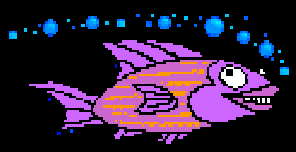
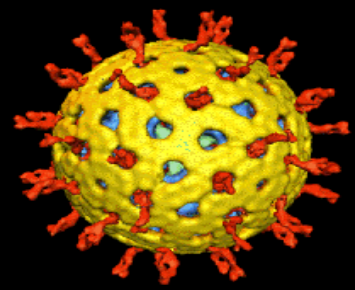


Microbiological Risk Assessment: Meat and Poultry Products



K.N. Bhilegaonkar

Division Of Veterinary Public Health

Indian Veterinary Research Institute

Izatnagar

I V R I

- Introduction: Microbial food safety

- Microbial Risk assessment steps with examples
  - –Hazard identification
  - –Hazard characterization
  - –Exposure assessment
  - –Risk characterization

- Appropriate level of protection, Food safety Objective

- Avian influenza

Food Safety

- Increased global trade
- Discerning and knowledgeable consumer

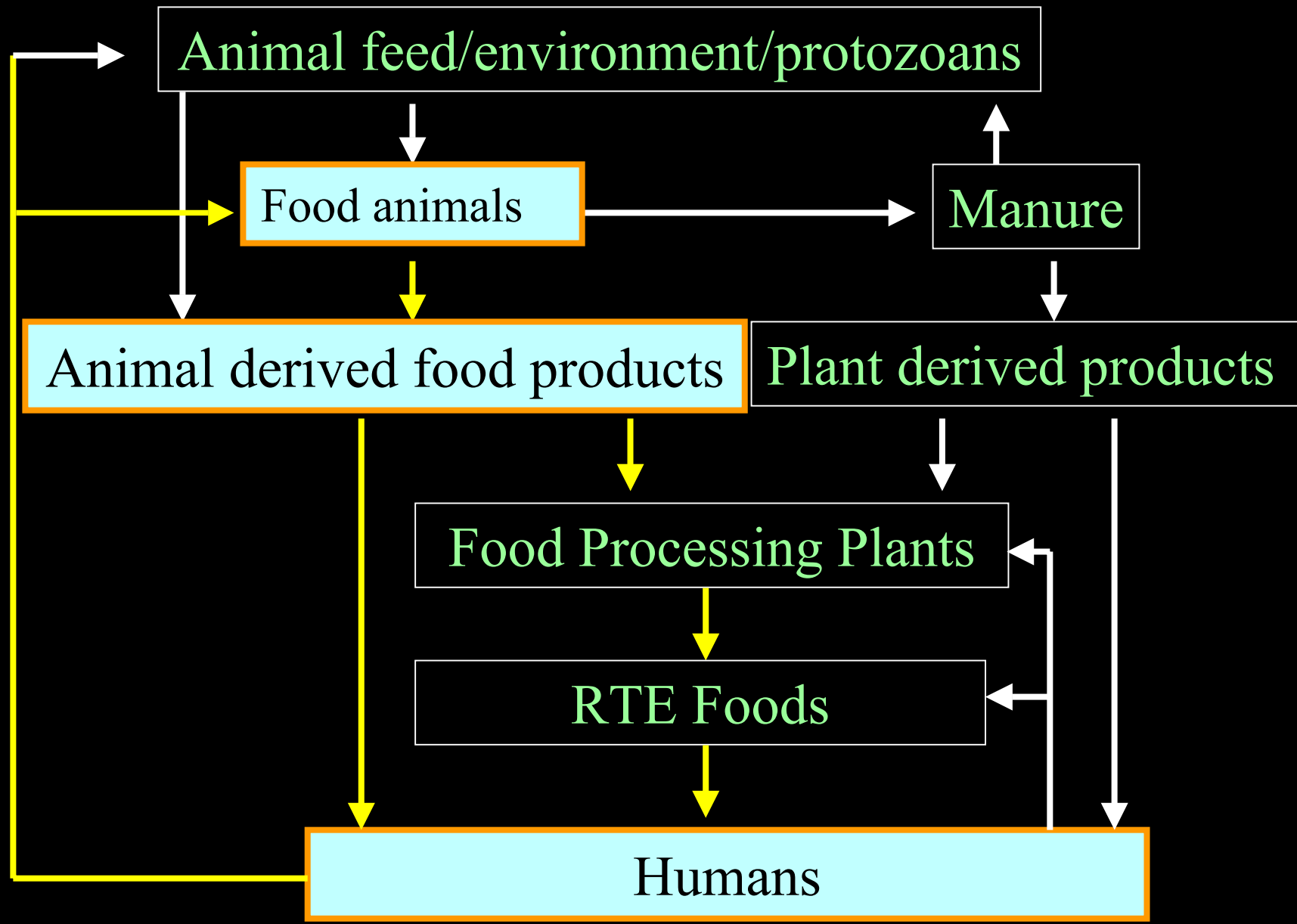
→ Important issue

- BSE & nv CJD: UK
- Melamine in milk/milk products: China
- Pesticide residues in cold-drinks: India
- Avian Influenza: India and Asian countries

Microbial Food Safety

- Foodborne illness : Global magnitude difficult to assess
  - Deaths due to diarrhoeal diseases in 2005: 1.8 million
  - Developed countries: 30% population (INFOSAN WHO 2008)
- USA: 76 million cases of foodborne diseases
  - 325,000 hospitalizations
  - 5,000 deaths
- India: Magnitude is unknown
  - Diarrhoeal diseases (Food and waterborne) : 18.6 million children under age of 5
  - 3,86,000 deaths in children (1 in every 5 global deaths in children)

Transmission of Foodborne Diseases



I V R I

Microbial Risk Assessment

I V R I

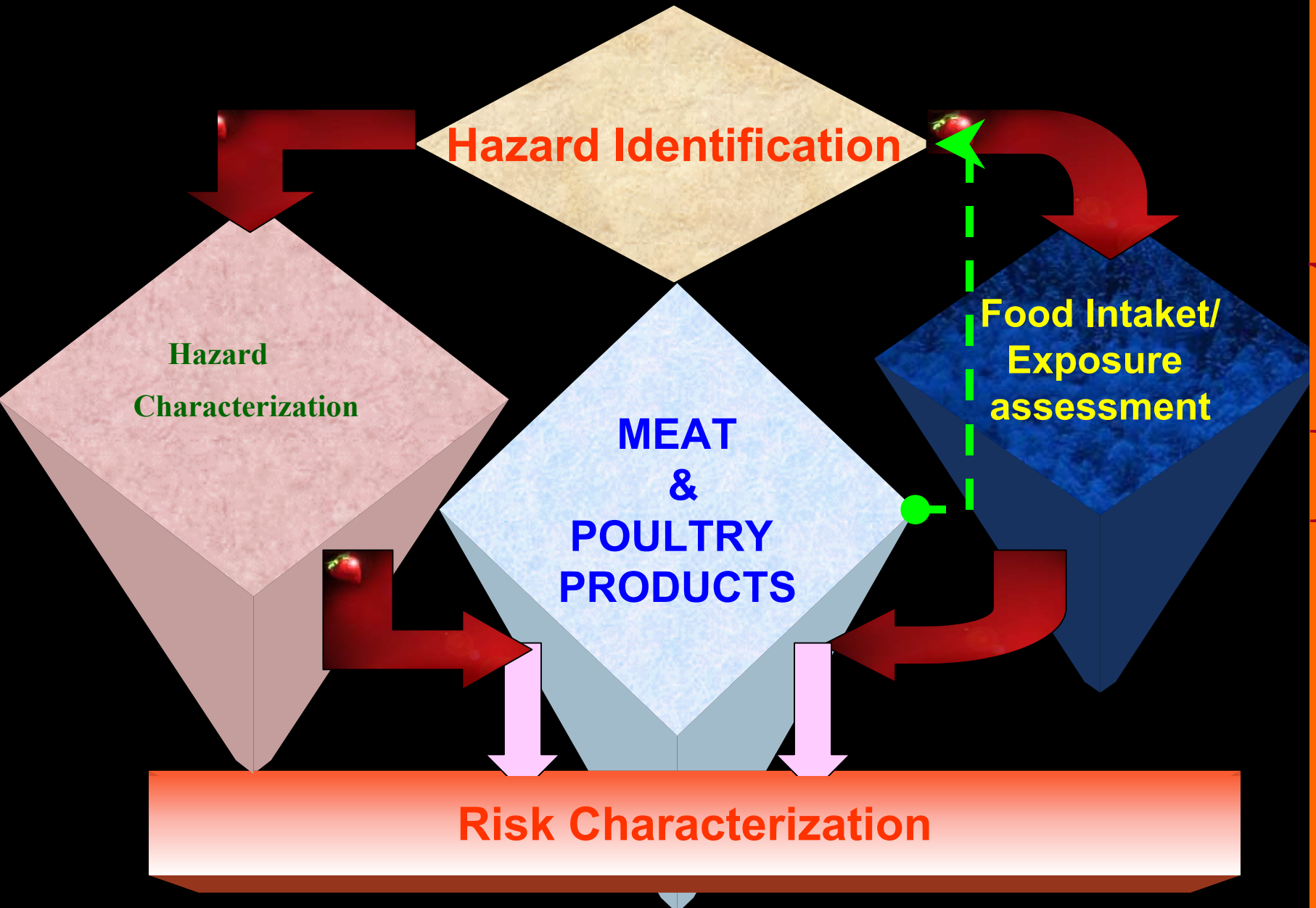
Microbial Risk Assessment

- A powerful tool for management of food safety
- Properly designed MRA: Objective and systematic evaluation of information
- Helps risk manager to take informed decision on food safety issue
- 1999: CCFH adopted principles and guidelines for the conduct of MRA
- Several Pathogen: commodity MRA : FAO & WHO, developed nations
  - *Salmonella* in eggs
  - *Listeria monocytogenes* in cheese

MRA: India

- No systematic MRA studies : Meat and poultry products
- Authentic and exhaustive base line data not available
- Vast country: Diverse culture/ethnicity
- Tremendous variations in eating habits and preferences
- Variations in risk patterns

Risk Assessment steps



IVRI

V
R
I

Hazard Identification

- **What are agents present in food?**
  - ***Salmonella* spp.**
  - ***Listeria monocytogenes***
  - ***Aeromonas* spp.**
  - **STEC**
  - ***Campylobacter* spp.**
  - ***Staph. aureus***
  - **Rotavirus**

Food-agent relationship

Food-agent relationship!

- *Campylobacter Jejuni*- Poultry
- *Listeria monocytogenes*: Cheese/milk products
- *Clostridium perfringens*: Meats
- *Aeromonas*: Fish

- No systematic surveillance studies in India
- Several scattered reports: but gives fair idea

Salmonella

- Prevalence in meat and poultry: 5 - 7%
- RTE meats and poultry products: 0 - 3%

Western World: Important pathogen

Indian context: Prevalence of non-typhoidal *Salmonella* less

- 2541 serotypes
- > 128 serotypes present in India
  - New added every year

IVRI

Salmonella from food of animal origin

Agarwal (2009)

Type of food sample	No. of samples examined	No. positive	(%) positive
Pork sausage	241	15	**6.22**
Cocktail sausage	208	9	**4.32**
Oxford sausage	33	1	**3.03**
Cooked ham	319	20	**6.26**
Ham garlic (salami)	346	18	**5.20**
Hot dog	137	3	**2.18**
Frankfurter	218	7	**0.03**
meat pie	75	8	**10.66**
Pork kebab	161	2	**1.24**
beacon	146	3	**2.05**
Luncheon meat	149	2	**1.34**
Fresh pork meat	132	12	**9.09**
Kofta	27	2	**7.4**
Total	2096	102	**4.86**

Foods	Samples examined	Samples positive	Percent positive
Goat Liver	50	0	0
Spleen	50	0	0
Lymph node	52	0	0
Fresh muscle	662	18	2.89
Total :	**814**	**40**	**4.91**

Foods	Samples examines	Samples positive	Percent positive
Sheep Mutton, shammi kebab	80	7	8.75
Mutton keema	21	1	4.76
Other products	18	0	0
Total :	**119**	**8**	**6.72**

Agarwal (2009)

Foods	Samples examined	Samples positive	Percent positive
Poultry Dressed frozen chicken	76	7	9.21
Poultry meat	209	21	10.04
Chicken-N-ham	119	3	2.52
Chicken sausage	75	0	0
Chicken salami	81	0	0
Chicken kebab	68	3	4.41
Chicken frankfurter	37	0	0
Egg	319	2	0.62
Other products	42	2	4.76
Total :	**1061**	**39**	**3.67**

Agarwal (2009)

Foods	Samples examined	Samples positive	Percent positive
Milk/milk product Raw milk	36	0	0
Dry milk powder	83	3	3.61
Milk chocolate	140	1	0.07
Khoya sweet	5	0	0
Cheese	51	0	0
Total :	**315**	**4**	**0.12**

Agarwal (2009)

E. Coli Prevalence

- Numerous reports
- One of the most Important foodborne pathogen in Indian context
  - Meat and poultry products : 20-50%; up to 100%
  - Human diarrhoea
  - Animal diarrhoea

- STEC (EHEC): Isolations in recent years
  - Animals : Important reservoirs
  - Isolations from meats : Buffalo, sheep, goat, kebabs, sausages

L. monocytogenes

Source	Prevalence	Place	Reference
Buffalo meat	< 10%	Gujarat	Brahmbhatt and Anjaria (1993)
Goat meat	< 10%	Bareilly	Banurekha *et al.*, (1998)
Goat meat	6.66%	Bareilly	Barbuddhe *et al.*, (2000)
Sheep meat	7.4%	Bareilly	Barbuddhe *et al.*, (2000
Various meats produts	3 - 8%	Bombay	Waskar (2005)
Poultry meat	8.5%	Nagpur	Kalorey *et al.*, (2005)

Campylobacter

Poultry : Most important transmitters

- Isolations from poultry meat: 20 - 50% up to 100%
- Other meats: 3 - 5%

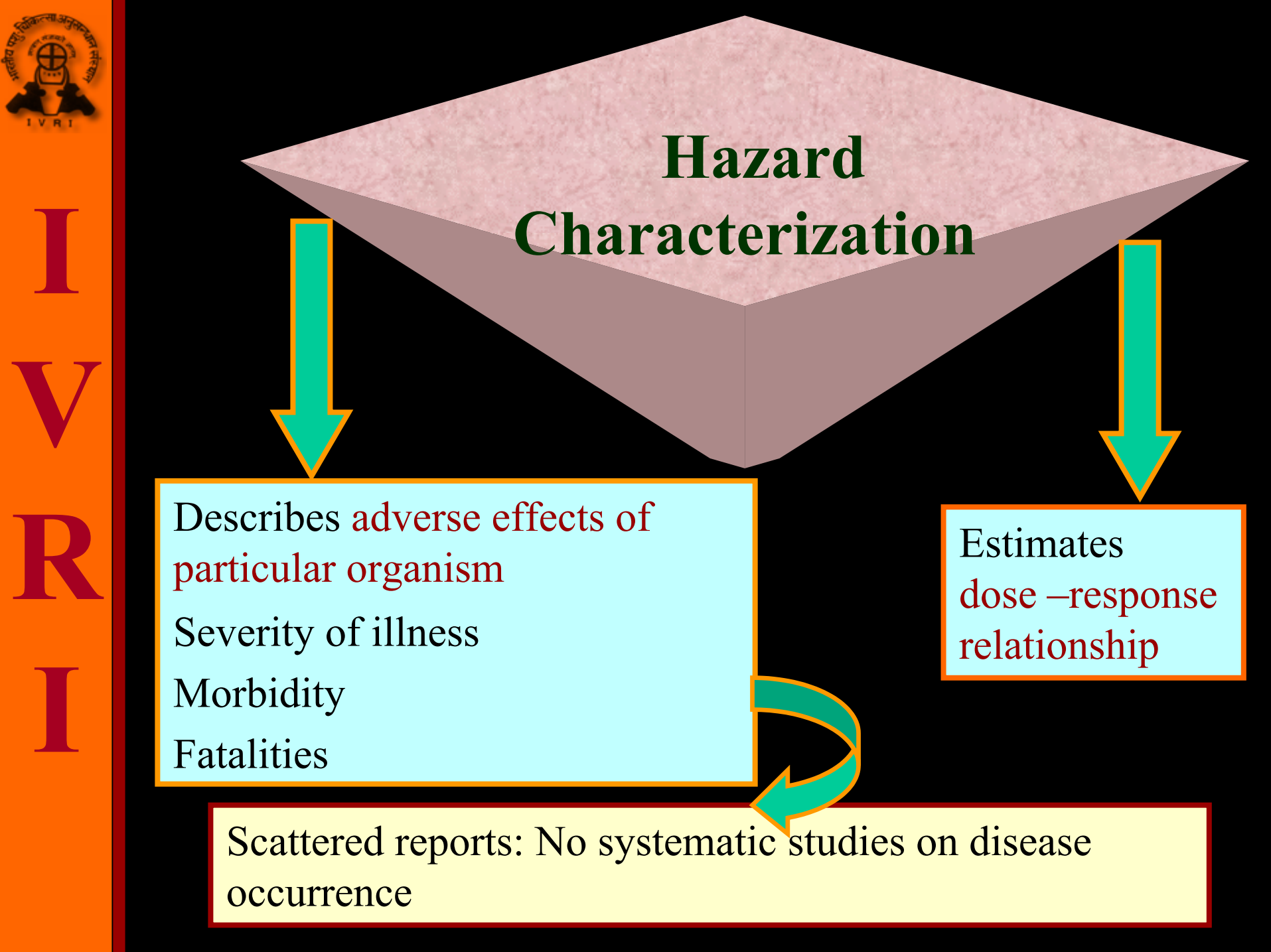
IVRI

Aeromonas

- Pathogen found in aquatic environment
  - Saline & brackish water
  - Drinking water
  - Treated & un-treated sewage
  - Abattoir waste water
  - Colonize slow sand filters
- Fish – Major source
- Poultry, Mutton, beef, milk, etc. are also found to be contaminated

Aeromonas: Prevalence in meats

Source	Prevalence	Place	Reference
Mutton	24-37%	Hisar	Khurana and Kumar (1997)
Poultry	32-38%	Hisar	Khurana and Kumar (1997)
Poultry meat	16%	Bareilly	Kumar (1998)
Poultry meat	16%	Bareilly	Ghatak (2005)
Eggs	12-22%	Bareilly	Agarwal (1997) Kumar (1998)
Goat meat	12%	Bareilly	Kumar (1998)
Various RTE meat products	14%	Bombay	Waskar (2005)


Hazard Characterization
Describes adverse effects of particular organism
Severity of illness
Morbidity
Fatalities
Estimates dose –response relationship
Scattered reports: No systematic studies on disease occurrence

Salmonella

- Prevalence in human (non typhoid) : 1 - 5%
- Prevalence in healthy carriers: 1 - 2%

L. monocytogenes : Prevalence in Human

Source	Prevalence	Place	Reference
Abortion (150 patients)	14%	Mumbai	Krishna *et al.*, (1966)
Abortion still births	3%	-	Bhujwala *et al.*, (1973)
Abortion	3.3%	Northern India	Kaur *et al.*, (2007)
Abortion	10%	Nagpur	Kalorey (2008)
Abortion	6%	Goa	Barbuddhe (2008)

Prevalence of STEC in Human

Source	No. of *E. coli*	STEC	Place	Reference
Diarrhoeal patients	1338	9	New Delhi	Pamchandran and Verghese (1987)
HUS patients	25	19	New Delhi	Kishore *et al.*, (1992)
Haemorrhagic enteritis	240	18	Bareilly	Kapoor *et al.*, (1995)
Diarrhoea and UTI infection	67	15	Bareilly	Banerjee *et al.*, (2001) & Ratore (2000)

Campylobacter

- Human diarrhoea: 10 - 15%
- Many asymptomatic carriers

- Industrialized countries: Manifestations are severe
- In Asian countries: Symptoms are milder.

Role in GB syndrome : needs to be explored

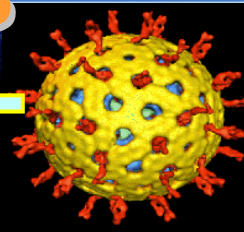
Aeromonas

- Emerging pathogen of importance : Dairrhoea
- Implicated in extra intestinal infections

Source	Prevalence	Place	Reference
Diarrhoea	6.5%	Chennai	Komathi *et al*., (1998)
Diarrhoea	8%	Kolkata	Chaterjee and Neogy (1972)
Diarrhoea	0.2%	Vellore	Jesudasan and koshi (1990)
Diarrhoea	6.5%	Bareilly	Ghatak (2005)



Rotavirus



- Major cause infant and children diarrhoea
- 100,000 to 150,000 deaths in children

(Broor *et al.,* 2003)

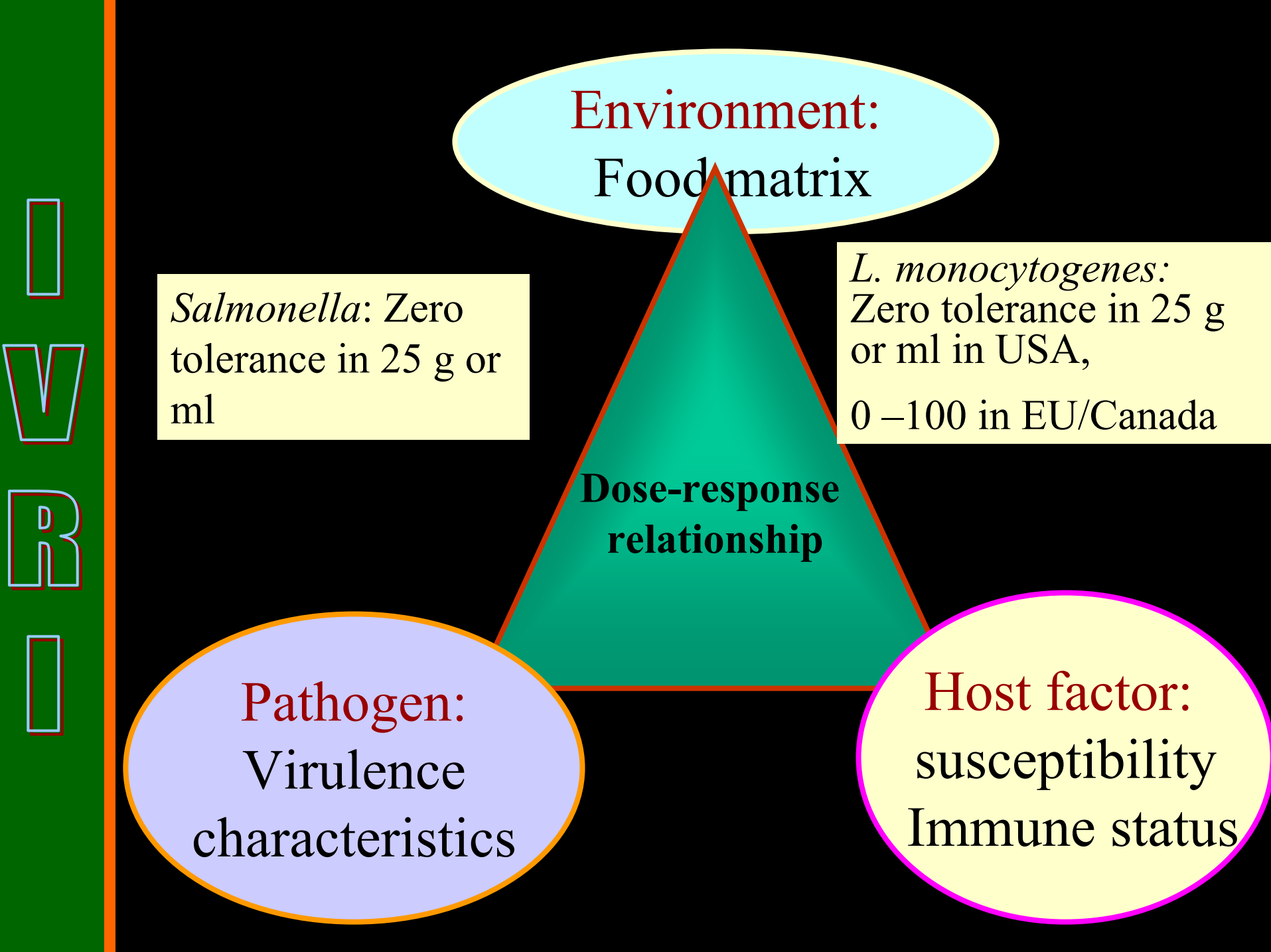
- Prevalence in diarrhoeal cases: 5-71%
  - In acute diarrhoea : 20-30%

☛ Incidence in animal diarrhoea: 10 -50%

  ☛ Cattle, goats, sheep, pigs

- Source studies in relation to food: Lacking
  - Occurrence in food : difficult to study

I V R I


Environment:
Food matrix
Salmonella: Zero tolerance in 25 g or ml
L. monocytogenes: Zero tolerance in 25 g or ml in USA,
0 –100 in EU/Canada
Dose-response relationship
Pathogen:
Virulence characteristics
Host factor:
susceptibility
Immune status

Food Matrix

- **Type of food, properties of food, storage conditions affects growth and survival of pathogen**

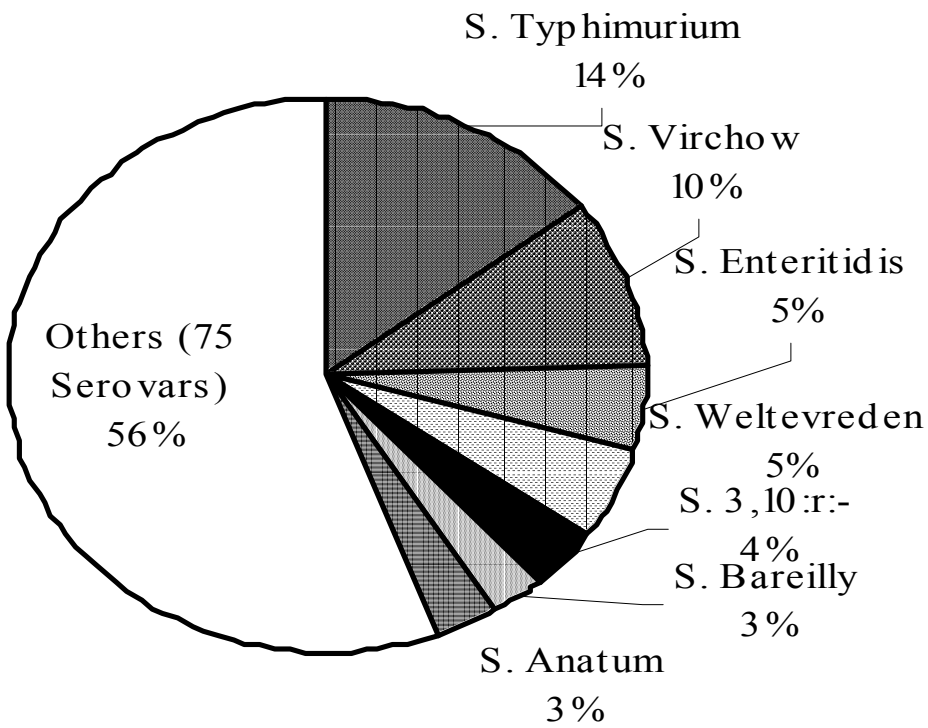
Salmonella

- **The pH optimum : around neutrality (6.8-7.5).**
- **Above 9.0 and below 4.0 being bactericidal.**
- **pH values > 10 *Salmonella* rapidly die.**
- **Temperature for optimum growth is 35°C-37°C ranging between 5°C and 46°C.**
  As low as 2°C : reported for *S.* Typhimurium.
- **Freezing arrests growth, but not bactericidal.**

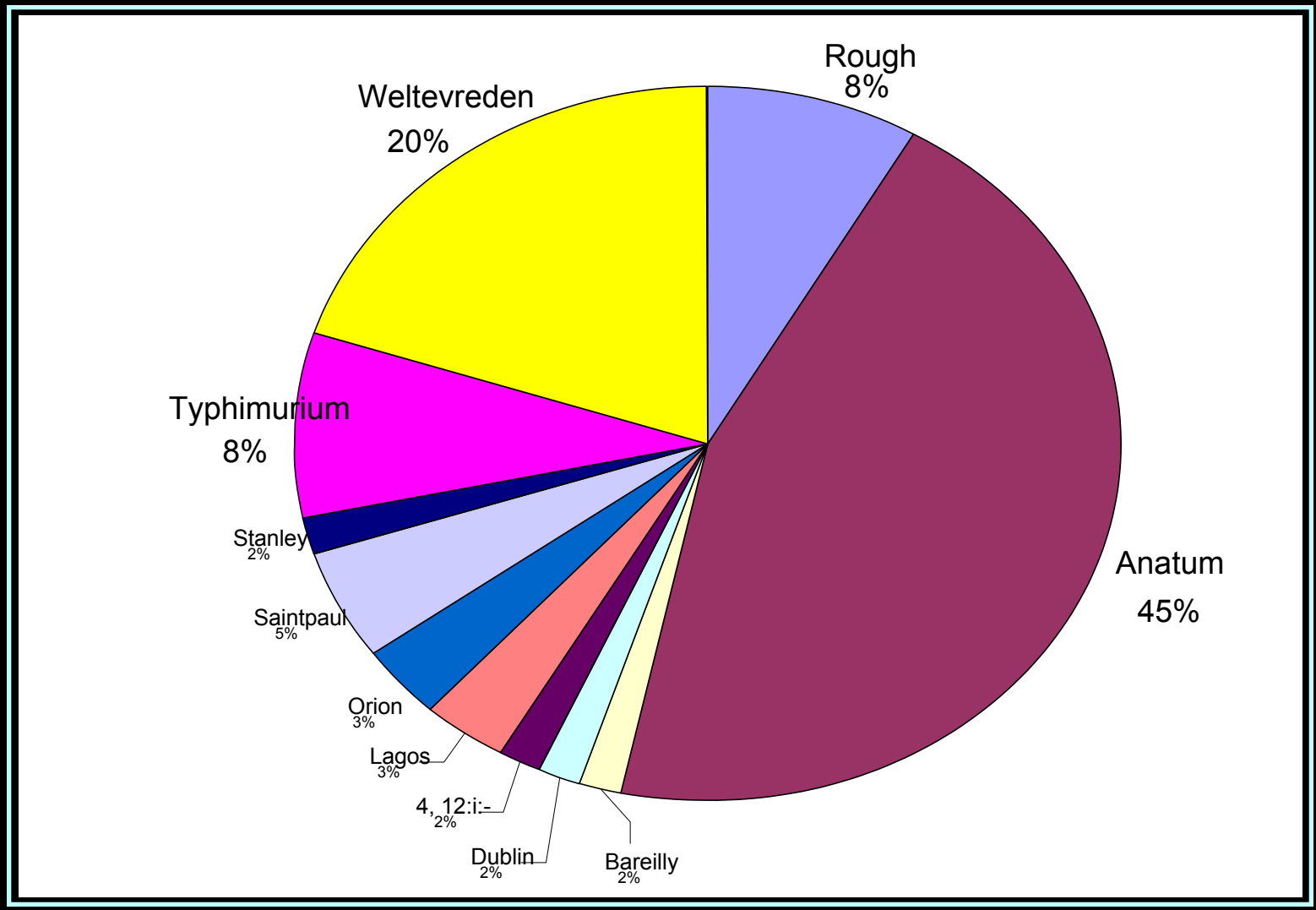
Listeria : Relatively sturdy to temperature and pH changes, survives various processes

Salmonella Characterization

Singh, 2004

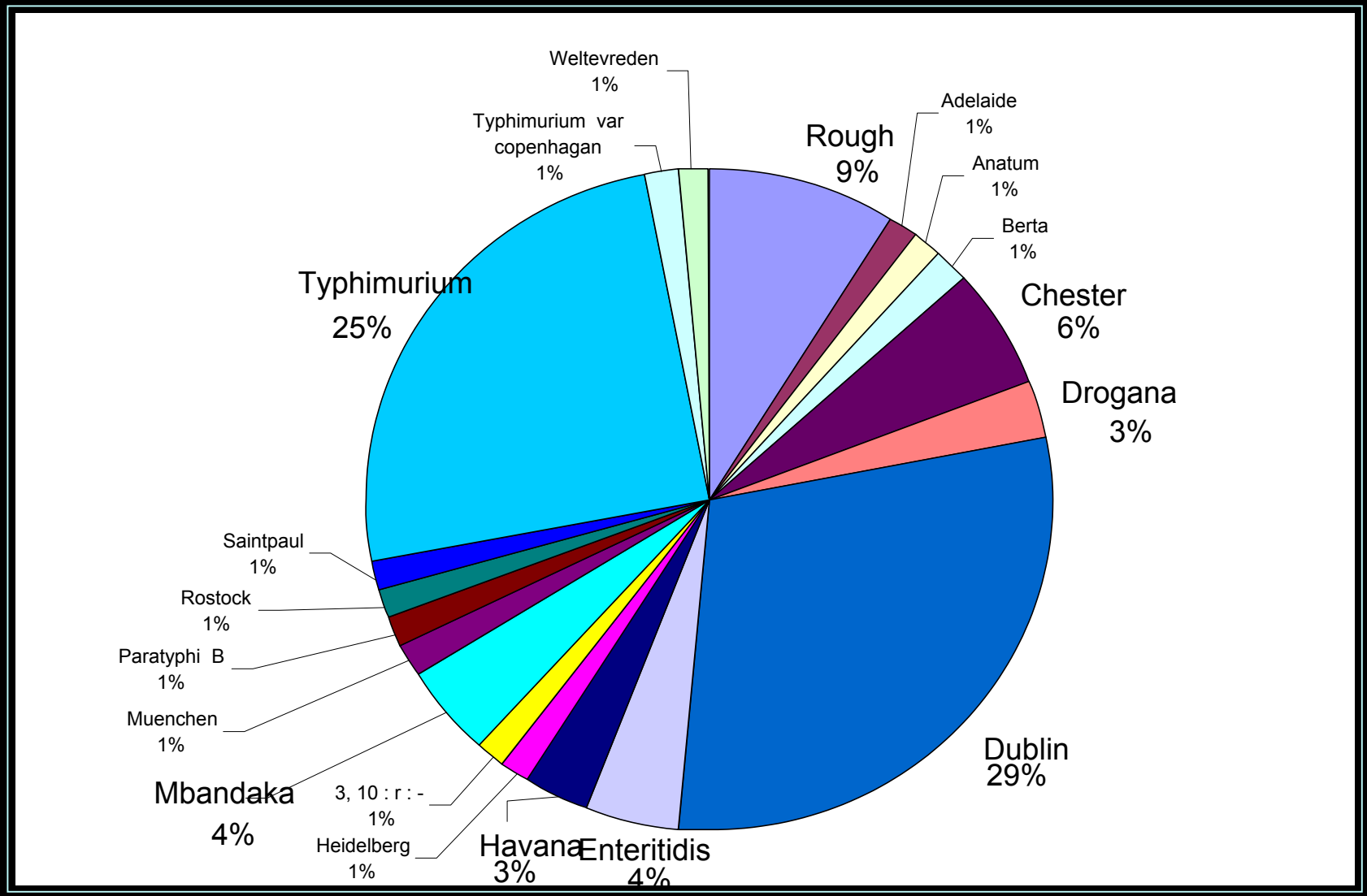


Salmonella serovars in buffaloes



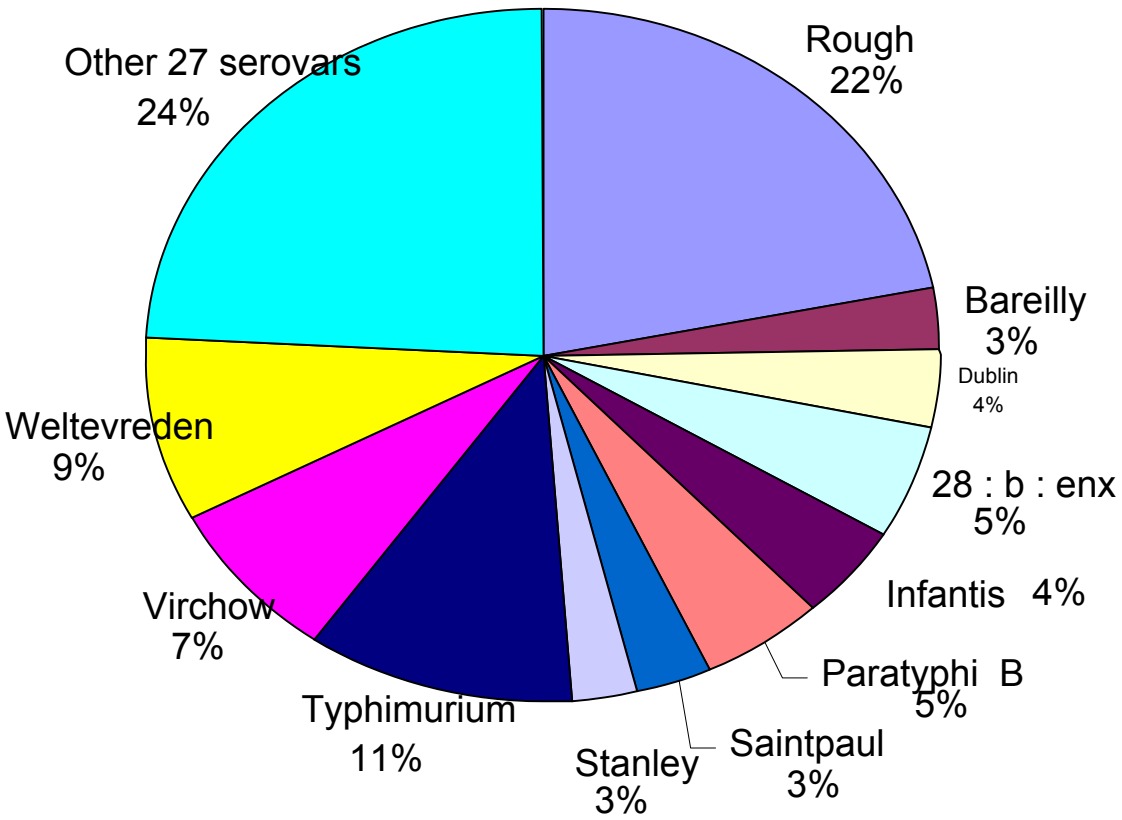
Singh, 2004

Salmonella serovars in Cattle



Singh, 2004

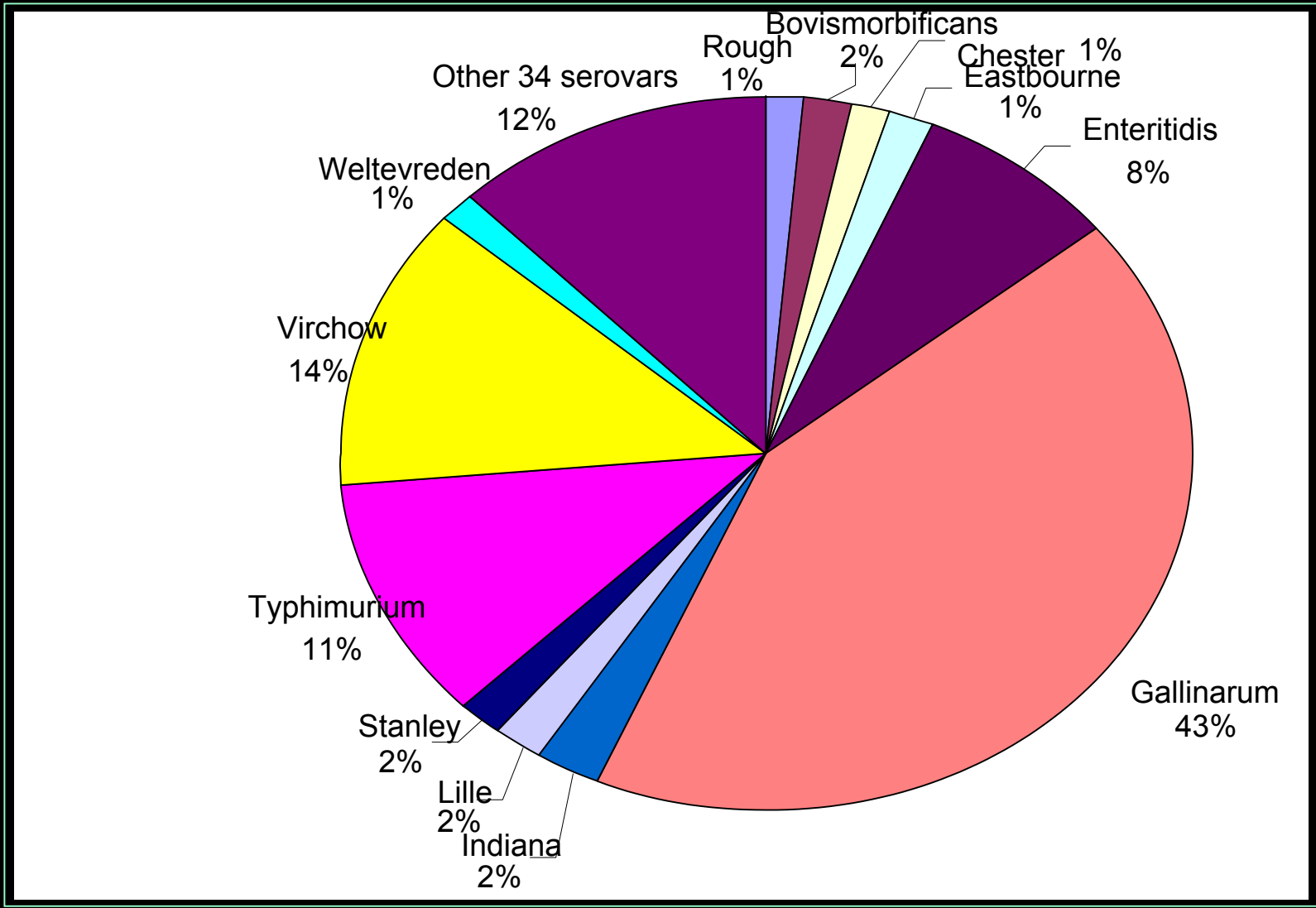
Salmonella serovars in Goats



Singh, 2004

Salmonella serovars in birds

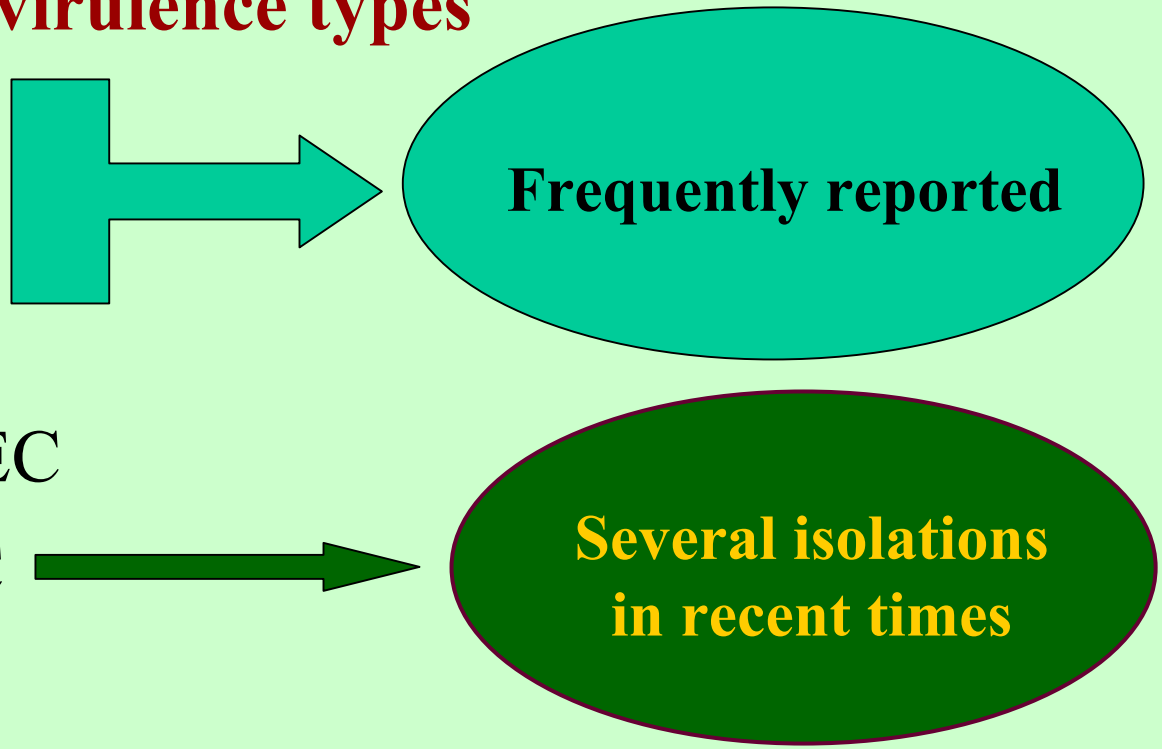
Singh, 2004



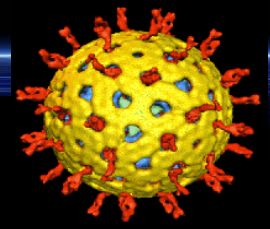
•Virchow and Typhimurium are more common than Enteritidis.

E. coli

- **Different virulence types**
  - EPEC
  - ETEC
  - EIEC
  - EAggEC
  - EHEC
  - DAEC
  - CDTEC



- Majority STEC are non O 157: H 7
  - O 157: H 7 : Rare
- Goat Isolates lack EAE gene: Significance not elucidated



Listeria monocytogenes

- Virulence depends on strain
- Studies on strain/serotype: virulence are available
- 1/2a, 4b serotypes: Implicated in > 90% outbreaks

Rotavirus

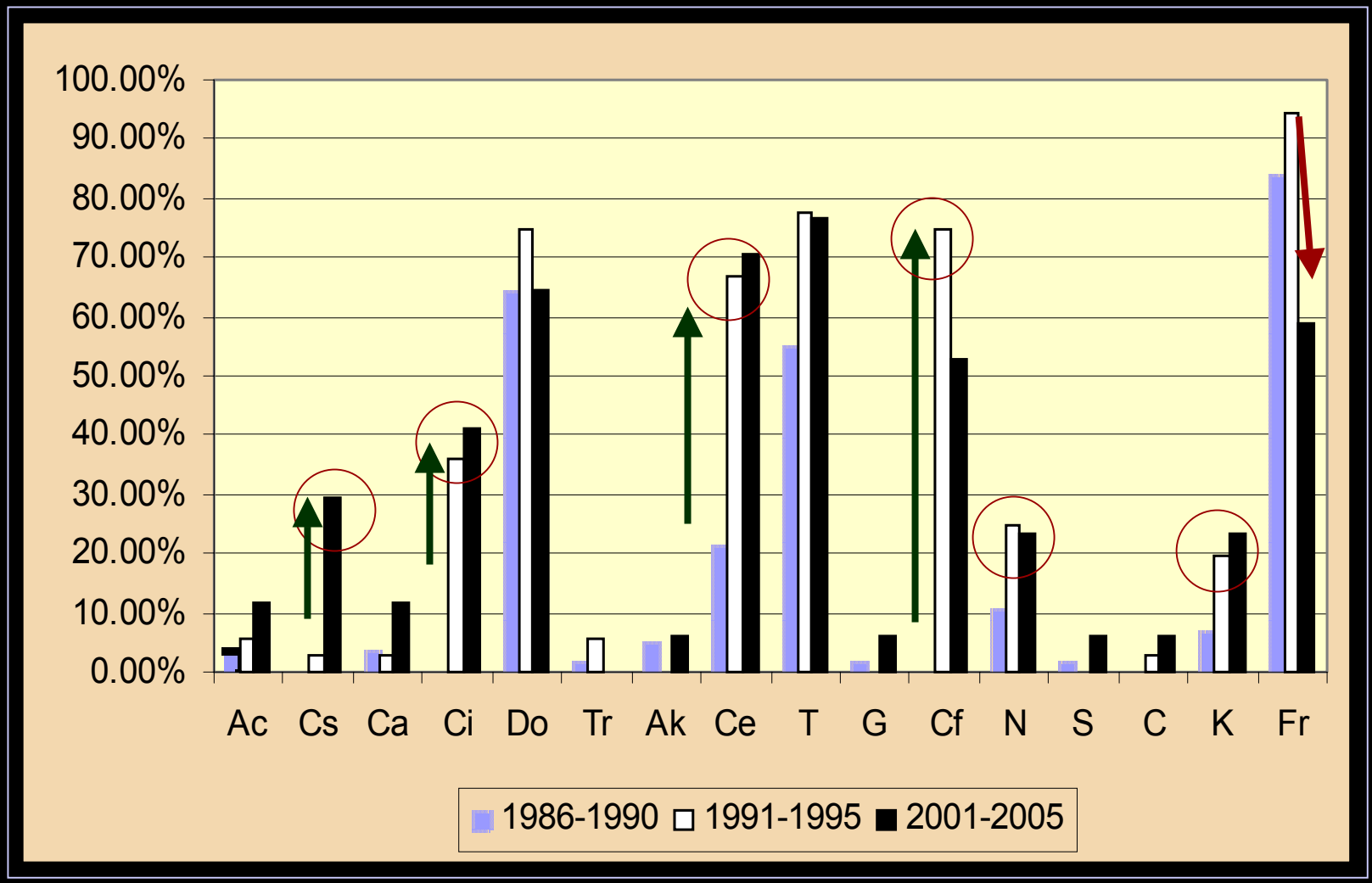
- Animal → Human : Evidence of Zoonoses
  - Bovine- human reassortants
  - Porcine –human reassortants
  - Atypical strains

Antibiotic Resistance

Increased antibiotic resistance of foodborne pathogens

- Health related issues associated with resistant bacteria
  - Treatment failures
  - Reduced therapeutic options
  - Increased severity of symptoms

Resistance profile of *Salmonella* Virchow



Cs: Cefoperazone; Ci: Ceftriaxone; Do: Doxycycline; Ce: cephotaxime; T: Tetracycline; Cf: Ciprofloxacin; N: Neomycin; K: Knamamycin; Fr: furazolidone

N= 109

Host Susceptibility

- Exposed population status
  - Normal adult
  - Infant
  - Elderly
  - Immuno-suppressed/diseased
- Severity of disease and dose required will vary

Food Intake/ Exposure Assessment

To consider appropriate food to include in model

Eg: *Listeria monocytogenes*:

Not linked

Bread, Cookies, cakes, soft drinks

Low risk

Meat/sea foods

Likely risk

Cheese/ milk products

Salmonella

- Eggs, poultry meat, pork and other meats and products are the common vehicles
- Improperly pasteurized fluid milk, ice-cream cheese and other milk products.
- Unpasteurized orange juice, uncooked tomatoes, raw alfa-alfa sprouts, etc.

IVRI

Characterize range of pathways by which food is contaminated

Sources of *Salmonella* in foods

- **Infected animals**
- **Animal excreta, cross-contamination in slaughter houses**
- **Contaminated water**
- **Contaminated soil**
- **Rodents, lizards and avifauna**
- **Insects**

Prevalence of *Salmonella*e in common house-pests/synanthropic animals.

Agarwal (2009)

Sources	No. examined	No. positive (%)
Cockroach	254	4 (1.57)
House-mouse	97	8 (8.24)
Shrew*	122	12 (9.83)
Fly	31	0
Bat	48	3 (6.25)
Ant	30	2 (6.66)
Wall-lizard	328	69 (21.03)
Total :	**910**	**98**

*Triple infection of *S.* Anatum, *S.* Hvittingfoss and *S.* Saintpaul in one shrew. Double infection of *S.* Saintpaul and *S.* Bareilly in one shrew and that of *S.* Typhimurium and *S.* Paratyphi-B in an another shrew.

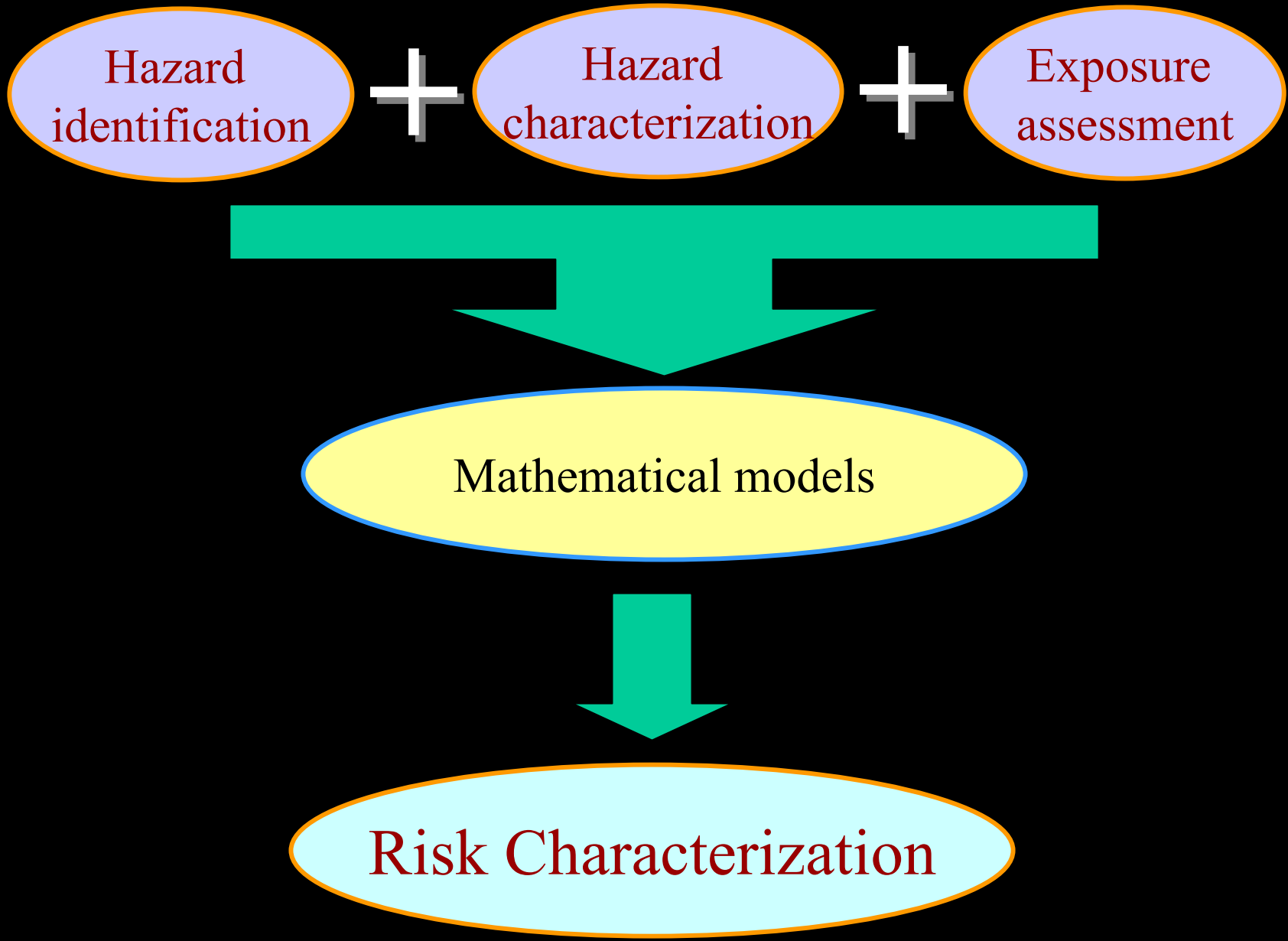
I V R I

Prevalence of *Salmonellae* in amphibians

Agarwal (2009)

Sources	No. examined	No. positive (%)
Snake*	187	16 (8.55)
Turtle	26	1 (3.84)
Toad	733	78 (10.64)

*Besides water snakes, these also include 20 cobra and other land snakes where form no *Salmonella* was isolated.


Hazard identification
+
Hazard characterization
+
Exposure assessment
Mathematical models
Risk Characterization

Risk Characterization

- Integration of Hazard identification, Hazard characterization and exposure assessment using mathematical models
- To obtain Risk estimates: Qualitative and Quantitative
- Severity of the adverse effects
- Uncertainties associated with estimates (biological variations s/a differences in virulence, susceptibility of population, etc.)

IVRI

- Based on MRA set the public health goals and targets
- Appropriate level of Protection (ALOP) is to be decided by authorities: Eg. Reducing incidence of Salmonellosis

from
100 per 1,00,000 population

To
10 per 1,00,000 population

ALOP

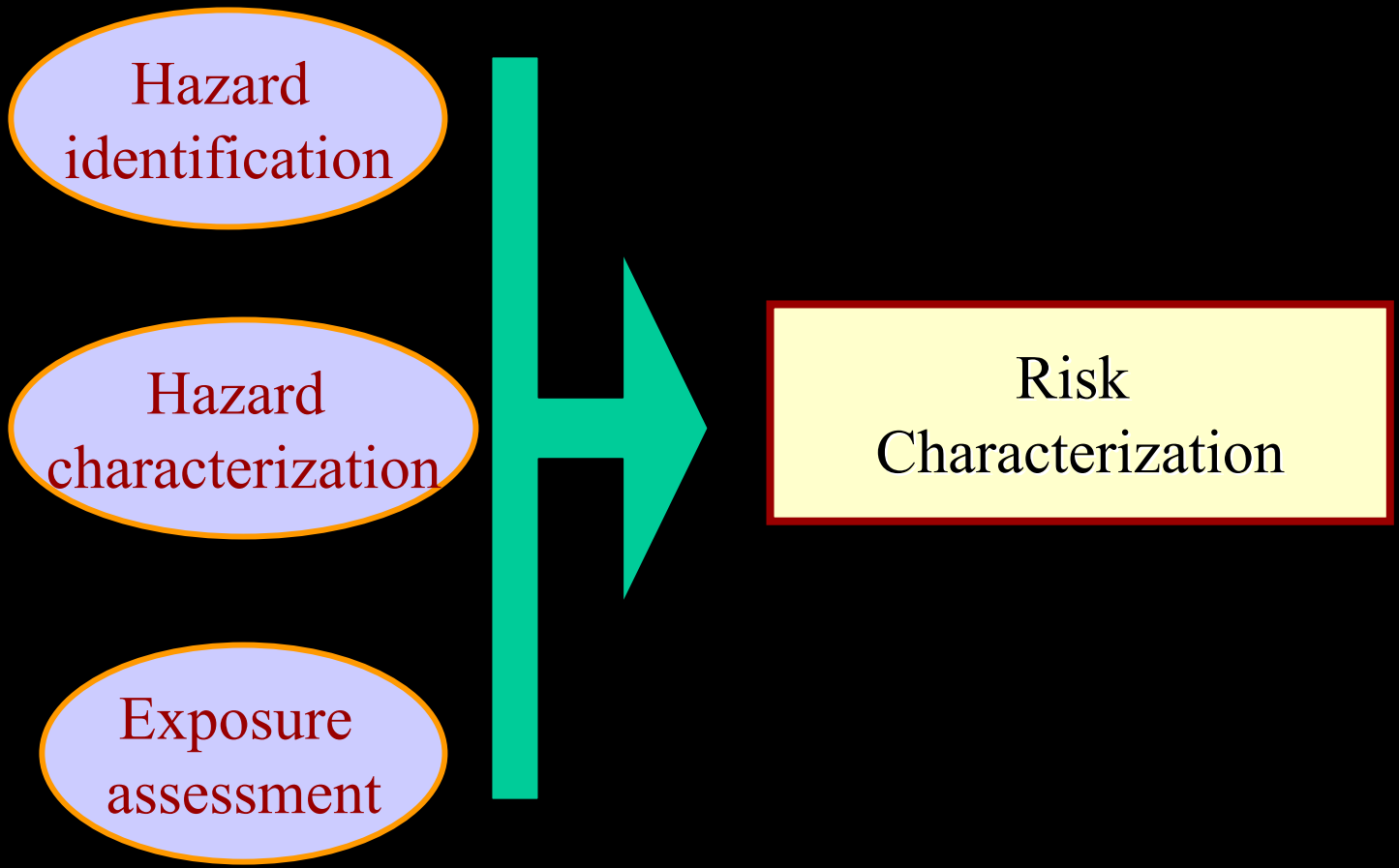
- Food Safety Objectives (FSO): The Maximum frequency and concentration of hazard in food at the time of consumption that provides ALOP to consumers

To Achieve ALOP of 10 per 1,00,000 Population of Salmonellosis

The incidence of Salmonellosis in a particular food should be brought down to 5% from 10% level

FSO

Avian Influenza



IVRI

Avian Influenza in India

- Tremendous loss to poultry Industry
- Maharashtra and Gujarat 2006
  - 3.45 lakh birds culled
  - 6 lakh eggs & 53 MT feed destroyed
- West Bengal and Tripura: 2008
  - > 40 lakh birds culled
  - 15 lakh eggs & 80,000 Kg feed destroyed

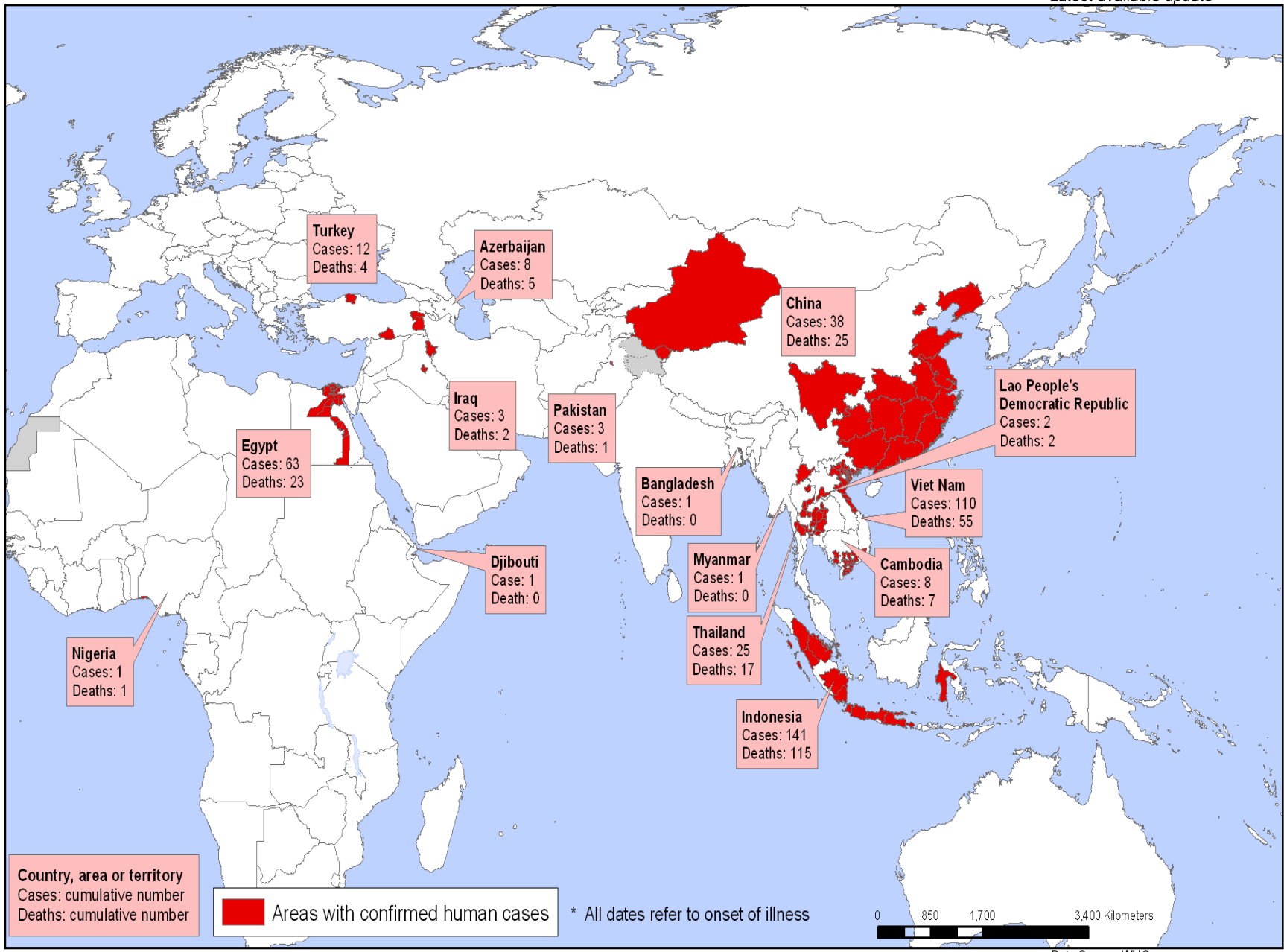
(10/32008, DAHD, Govt. India Media notification)

- Assam, West Bengal (Darjeeling) and Sikkim: 2009: Exact estimates not available

H5N1	02/01/2009	Follow-up report No. 4
	13/01/2009	Follow-up report No. 5
	21/01/2009	Follow-up report No. 6
	02/02/2009	Follow-up report No. 7
	19/02/2009	Follow-up report No. 8
	04/03/2009	Follow-up report No. 9
	16/03/2009	Follow-up report No. 10
	30/03/2009	Follow-up report No. 11

Areas with confirmed human cases of H5N1 avian influenza since 2003 *

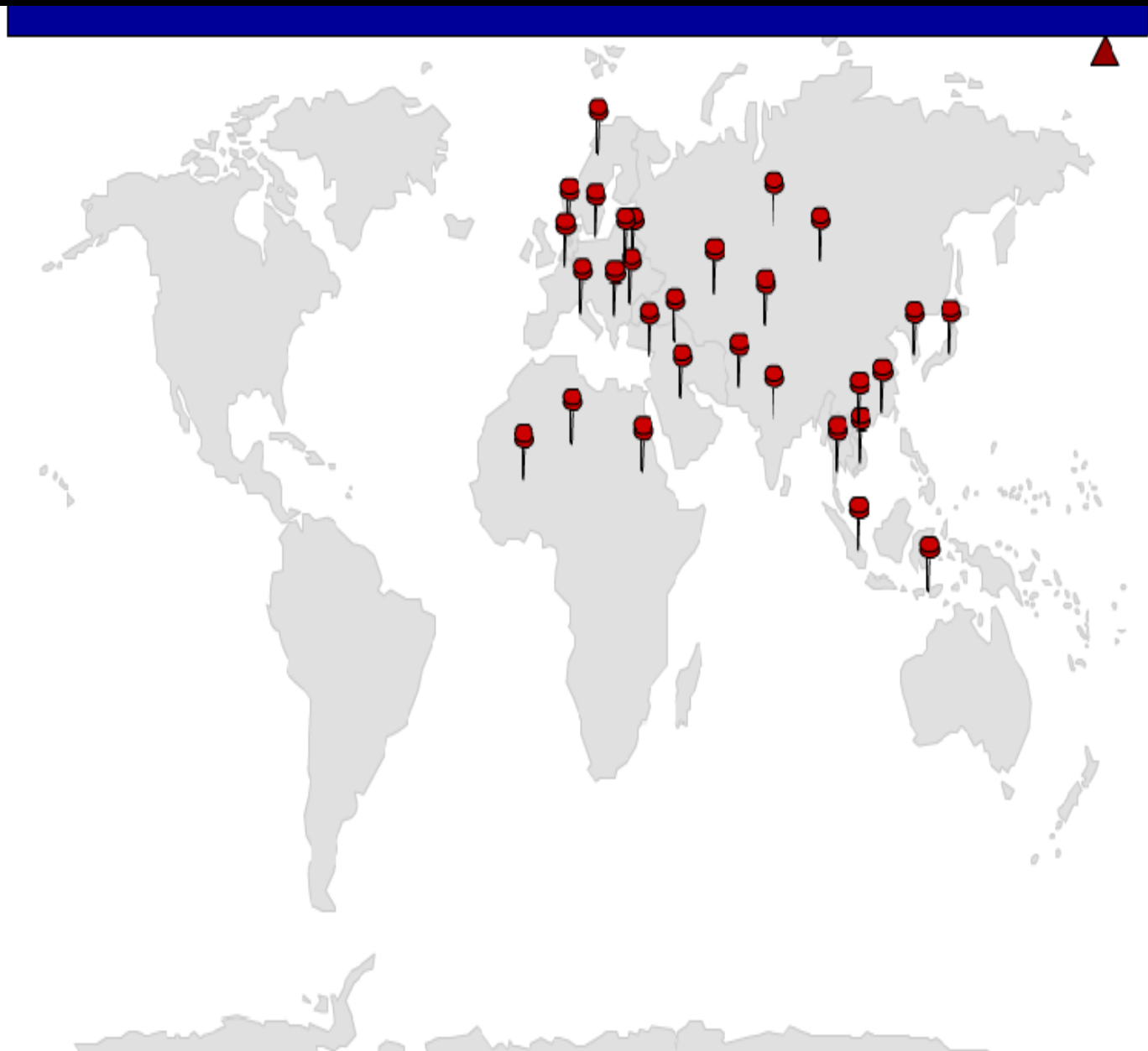
Status as of 08 April 2009
Latest available update



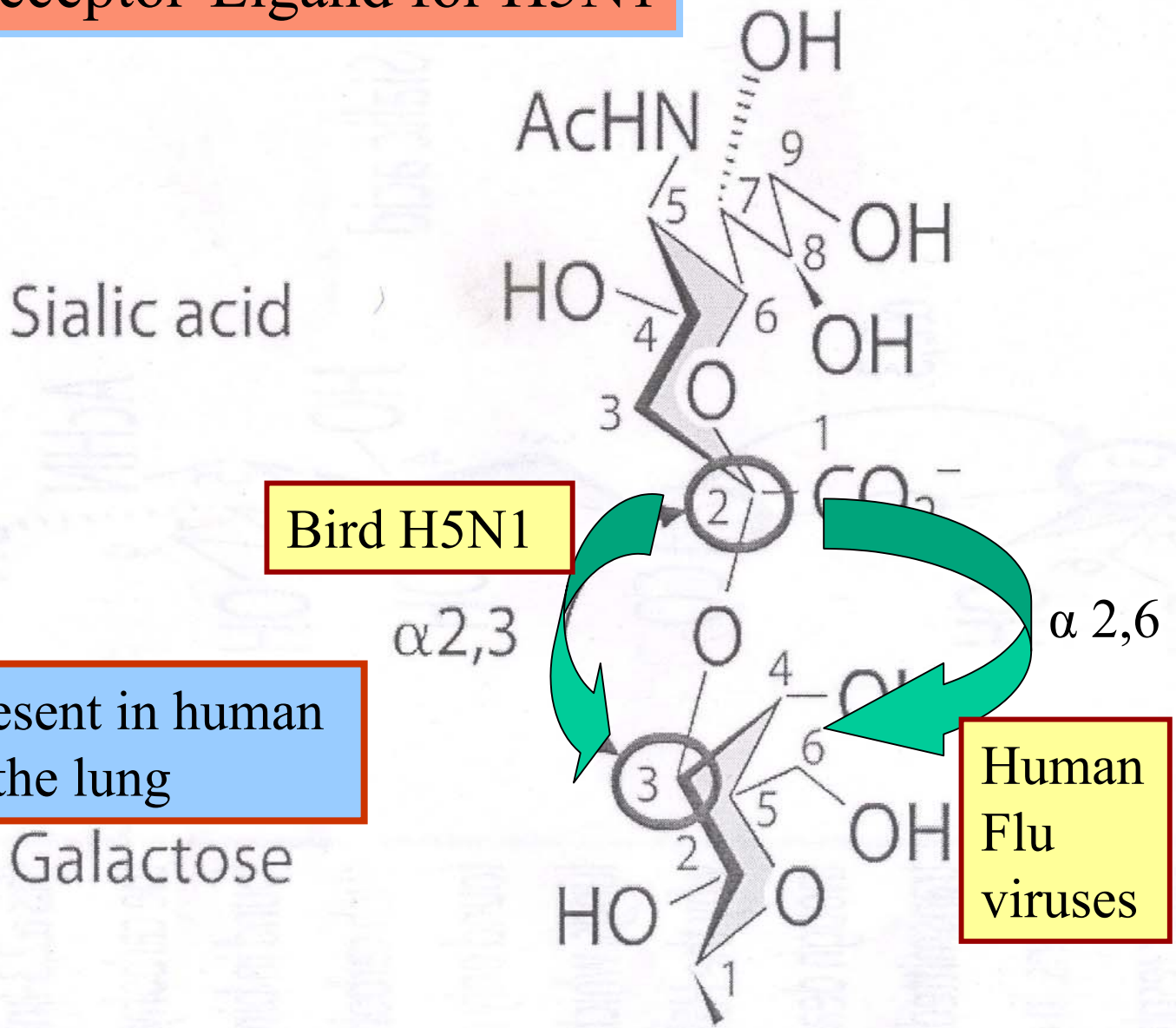
World Health Organization

Data Source: WHO
Map Production: Public Health Information and Geographic Information System (GIS)
World Health Organization

Avian influenza in birds

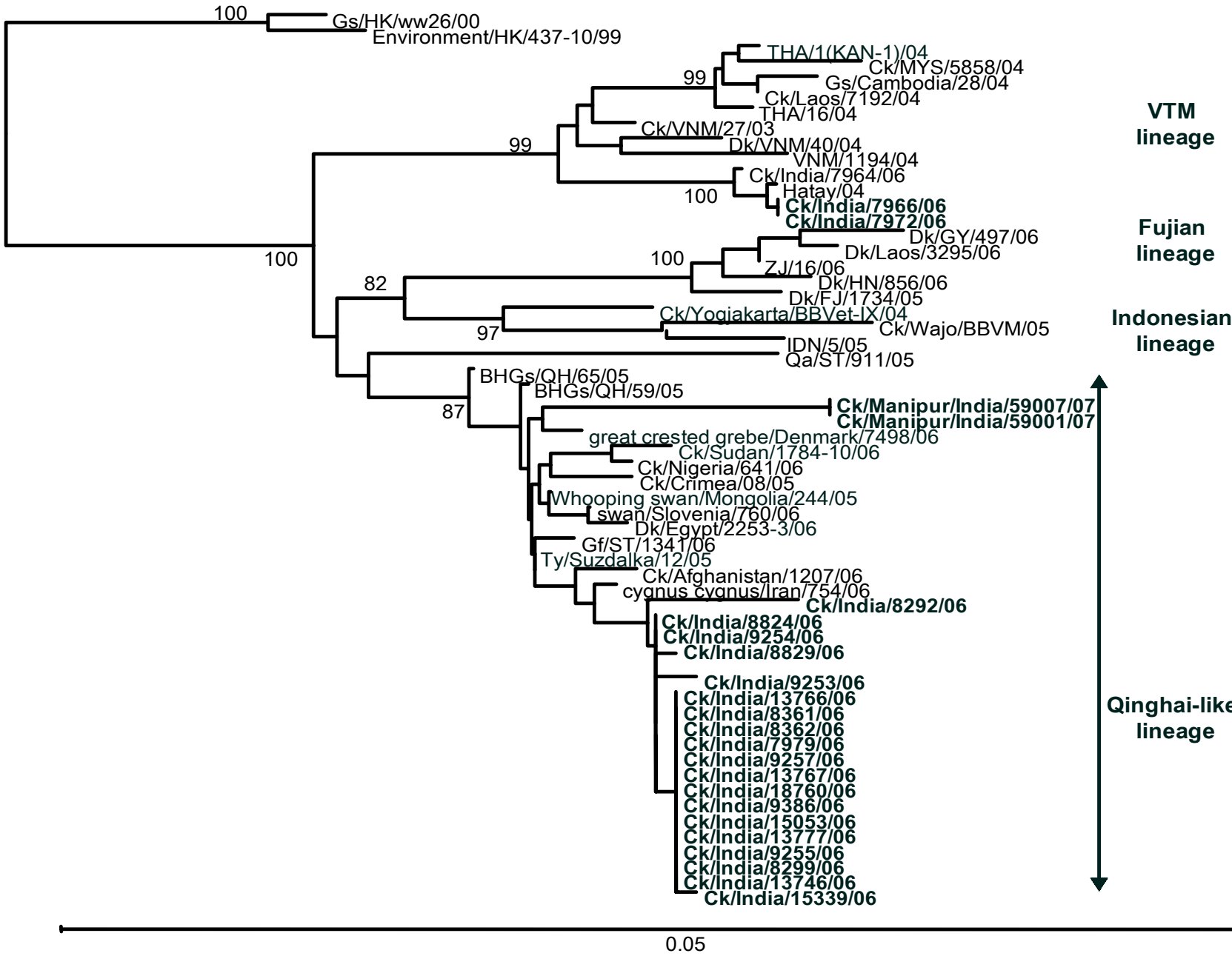


Receptor-Ligand for H5N1



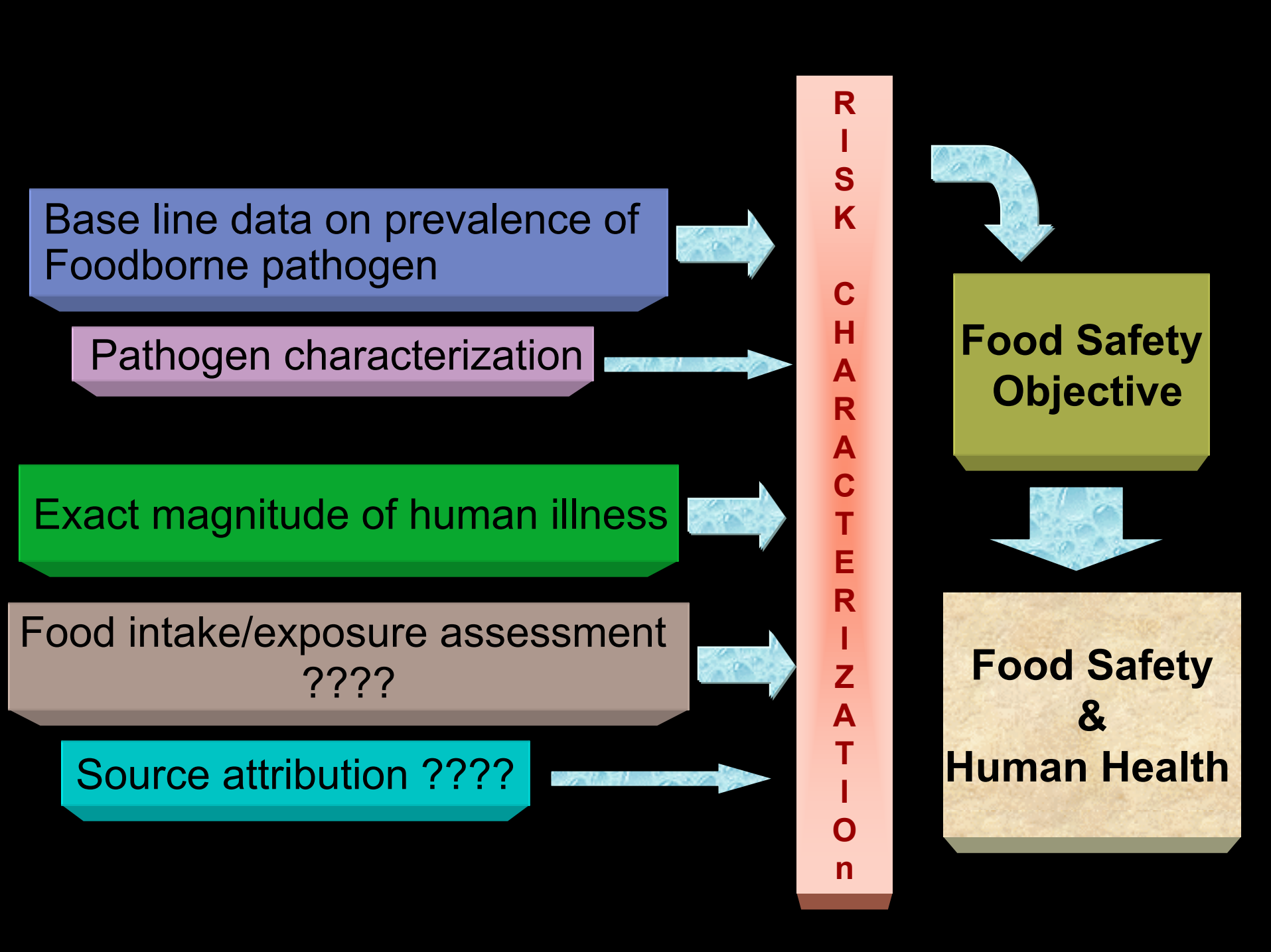
α 2,3 present in human deep in the lung

Phylogenetic analysis of NA gene of H5N1 viruses



WHO Food safety information Avian Influenza

- Conventional cooking (> 70^0C) safe : Inactivate virus
- In poultry meat: not killed by refrigeration or freezing
- Home slaughtering and preparation is hazardous
- Eggs contain virus both on outside and inside of shell
- No evidence of infection: If consumed properly cooked poultry and eggs
- Greatest risk in handling and slaughter of live infected birds


Base line data on prevalence of Foodborne pathogen
Pathogen characterization
Exact magnitude of human illness
Food intake/exposure assessment ????
Source attribution ????
RISK CHARACTERIZATIOn
Food Safety Objective
Food Safety & Human Health


Thanks