing at 37°C on lactose-bile salt containing agar (called tergitol 7 TTC agar), acid producing in 24h
and oxidase negative .
. The selective agar medium Tergitol 7 TTC contains molecules that inhibit cocci gram positive growth (like streptococci…)

Thermoduric coliform :
Same proprieties than coliforms, but at 44°C.

There are two steps : presumption andthen confirmation
- growing ontergitol 7 TTC
- inoculating each colony from Tergitol 7 on nutritive agar in order to examine the presence of oxidase :

Streptococci :
Micro-organism growing at 37°C on glucose- azide containing agar (called Slanetz Bartley agar), generating typical TTC reducing colonies, and generating positive reaction in 24h at 37°C on esculine –bile containing agar. The selective agar medium Slanetz Bartley contains molecules that inhibit rod shaped gram negative growth (like coliforms…)

5.2.3. Operation

First day :
- Prepare aseptically the filtration apparatus and the membrane : page 1 and page 2 : figures 1 to 5 (but no absorbent pad)
- filtrate 100ml of water sample
- place the membrane : (figures 6 to 9 page 2 but between figure 7 and 8, rinse the funnel with sterile water)

- on Tergitol 7 TTC agar plate and incubate at 37°C in order to analyse total coliform
- on Tergitol 7 TTC agar plate and incubate at 44°C in order to analyse thermoduric coliform
- on Slanetz Bartley agar plate and incubate at 37°C in order to analyse fecal streptococci

Duration of incubation : 24h

Second day : Count positive colonies :
- on Tergitol 7 : yellow colonies surrounded by a yellow halo
- on Slanetz : red pink or brown colonies

5.2.4. Results

Each colony is issued from one bacterium growth :

Bacteria concentration = Number of colonies present on selective medium / Volume of filtrated water

5.3. Numeration of FCI in surface water by MPN technique

5.3.1. Principle

FCI are researched in surface water as contaminants issued from waste water or other contaminated discharge ; these waters are turbid and the numeration can't be performed by means of filtration.
The following statistic method is applied :
Sets of sterile nutritive containing tubes are inoculated by different serial dilutions of the tested water ; once a high enough dilution is inoculated, only one bacterium is introduced in the tube, and after incubation, the growth is detected as the medium aspect turns (becomes turbid…)

The further dilutions don't incorporate any bacterium in the tubes and they remain sterile.

At the end of the incubation, we note the number of positive tubes, per dilution.

A statistic table, called Mac Grady Table, and by means of the precedent result, indicates us a number, the MPN ; a formula allows us to calculate the concentration of bacteria in our sample.

Example of result : inoculation of set of three tubes with serial dilution of the sample

Dilution	10^1	10^2	10^3	10^4	10^5	10^6
Result : number of positive tubes	3	3	2	1	0	0

↑ Highest dilution of the three

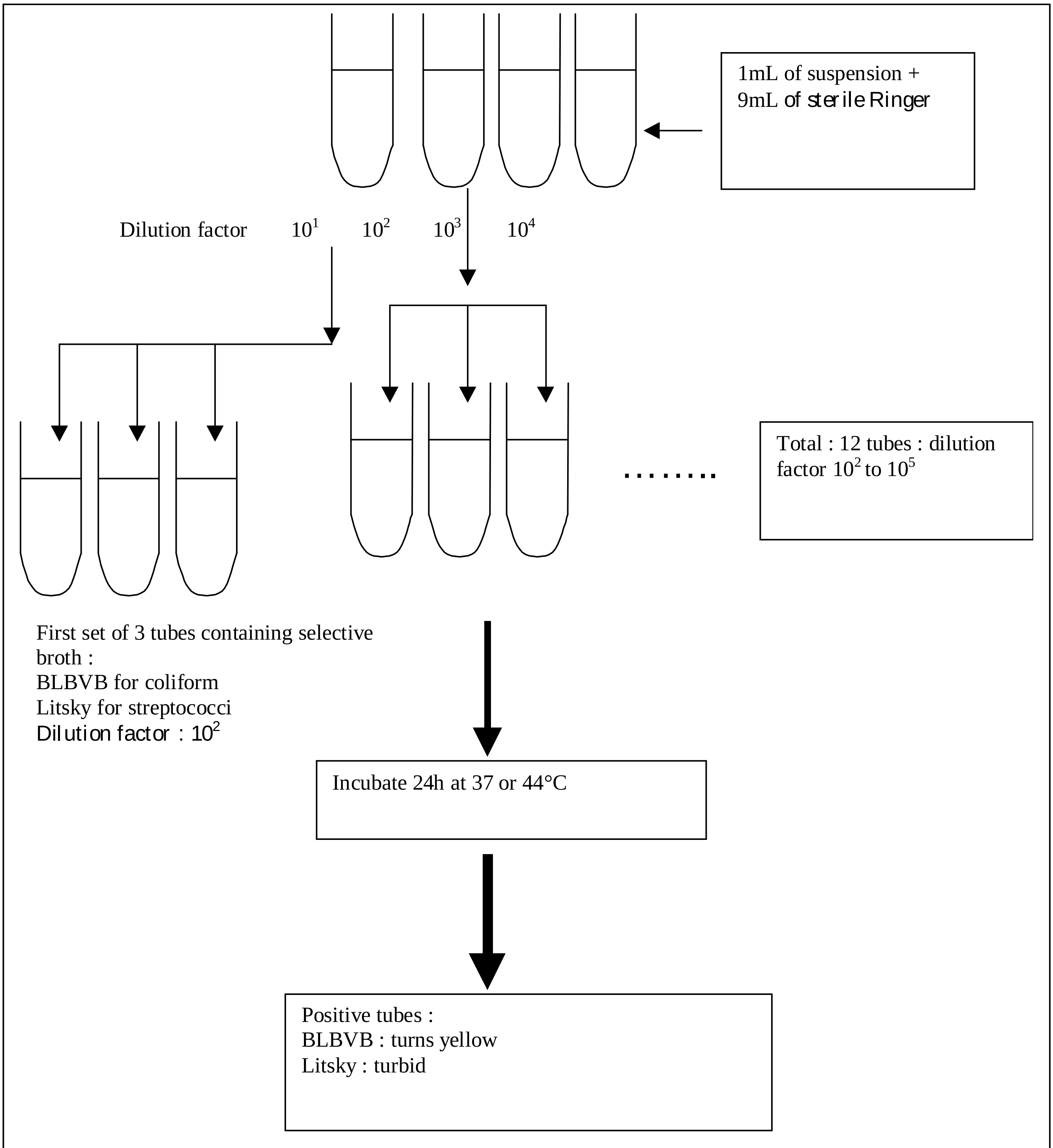
Characteristic Number = 321 (see the Mac Grady Table rules)

For 321, the Table indicate
MPN = 15
Then the concentration is $15x10^{\text{higest dilution}}$ / 100 mL = $15x10^{4}$ / 100mL. (150,000)

The used medium for determination of coliform by MPN technique is called BLBVB ; this selective broth contains chemicals that inhibit cocci Gram positive growth (like streptococci…)
In the same way, selective Litsky broth contains chemicals that inhibit rod shaped Gram negative (like coliforms…) growth.
There should be two steps : presumption and confirmation ; we only use confirmative broth.

5.3.2. Operation

Carry out a first set of water sample dilutions by means of the 10 tubes containing 9mL of sterile Ringer :
Page 1 : pipetting helpers and how to use a pipette
Page 2 : the use of a pipette pump
Page 3 : General steps in a dilution procedure (the method, i.e. Serial dilution and spread technique, will not be used for this lesson)



<u>Figure 12 : Numeration of FCI in surface water by MPN technique</u>

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