

Prevalence of *Mycoplasma gallisepticum* Infection in Indian Poultry Farms

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(ISO 9001:2008 Certified Institution)

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Background

- Mycoplasmas are highly versatile and successful pathogen
- **Chronic Respiratory Disease:** Once infected, infection remains for life
- Mycoplasma lack a cell wall: resistant to penicillin group of antibiotics
- Antimicoplasmal drugs are bacteriostatic
- Antibodies can not eliminate infection
- Field infection (MG/MS) level is high due to vertical transmission and low level of biosecurity
- Raising mycoplasma clean flocks is not practicable

Major pathogenic species

M. gallisepticum - Chickens, Turkeys

M. synoviae - Chickens, Turkeys

M. meliagridis - Turkeys

M. iowae - Turkeys

Smallest self replicating prokaryotes

Lack of cell wall, bounded by cell membrane

Fragile – easily killed outside its host by disinfectants

Economic significance

- **Costliest Disease in Poultry**
- Mortality
- Reduced feed conversion
- Loss of weight
- Complications with – *IB, ND, E coli*
- Condemnations
- Drop in egg production
- Reduced hatchability & chick viability
- Cost of eradication and control programs

MG related losses

Excluding medication costs

Effect	Per cent
Body weight gain	20-30
FCR	10-20
Egg production	5-10
Embryo mortality	5-10
Mortality	5-10
Carcass down grading	10-20

Stipkovits and Kempf (1996)

Transmission

- Horizontal
 - Within a flock - contact or aerosols
 - Between flocks - windborne
- Vertical
 - Parent to the chick through egg
- Venereal

Target organs:

- Respiratory system
- Synovial membranes
- Reproductive system

Incubation period:

- Chronic slow spreading
- Varies from 6-21 days

Clinical Signs

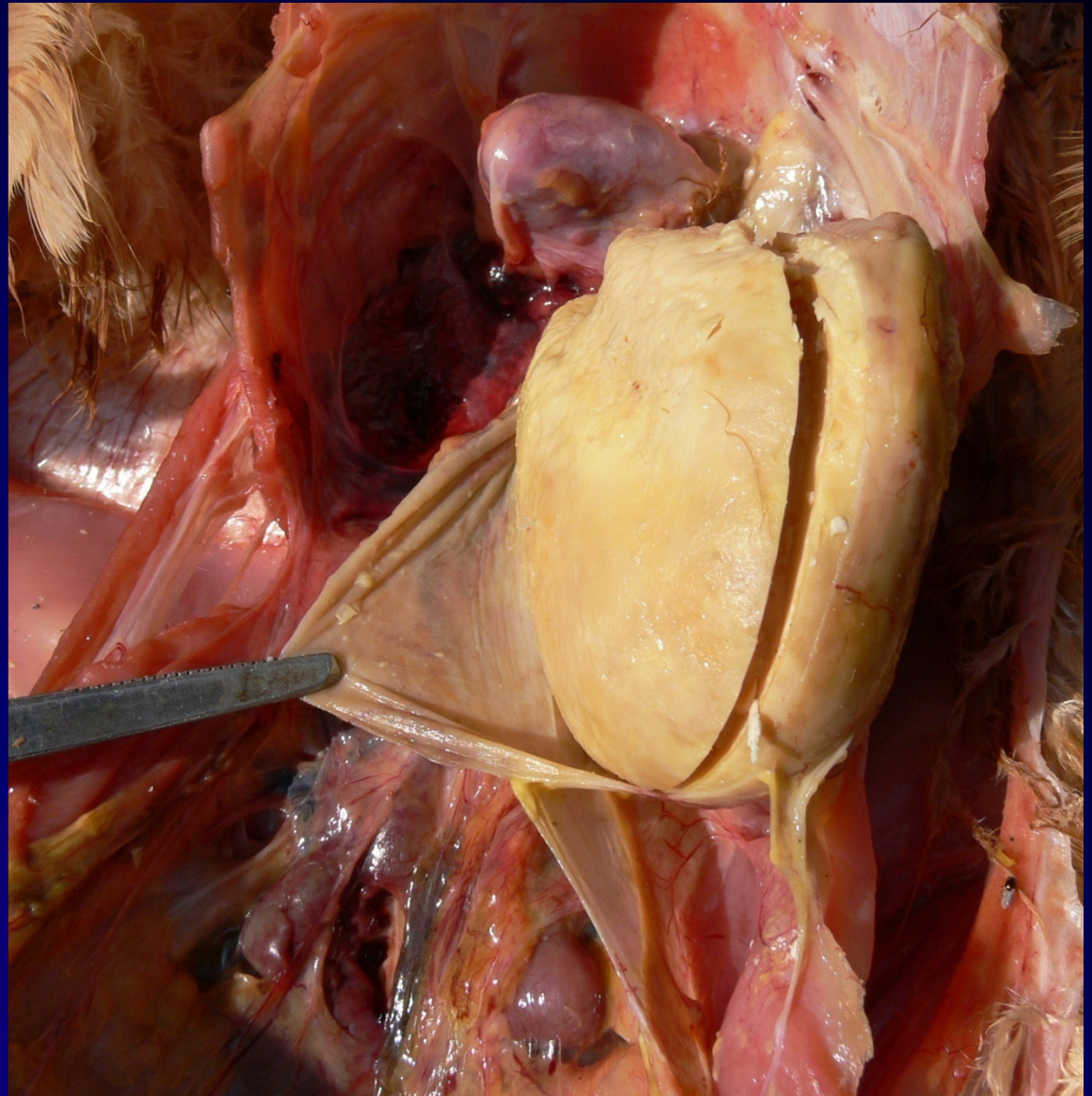
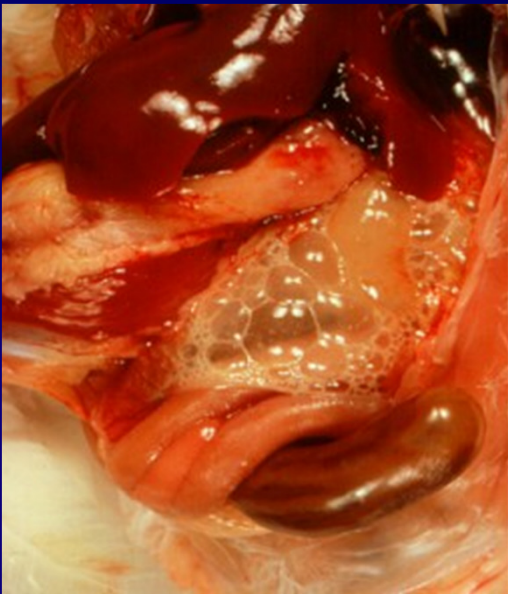
- Coughing, sneezing, Nasal discharge
- Foamy secretions in the eye
- Open mouthed breathing
- Tracheal rales
- Reduced feed consumption
- Loss of weight – more stunted chicks
- Drop in egg production – layer, breeder
- Reduced hatchability, chick viability
- Lameness
- Morbidity – up to 100%
- Mortality – up to 30% in young, negligible in adults

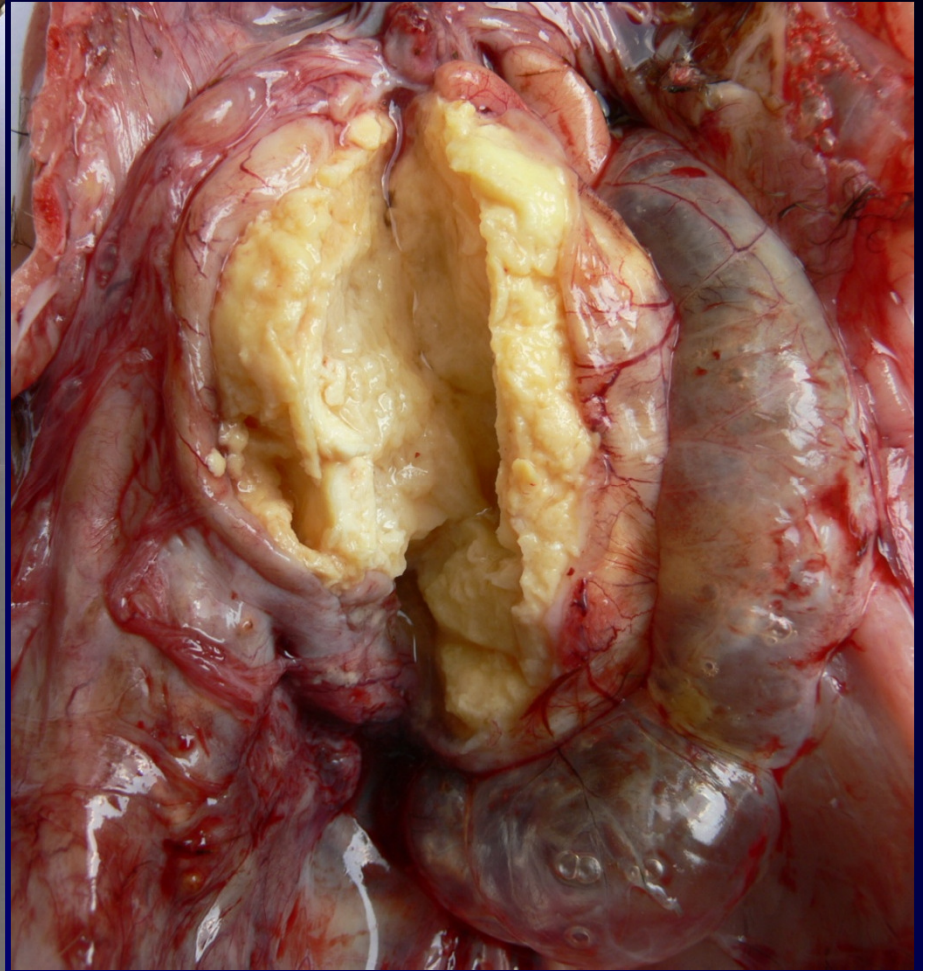
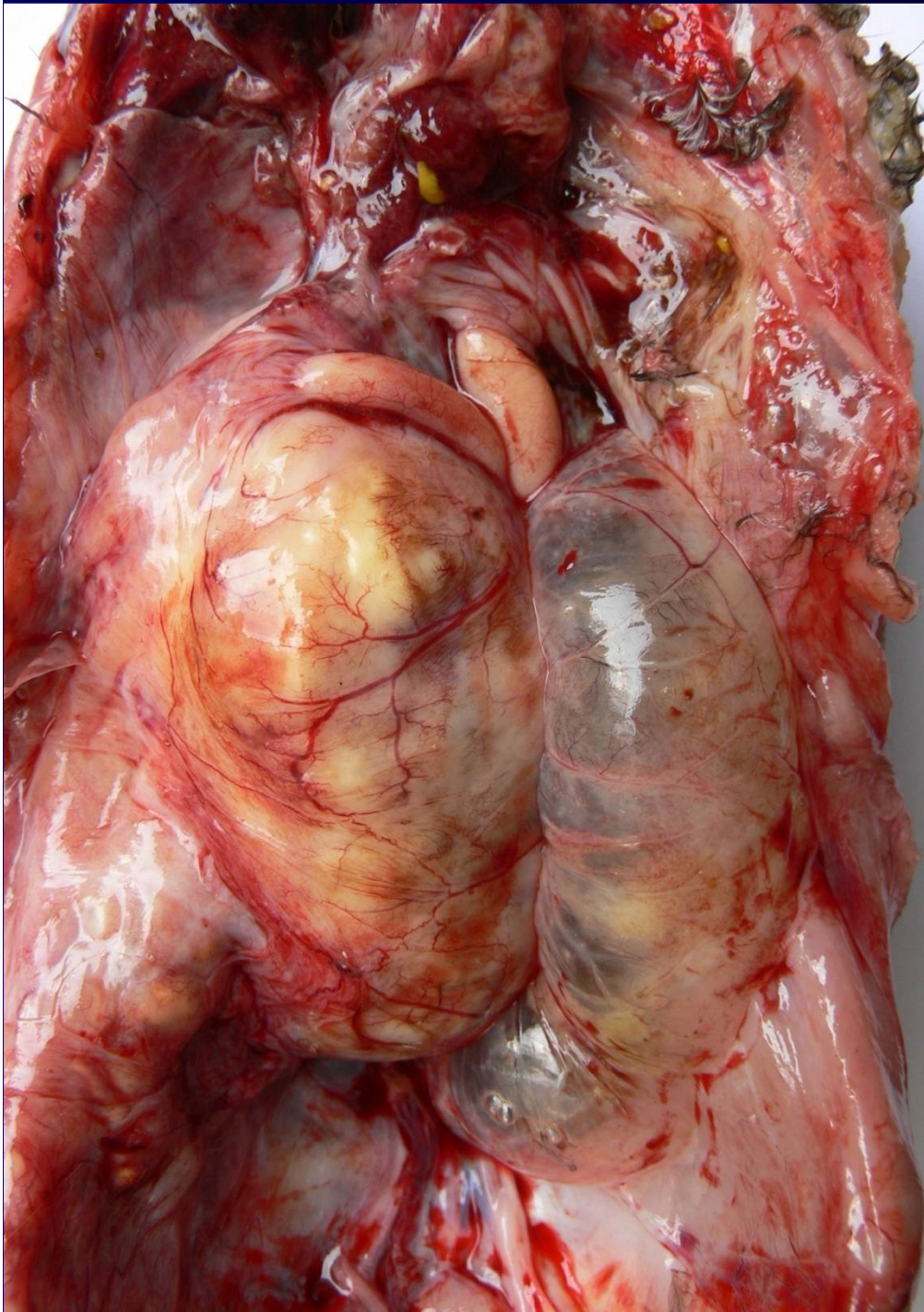
Gross Lesions

- Sinusitis and conjunctivitis
- Tracheitis with excessive mucus
- Airsacculitis
- Pneumonia
- Synovitis
- Osteomyelitis
- Salpingitis

Accumulation of Cheesy mass in thoracic air sac

Frothy exudate
in Air sac







**Complicated Chronic Respiratory Disease
Air-sacculitis, Pericarditis, perihepatitis**



STUDY OBJECTIVE

To determine the prevalence of *M. gallisepticum* from different regions in Indian poultry industry

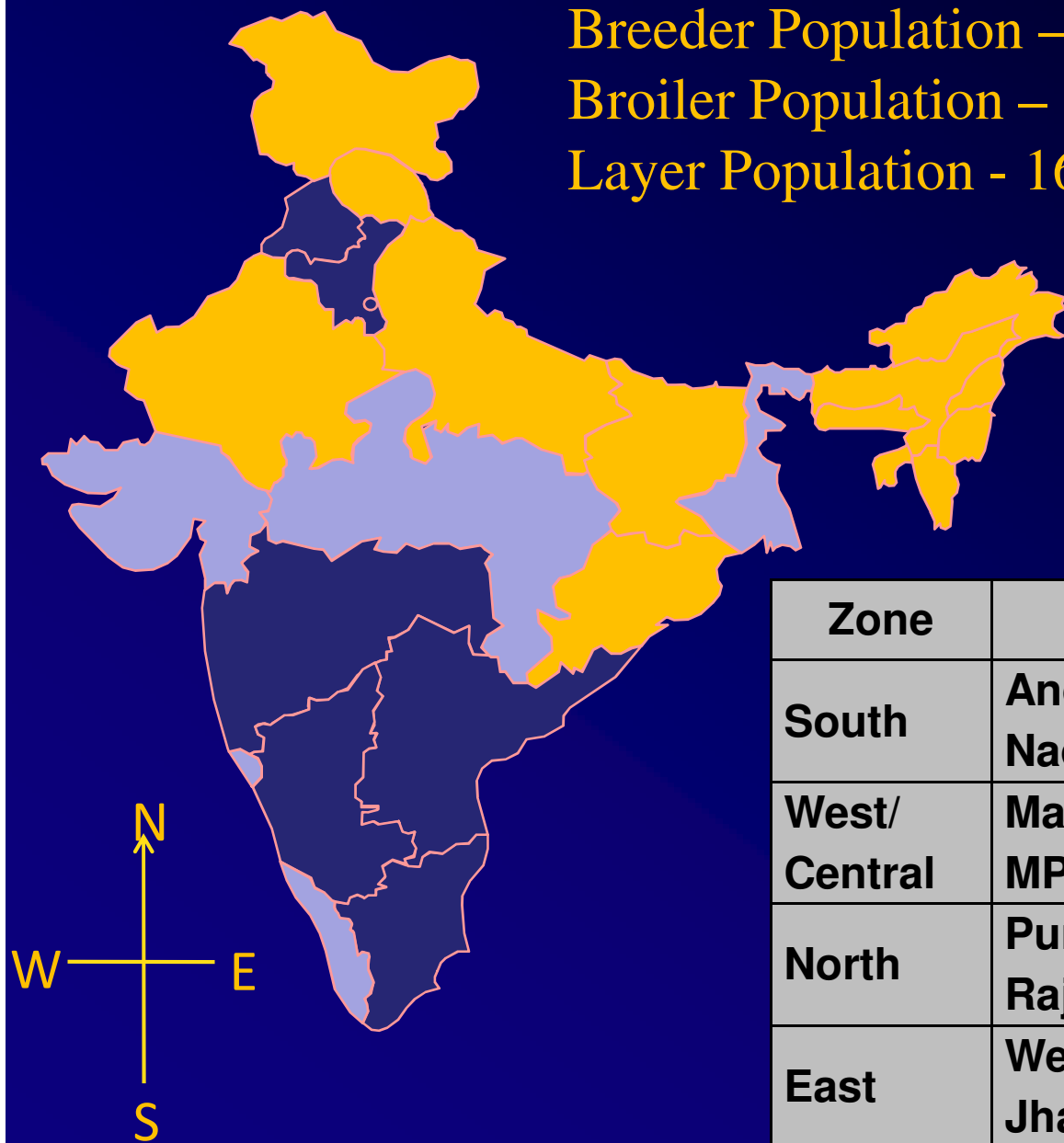
Methodology

- Sampling
- Antibody detection by ELISA
- Isolation and Identification of MG

Poultry map of India

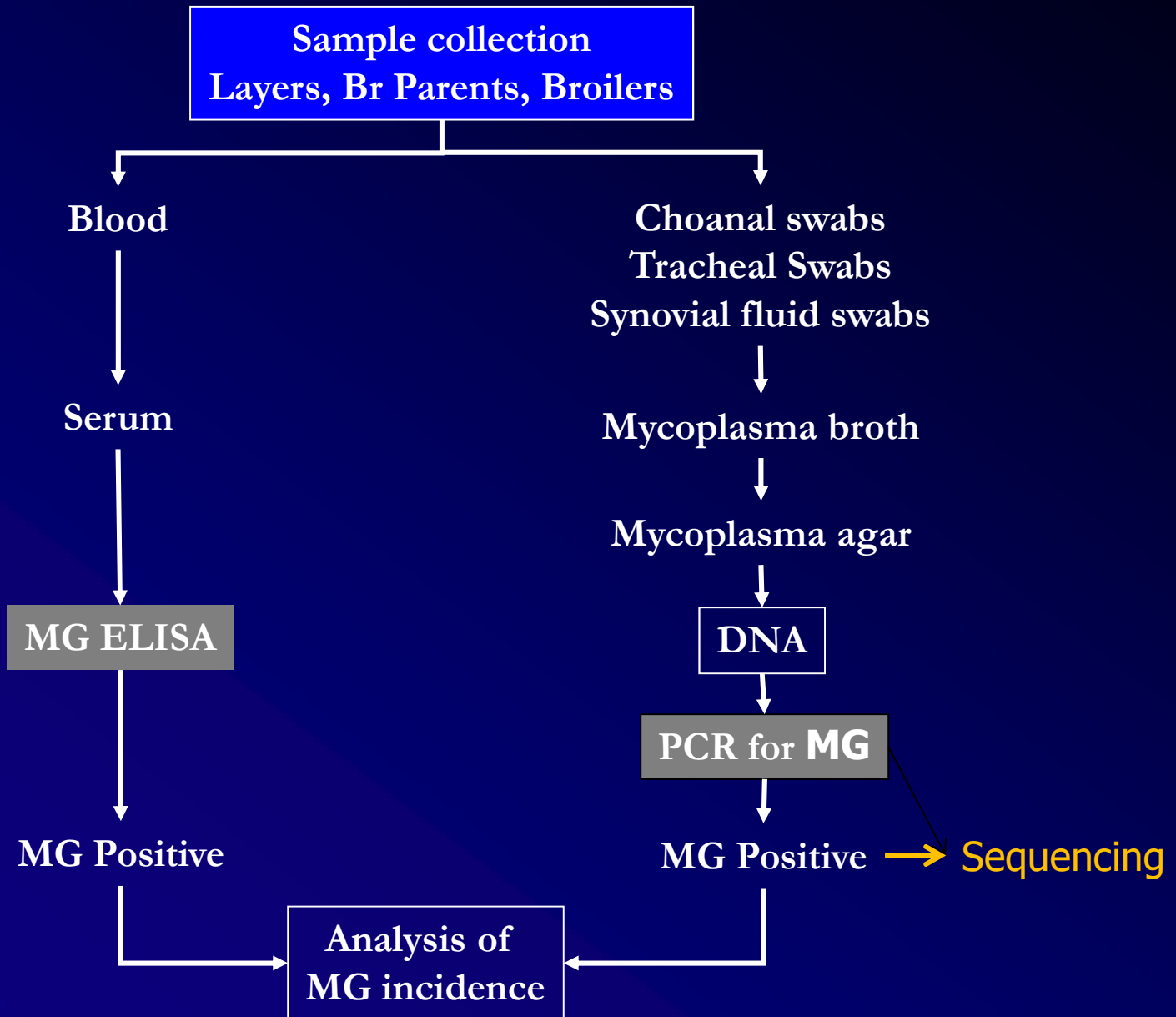
Breeder Population – 15.6 m
Broiler Population – 2000 m
Layer Population - 165 m

- High population
- Moderate population
- Low population



Zone	State	Share
South	Andhra Pradesh, Tamil Nadu, Karnataka, Kerala	40%
West/ Central	Maharastra, Gujarat, MP, Chattishgarh	25%
North	Punjab, Haryana , Rajasthan, HP, UP, J&K	20%
East	West Bengal, Orissa, Jharkhant, North East	15%

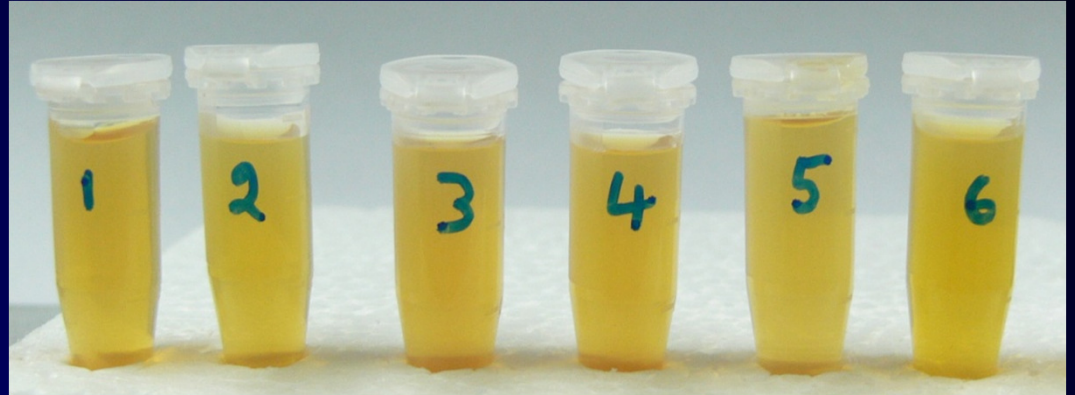
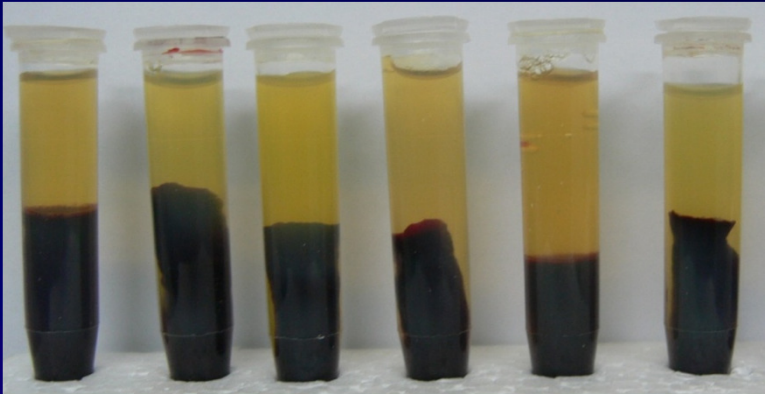
Flow Chart for
Serology, isolation and identification of
Mycoplasma gallisepticum



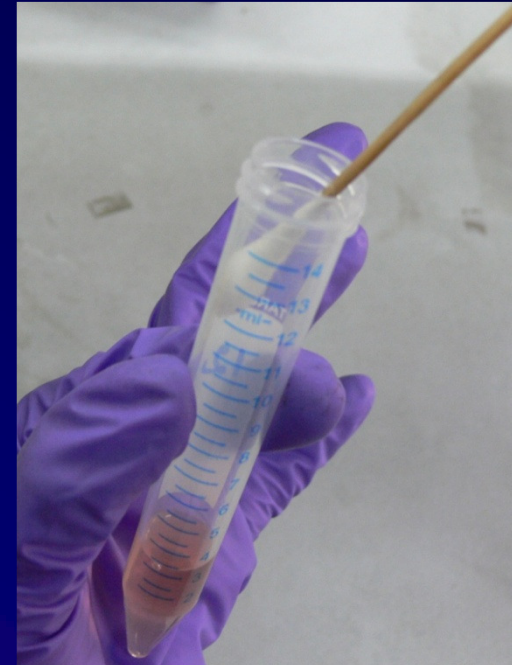
Target Number of Samples and Geographic Regions

Geographic Location	<i>Samples</i>	
	Serum	Swabs
North	250	250
South	500	500
Central	300	300
East	100	100
TOTAL	1150	1150

Serum separation for ELISA



Collection of choanal swab



Filling Sample Submission Form

PROJECT DIRECTORATE ON POULTRY
INDIAN COUNCIL OF AGRICULTURAL RESEARCH
RAJENDRANAGAR, HYDERABAD-500 030

Pfizer-PDP (ICAR)
 Contract Research Project

Name of the Farm : TARGET POULTRY
 Name of the Owner : Shri MOHAN SINGH
 Address of the Farm : Shankar Pally, Golconda Military area
 State: Andhra Pradesh
 Phone: 040-24557007 Pin: 500 037
 Fax: 040-24073946

SAMPLE SUBMISSION FORM

Sample ID	Sample Description	Breed/type of chicken	Age	Sex
1	Serum	Layer Parent	32 wks	♀
2	Serum	do	32 wks	♀
3	Serum	do	32 wks	♀
4	Serum	do	32 wks	♀
5	Serum	do	32 wks	♀
6	Serum	do	32 wks	♀
7	Serum	do	32 wks	♀
8	Kidney	do	32 wks	♀
9	Kidney	do	32 wks	♀
10	Choanal Swab	Broiler Parent	8 wks	♂
11	Choanal Swab	Layer	8 wks	♂
12	Choanal Swab	Layer	13 wks	♀
13	Tracheal Swab	Layer	13 wks	♀
			19 wks	♀

Sender's Name : My. SRIKA
 Sender's Address : Area mama
Pfizer ph
Secundera
 Phone No : 040-94
13
13 wks limited
20047
06

Sender's Signature

Number of Samples processed from different Geographic Regions

Region	Choanal Swabs		Serum Samples	
	Number of birds sampled	Number of flocks sampled	Number of Birds sampled	Number of flocks sampled
Central	440	22	441	22
East	200	10	200	10
North	284	15	368	26
South	791	27	818	28
Total	1715	64	1827	86

Sample Distribution

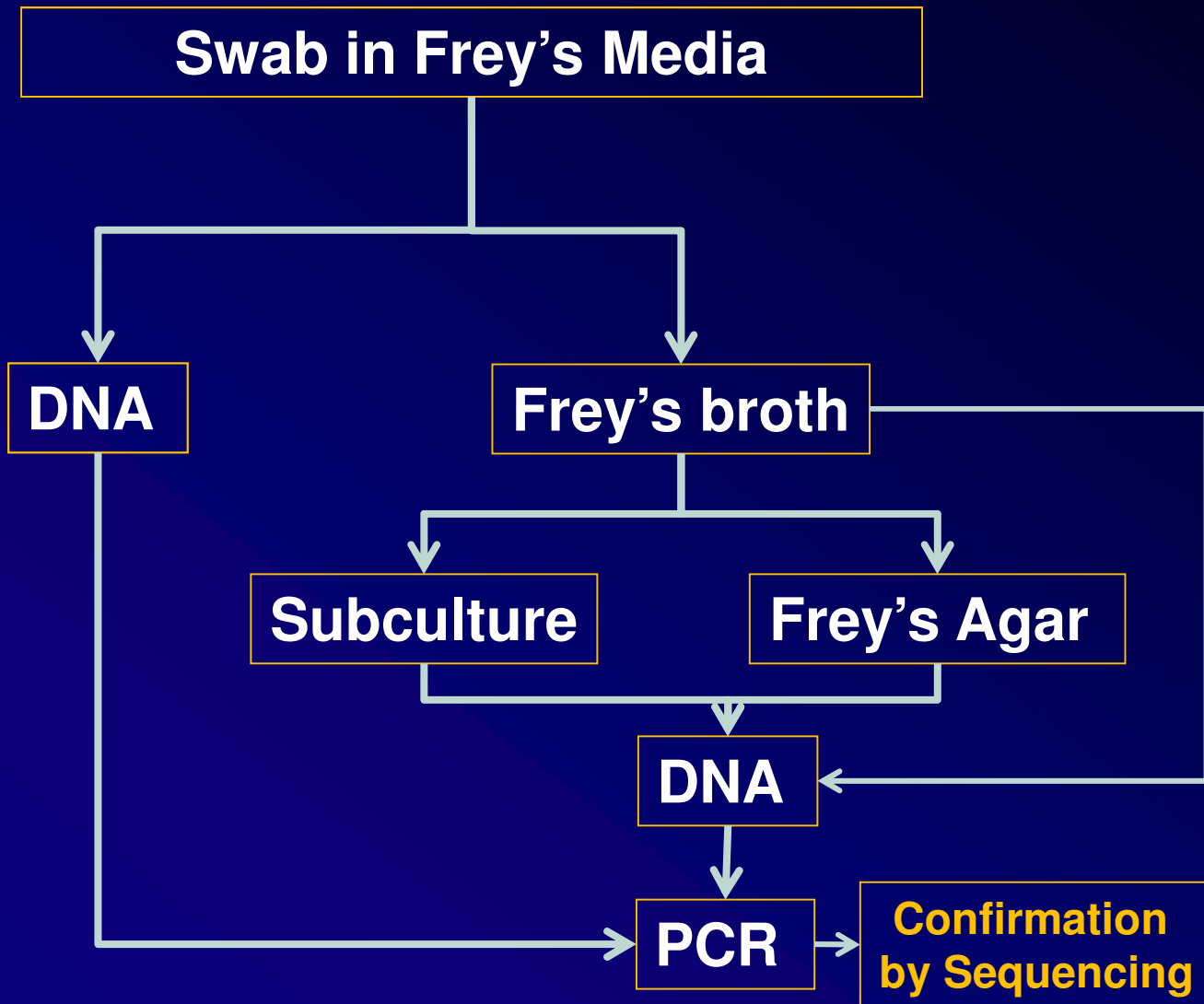
Region	Sample (%)
Central	24.1
East	10.8
North	20.0
South	45.1

Flock Type	Sample (%)
Br Parent	41.5
Broilers	13.1
Layers	45.5

Antibody ELISA

- Serum Dilution: 1:500
- Addition of diluted sera onto a MS/MG antigen-coated plate,
- Incubation 30min, then wash
- Addition of HRP labeled, anti-chicken antibody (conjugated antibody)
- Incubation 30 min, then wash
- Addition of substrate
- Incubation 15 min, addition of Stop solution
- Reading absorbance at 650nm
- Calculation of titer (≥ 1076 positive)

Isolation and Identification



MG PCR Primers

Primer name	Sequence	Product size (bp)
MGC2 2F	5'-CGCAATTTGGTCCTAATCCCCAACA-3'	300
MGC2 2R	5'-TAA ACCCACCTCCAGCTTTATTTCC-3'	
MG14F	5'-GAGCTAATCTGTAAAGTTGGTC-3'	185
MG13R	5'-GCTTCCTTGCGGTTAGCAAC-3'	

Frey's Media (1000ml)

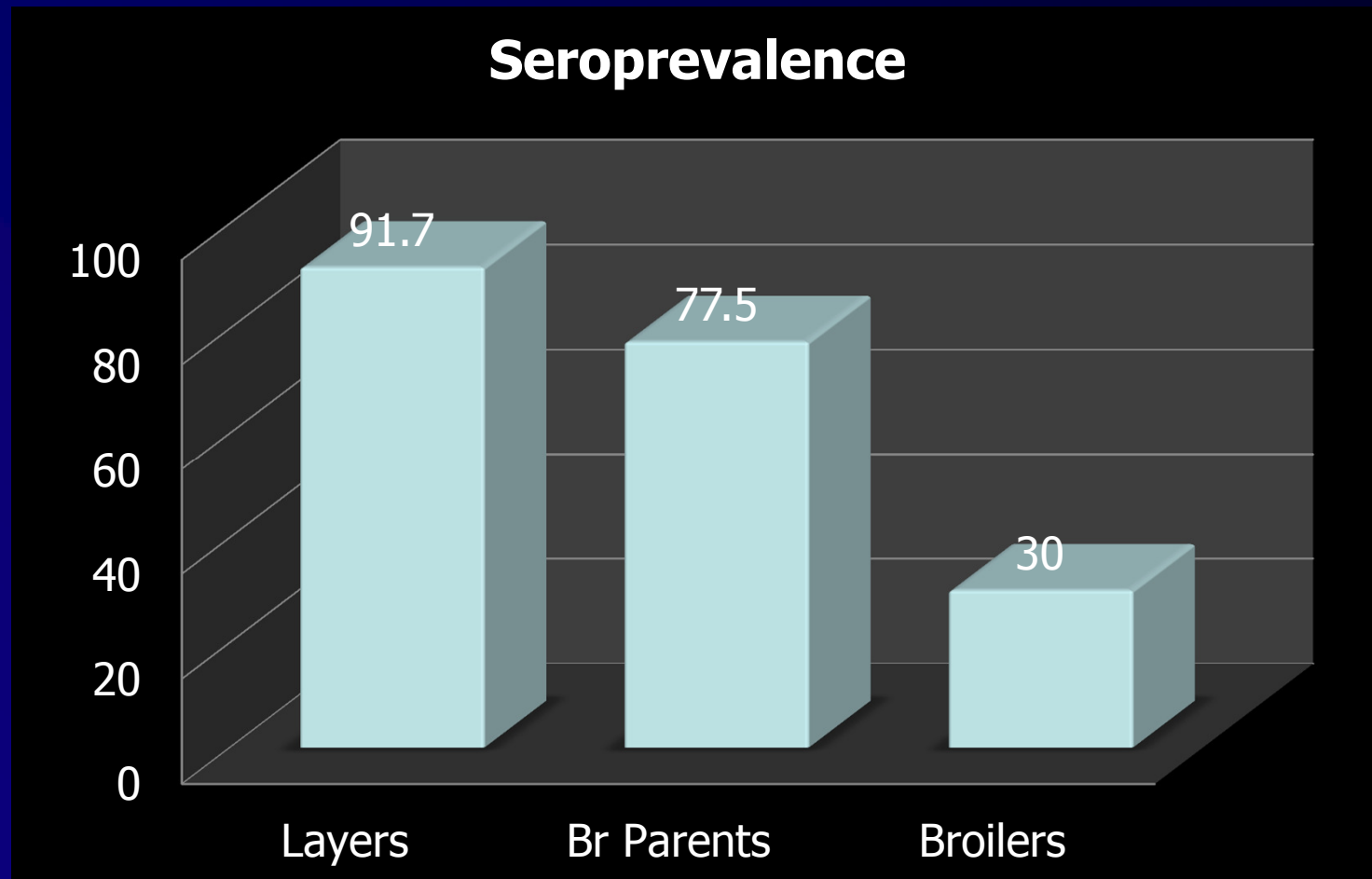
- Frey's broth base 22.3 g
- Glucose (10%) 10 ml
- Thallous Acetate (5%) 10 ml
- NAD (1%) 10 ml
- Cysteine HCL (1%) 10 ml
- Phenol red (0.1%) 20 ml
- Pig serum 120 ml
- Penicillin 2 lakh IU/ml 5 ml

RESULTS

Seroprevalance of *Mycoplasma gallisepticum* by ELISA (Based on Flocks)

Region	No. of Flocks Tested	No. of Flocks Positive	% Positive
Central	22	17	77.3
East	10	07	70.0
North	26	22	84.6
South	28	21	75.0
Total	86	67	77.9

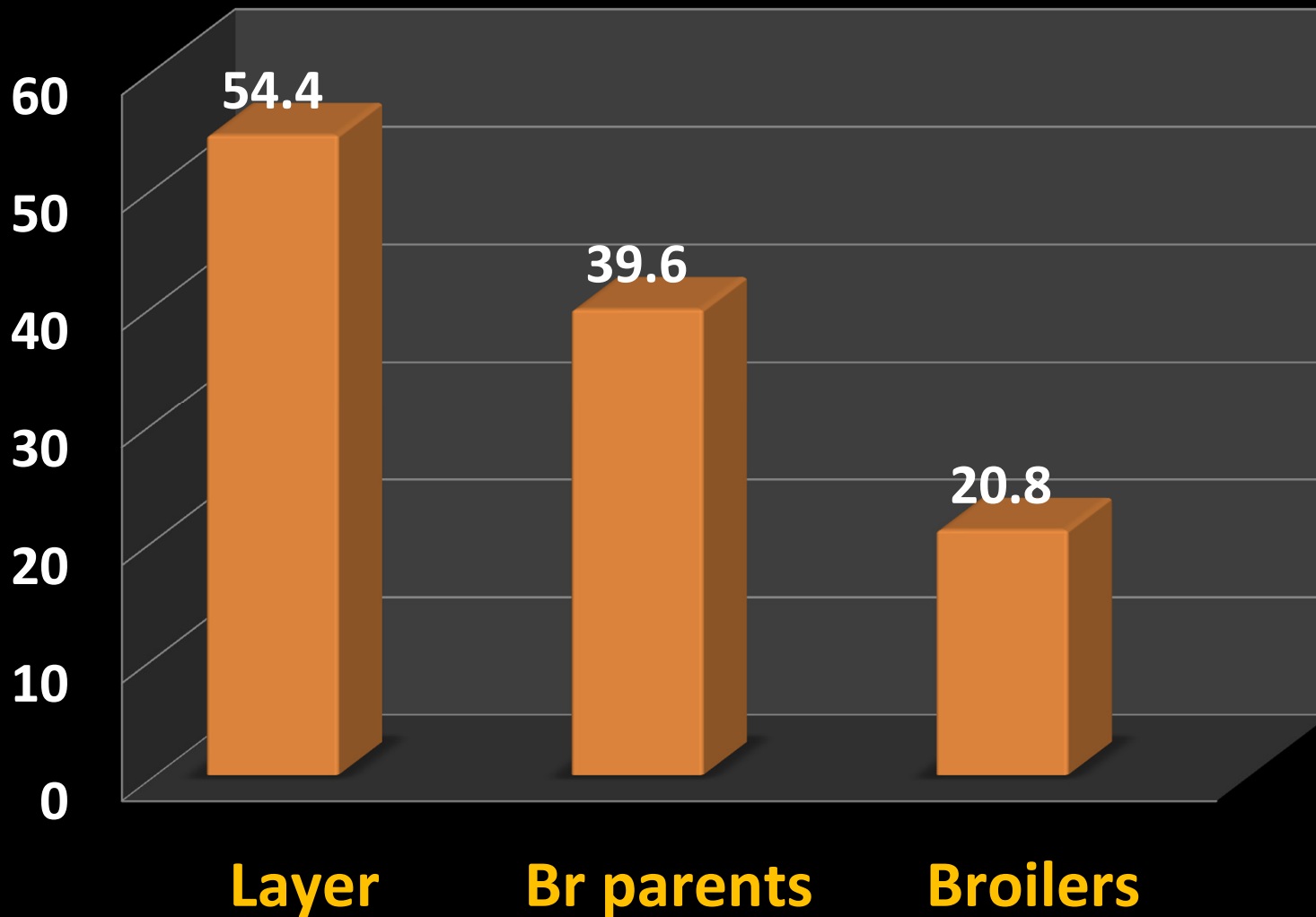
Seroprevalance of *M. gallisepticum* by ELISA (Based on Flocks)



Seroprevalance of *M. gallisepticum* by ELISA (Based on Samples tested)

Region	No. of Flocks Tested	No. of Flocks Positive	% Positive
Central	441	220	49.9
East	200	113	56.5
North	368	212	57.6
South	818	258	31.5
Total	1827	803	43.9

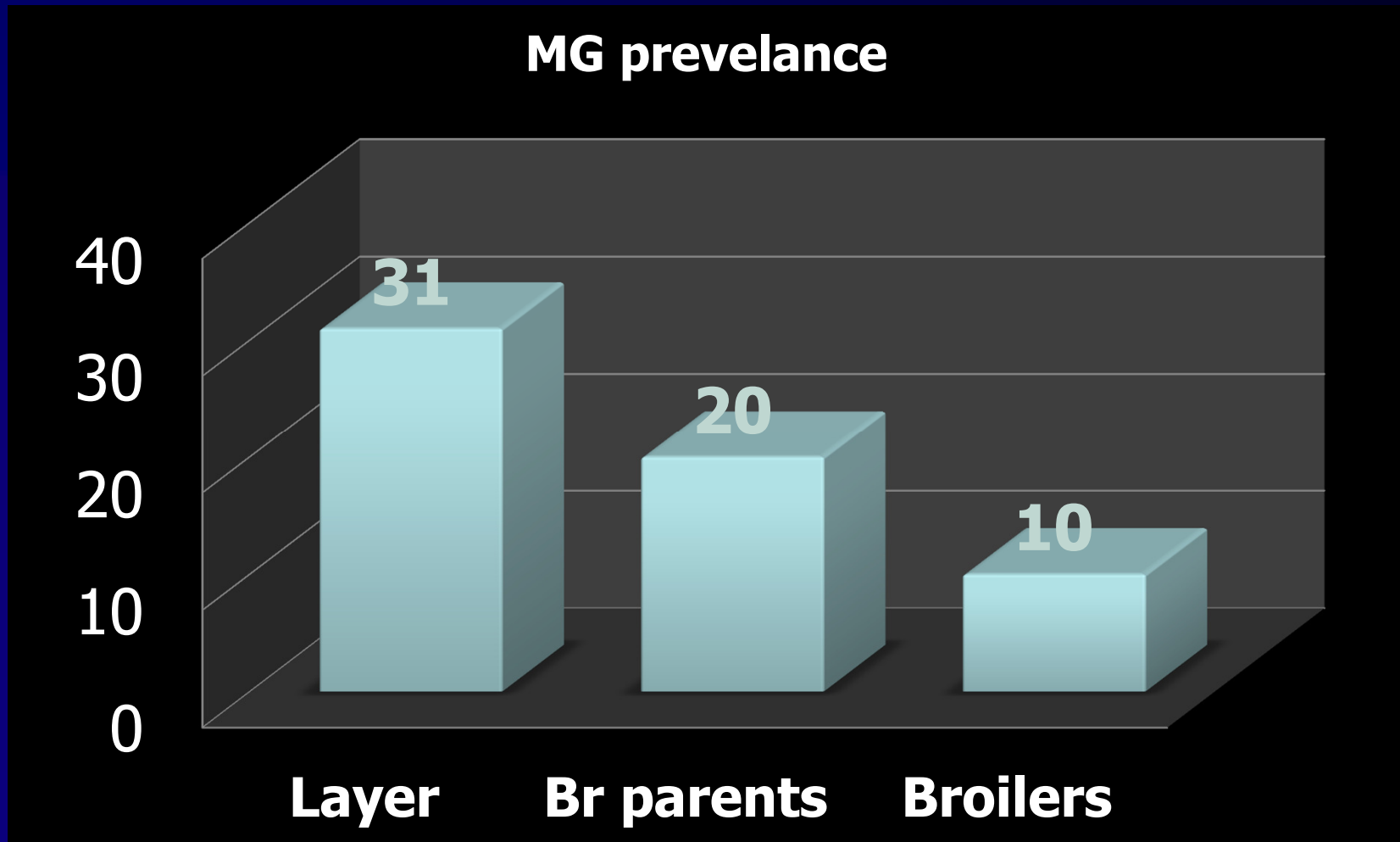
Seroprevalance of *M. gallisepticum* by ELISA (Based on samples tested)



Prevalance of *M. gallisepticum* by Isolation and Identification (Based on Flocks)

Region	No. of Flocks Tested	No. of Flocks Positive	% Positive
Central	22	7	31.8
East	10	1	10.0
North	15	2	13.3
South	27	7	25.9
Total	64	17	26.6

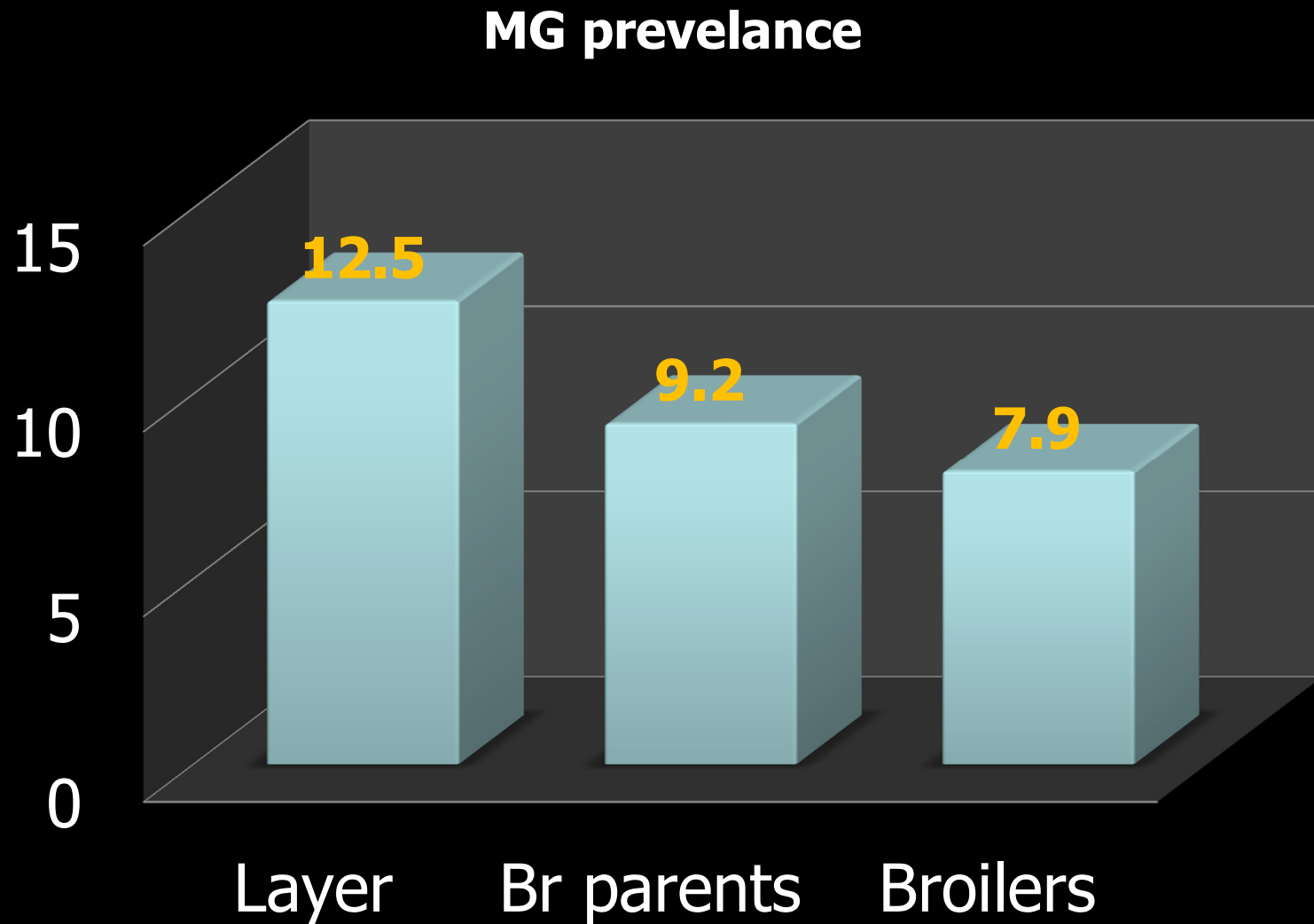
Prevalance of *M. gallisepticum* by Isolation and Identification (Based on Flocks tested)



Prevalance of *M. gallisepticum* by Isolation and Identification (Based on Samples tested)

Region	No. of Samples Tested	No. of Samples Positive	% Positive
Central	440	82	18.6
East	200	2	1.0
North	284	5	1.8
South	791	89	11.3
Total	1715	178	10.4

Prevalance of *M. gallisepticum* by Isolation and Identification (Based on samples tested)



Conclusions

- This study demonstrated the high prevalence of *M. gallisepticum* infection in Commercial Layers, Broiler Parents and Commercial Broilers in all major poultry growing areas of the country.
- Because of high value of individual parent breeder hen and their ability to infect progeny by vertical transmission, economic losses are potentially more severe when MG infection occurs in breeder flocks.
- The high prevalence and wide distribution of MG infection warrants development and adaptation of strategies to prevent or minimize economic impact of MG infection.

Thank you