

# IB 4/91 vaccine development and the concept of broadening protection (“protectotypes”)

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# Plan of talk

- **Safety studies**
- Efficacy studies
- Field trials
- Improving protection against IB challenges

# Live IB vaccine - requirements

- Sufficiently attenuated, but not over attenuated (i.e. not protective)
- Safe even at a high over dose
- The attenuation is stable (no reversion to virulence)
- Compatible with other vaccines to be used

# Attenuation of the virus

- Passage in embryonated chicken eggs
- Tested for level of attenuation a regular intervals (damage to tracheal epithelium of young SPF chickens)
- Safety evaluated
- Efficacy evaluated

# Safety of high and repeat doses of vaccine

For all possible routes of vaccination, the 4/91 vaccine was administered at:

- 10 times the recommended dose at day-old
- The recommended dose on two occasions (2 weeks apart)
- Safety assessed by histology and low power microscopy (damage to ciliated epithelium)

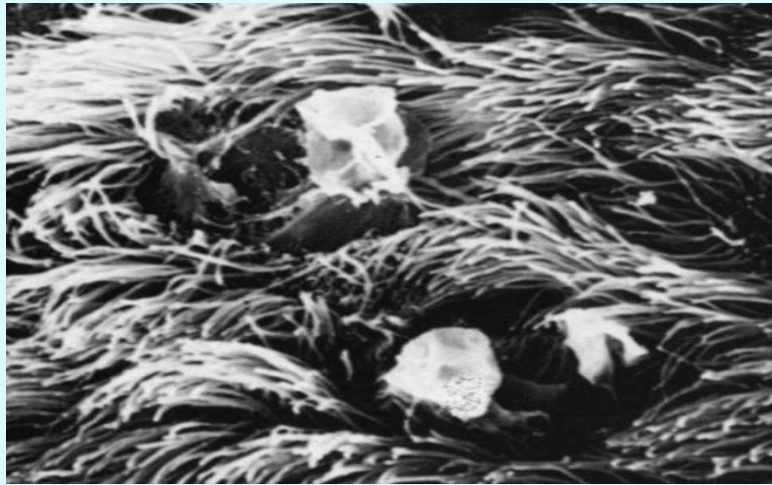


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- The recommended dose on two occasions (2 weeks apart)
- Safety assessed by histology and low power microscopy (damage to ciliated epithelium)
- **No adverse effects were observed**

# Assessment of tracheal damage by low power microscopy



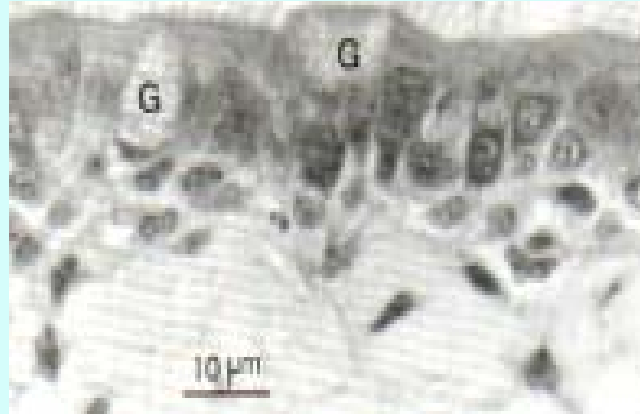
Normal trachea

A Malo

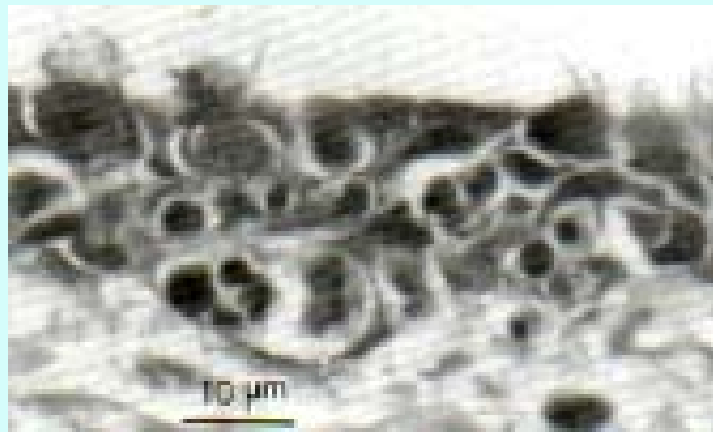
IB inoculated trachea



# Histology



Normal trachea (G= goblet cell)



Trachea 2 days post inoculation



Trachea 5 days post inoculation

# No reversion to virulence

- Vaccine virus passaged 10 times in one-day-old SPF chickens
- Damage caused by virus after 6 & 10 passages compared to starting virus and original "field" viruses (histology & low power microscopy)



# No reversion to virulence

- Vaccine virus passaged 10 times in one-day-old SPF chickens
- Damage caused by virus after 6 & 10 passages compared to starting virus and original "field" viruses (histology & low power microscopy)
- **No evidence for reversion to virulence**

# Genetic stability of 4/91 vaccine

- Isolates of the 4/91 serotype collected over 7- year period showed "remarkable genetic stability" (Cavanagh *et al.*, 1998)
- S1 gene sequence of 5 different isolates showed >97% nucleotide similarity (Adzhar *et al.*, 1997)
- S1 gene sequence of 4/91 field isolates & the vaccine virus showed no detectable differences (Cavanagh *et al.*, 1999)

# Compatibility with other poultry vaccines

The vaccine can be administered safely at the same time as:

- Gumboro vaccines D78 and 228E
- IB vaccines Ma5 and H120
- ND vaccine Clone 30
  
- With live TRT vaccine, 14 days should be left between these two vaccines

# Compatibility of 4/91 and live TRT (APV) vaccine

- Recommended not to give IB vaccines at same time as APV vaccine
- Protection is **NOT** affected, but IB vaccines may affect the antibody response to APV

# Plan of talk

- Safety studies
- **Efficacy studies**
- Field trials
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# Efficacy studies

- Chicks (SPF & MDA+) challenged under controlled conditions - known virus dose
- *Protection determined by :*  
virus re-isolation from trachea and/or assessing damage to the tracheal epithelium

# Efficacy studies

Minimum recommended dose given to SPF or MDA+ chicks by:

- Eye drop (day-old)
- Spray (day-old)
- Drinking water (7 day-old)

Challenged with virulent virus 3 weeks later

-

# Efficacy studies - SPF

Vaccination route	Tracheal damage		Challenge virus re-isolated	
	Vaccinated	Control	Vaccinated	Control
Eye drop	0/20	10/10	0/20	10/10
Spray	0/20	10/10	0/20	10/10
Water	0/20	10/10	0/20	10/10

# Efficacy studies - MDA+

Vaccination route	Tracheal damage	Challenge virus re-isolated
Eye drop	100% protected	None
Spray	100% protected	None
Water	90% protected	None

# Efficacy studies

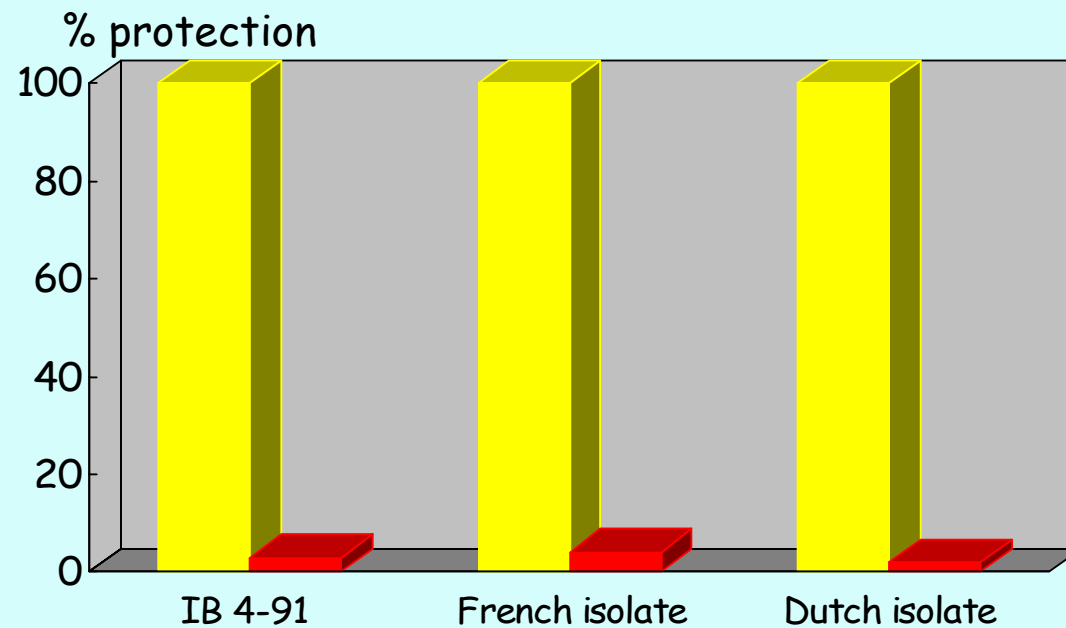
Minimum recommended dose given to SPF or MDA+ chicks by:

- Eye drop (day-old)
- Spray (day-old)
- Drinking water (7 day-old)

Challenged with virulent virus 3 weeks later

- **Good protection by all routes of vaccination**

# Challenge with other 4/91 viruses



■ Vaccinated ■ Not vaccinated

# Duration of immunity

- At least 6 weeks (longer than the life time of a broiler)
- For prolonged immunity (future breeders & layers) chickens should be revaccinated about every 6 weeks

# Plan of talk

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# IB 4/91 field trails (UK and France)

Sheds of commercial broilers vaccinated by coarse spray:

Shed	Vaccination schedule	
	Day-old	14 days
1	H120	-
2	H120	4/91
3	H120	H120

# Field trial - field results

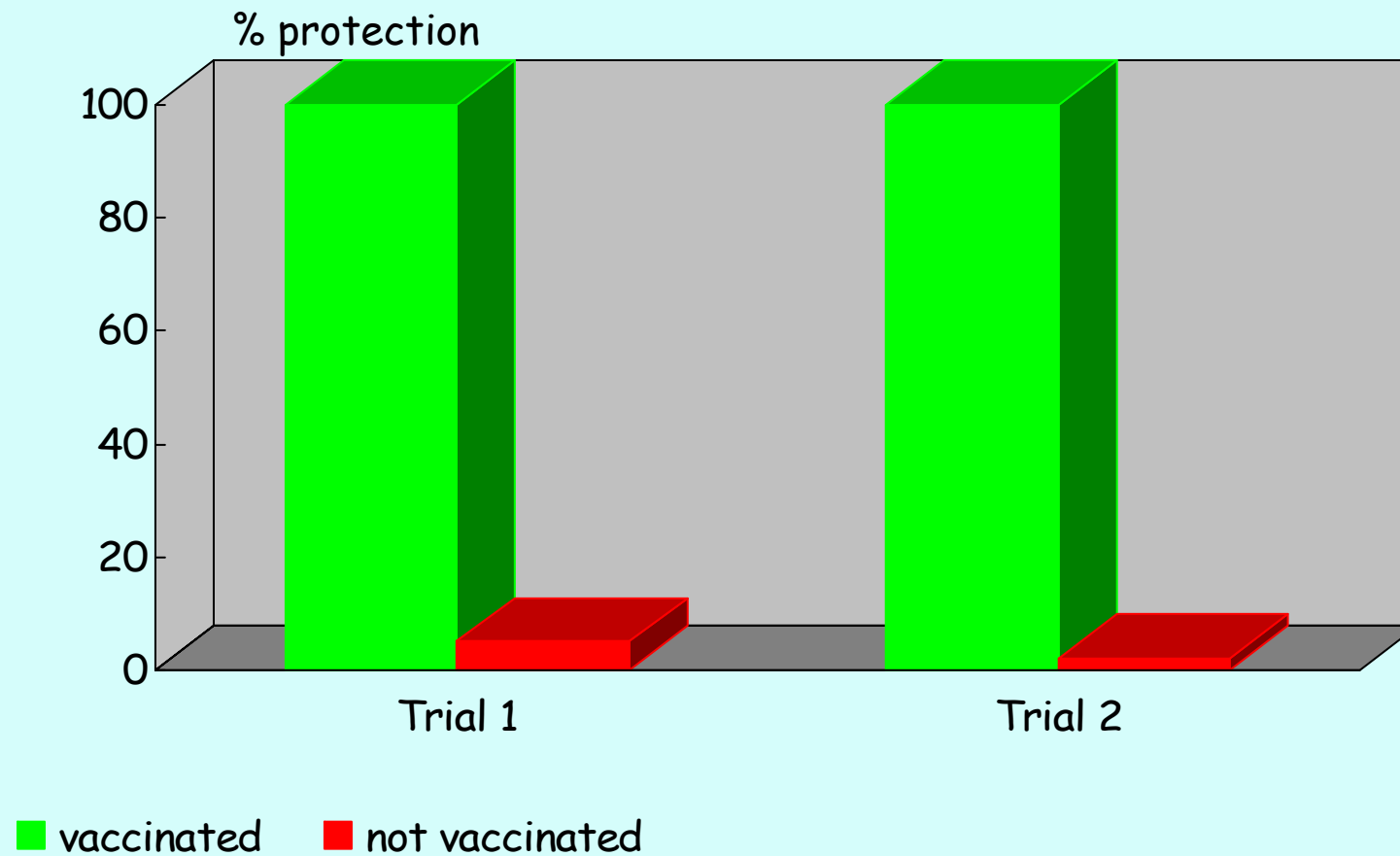
- Serum samples taken in the first 10 days of age confirmed the presence of 4/91 MDA
- The mortality and condemnation rates between the groups were similar
- The 4/91 vaccinated flocks showed a **significant improvement in economic return compared to the other groups**

# IB 4/91 field trails - UK

- Since natural field challenge cannot be guaranteed, groups of broilers taken to the laboratory at 14 days (pre 4/91 vaccination)
- Other broilers, vaccinated with 4/91 under field conditions, taken to laboratory 3 days after vaccination
- Both groups challenged with pathogenic IBV 4/91 3 weeks post vaccination and resistance to challenge assessed using the ciliostasis test

# Laboratory challenge

the vaccinated birds were well protected



# Vaccination strategy

- From day-old by coarse spray or eye drop route
- From 7 days via drinking water
- Future layers & breeders: from 3 weeks of age by eye drop, coarse spray or via drinking water

# Suggested vaccination schedule

Age at vaccination	1 day	14 days	6-10 weeks	16-18 weeks
Broiler	Ma5	4/91	-	-
Layer/ breeder	Ma5	-	4/91	Mass type inactivated

# Conclusions so far

## *Nobilis 4/91 vaccine is:*

- Safe & efficacious in the laboratory
- Stably attenuated
- Effective under field conditions

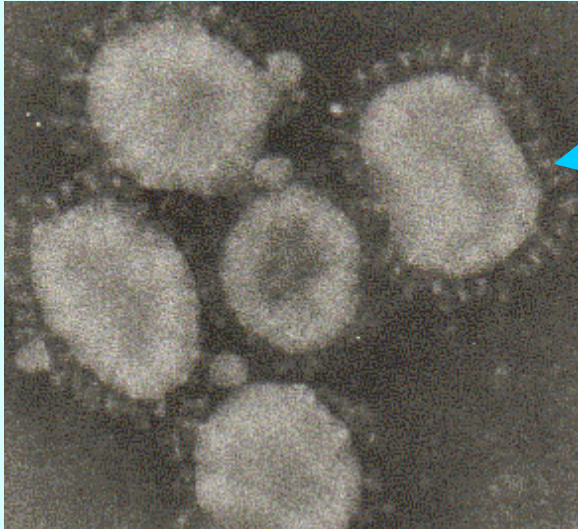
# Plan of talk

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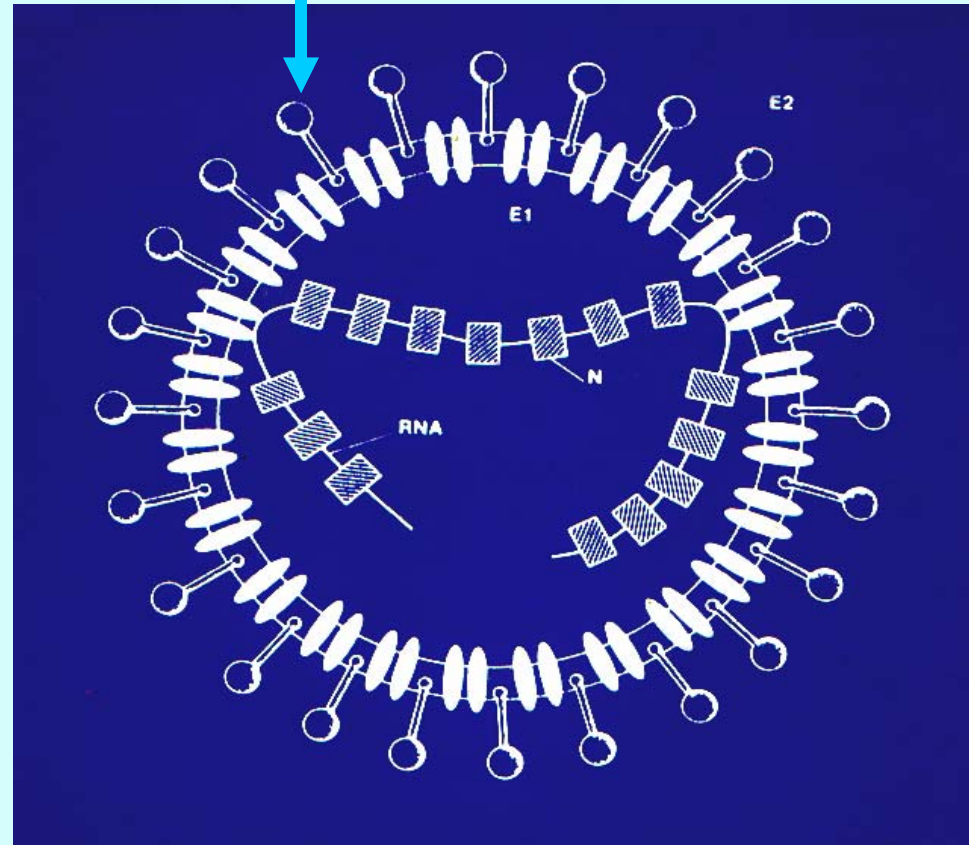
# What is the problem?

- Number of IB variants is increasing
- Different in each part of the world
- Important ones change with time
  
- Do we need new vaccine for each new variant!!
- *No!* Because protection is broader than typing methods suggest

# Infectious bronchitis virus



The spike protein



# Spike glycoprotein of IBV

- Induces neutralising antibody and therefore protective immunity
- Important in emergence of new variants, *because: A few amino acid changes may result in new serotype either by mutation or recombination*
- *Therefore- most of genome is unchanged*

# Awareness of variants is crucial for control of IB infections

Second important point to remember:

- Vaccinal antibodies must be able to recognise the epitopes on the spike if they are to be effective and neutralise the virus

These 2 points are very important for control strategies

# Ways to classify IB viruses

- Serology → Serotype
- Molecular techniques → Genotype
- Disease caused → Pathotype
- What happens in the bird → **Protectotype**

# Which typing method is most useful?

**Serotyping/genotyping is useful for:-**

- epidemiology
- research

**For control in the field:-**

- **protection studies** are more important

# Broadening protection

- Modify vaccination programme to include the use of two **different IB vaccines**
- Experimental data (using "ciliostasis test") to demonstrate this

# Protection studies in young chickens

## *Objective*

- Protection against different IB variants (including newly emerging ones) using Nobilis Ma5 & 4/91 vaccines

# IB Ma5 vaccine

## *Characteristics*

- Massachusetts serotype
- Plaque picked

## *Benefits*

- Improved immunogenicity
- Breaks through MDA, but safe with low MDA
- Use at any age
- Can combine with ND vaccine

# Ciliostasis test

*10 rings from each trachea*

3 x top

4 x middle

3 x bottom

***Score***

0 = all cilia beating

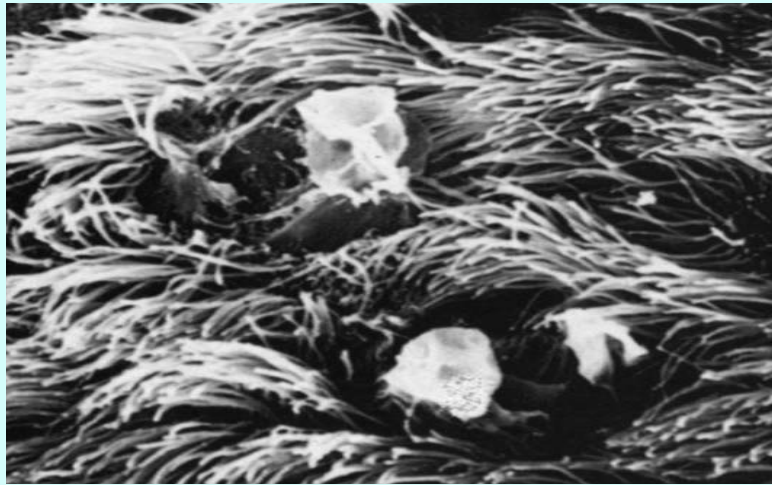
1 = 75% beating

2 = 50% beating

3 = 25% beating

4 = all cilia stopped

# Assessment of protection "ciliostasis test"



Normal trachea

A Malo

IB inoculated trachea

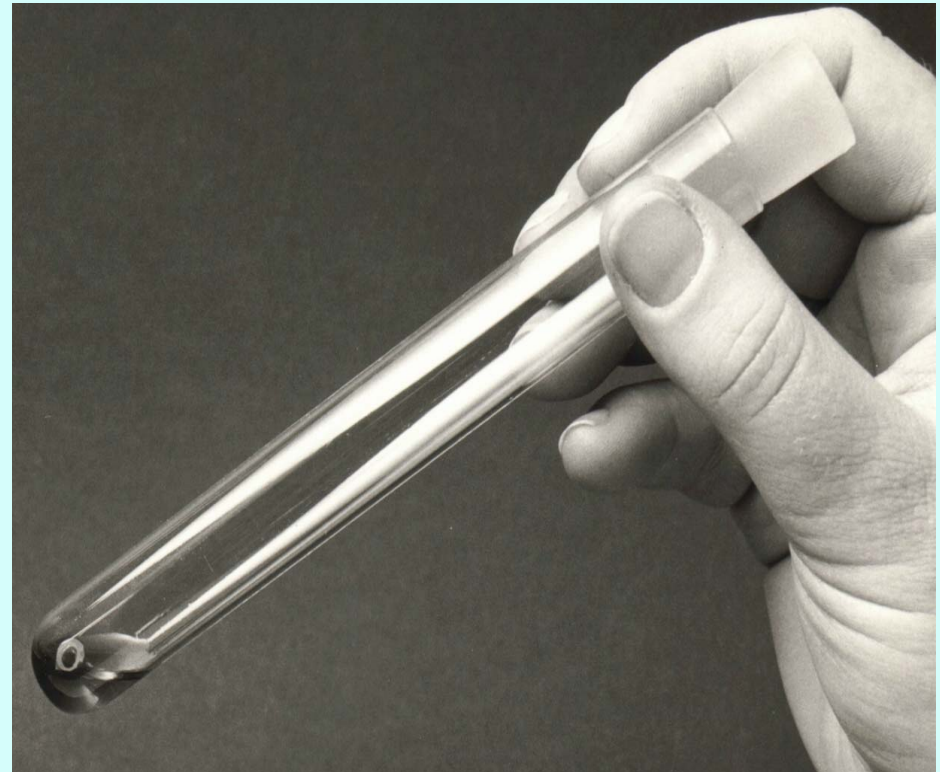


# Preparing tracheal organ cultures



Chopping the cultures

Ready for incubation



# Experimental design

Vaccination-	Day-old	2 weeks
	Ma5	none
	None	4/91
	Ma5	Ma5
	Ma5	4/91
	none	none

Challenge - 5 weeks of age

Protection assessed - 5-7 days later

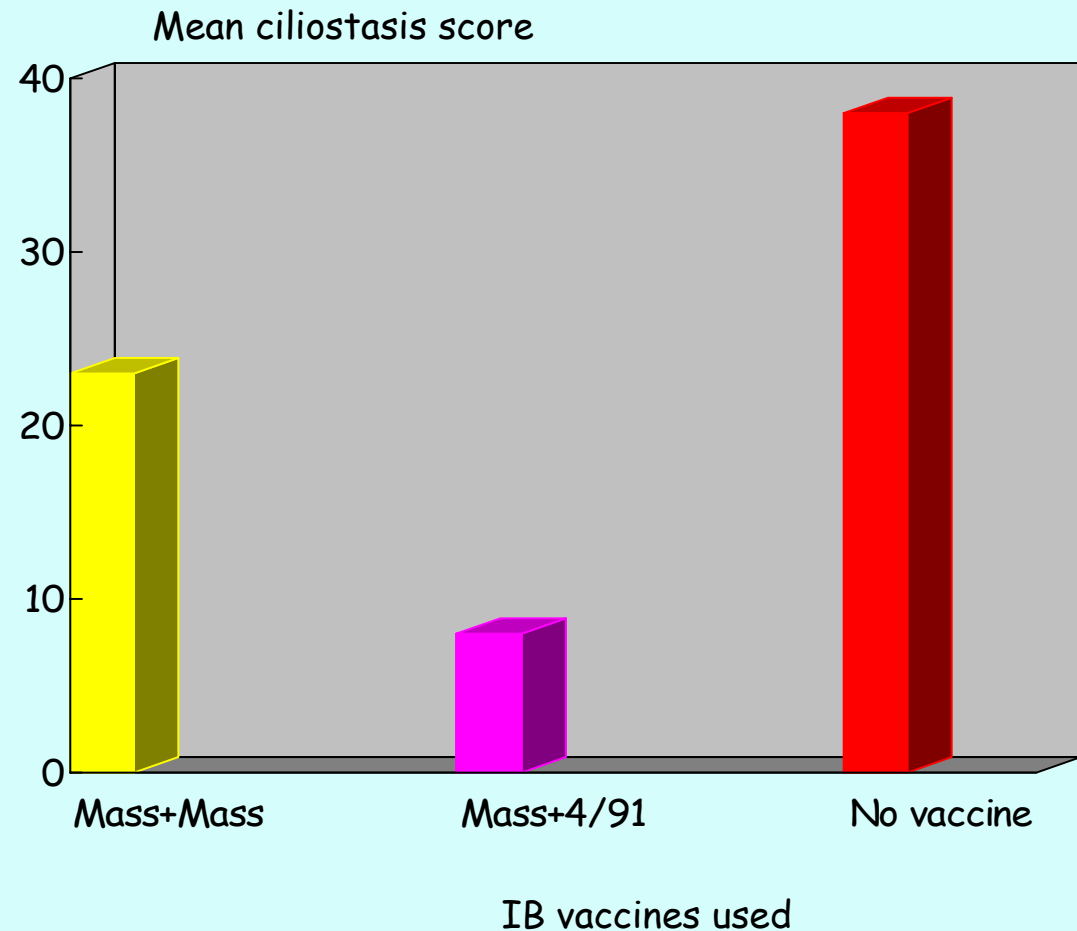
# Improved cross protection

## 1st experiment:

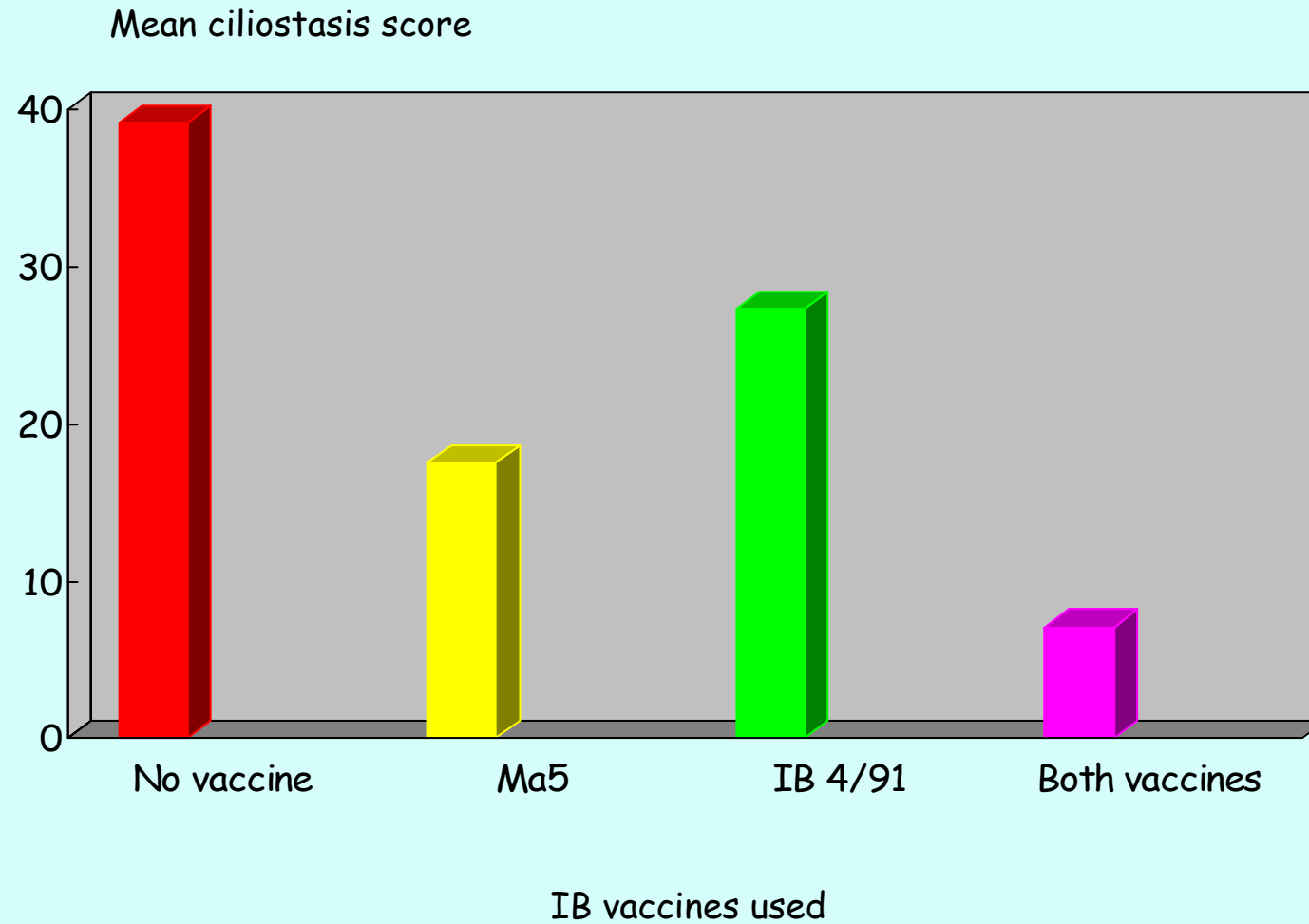
Ma5 at day old and at 14 days or Ma5 at day-old & 4/91 at 14 days

## Conclusion:

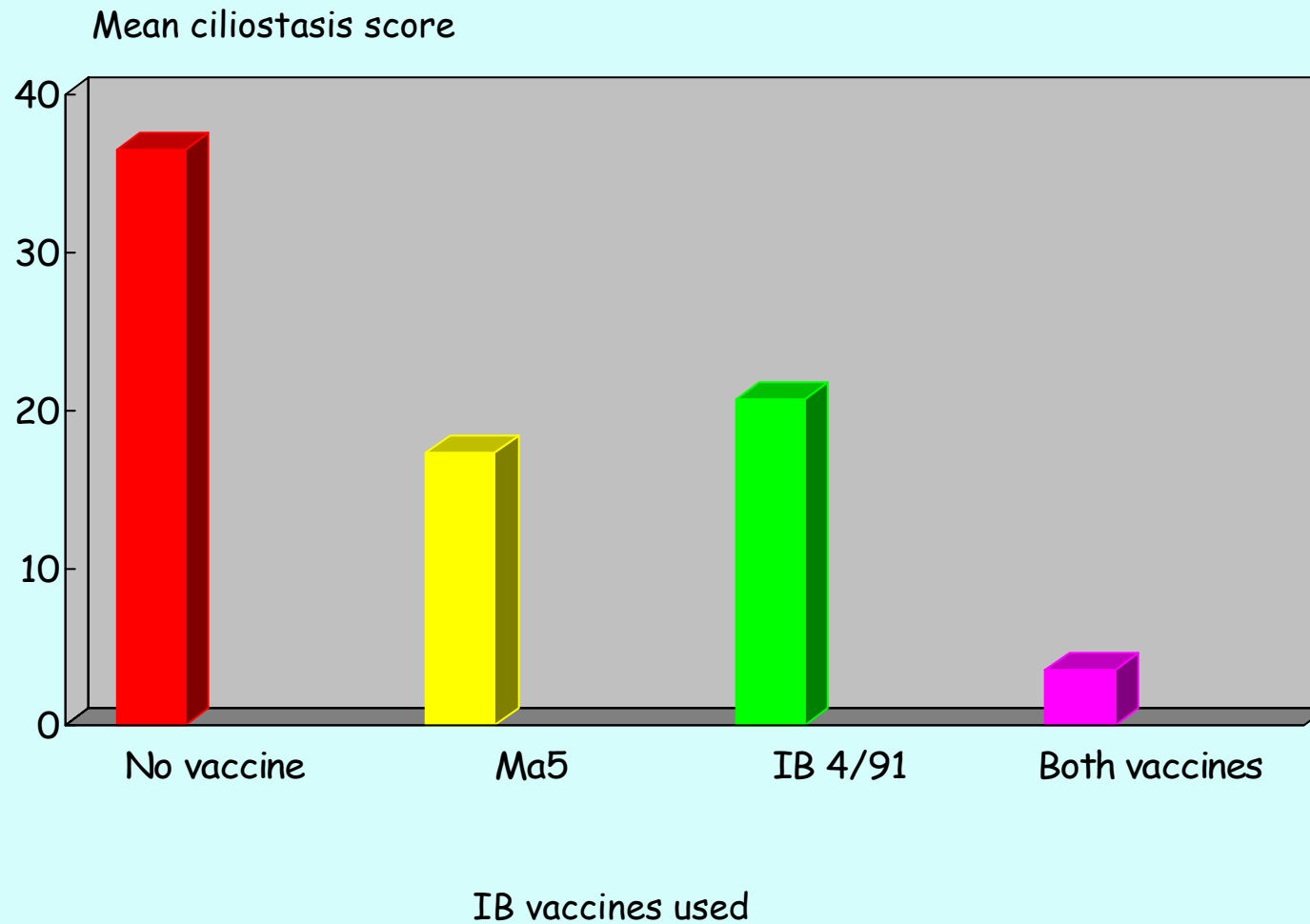
Two doses of the same vaccine not as effective as *using two different vaccines*



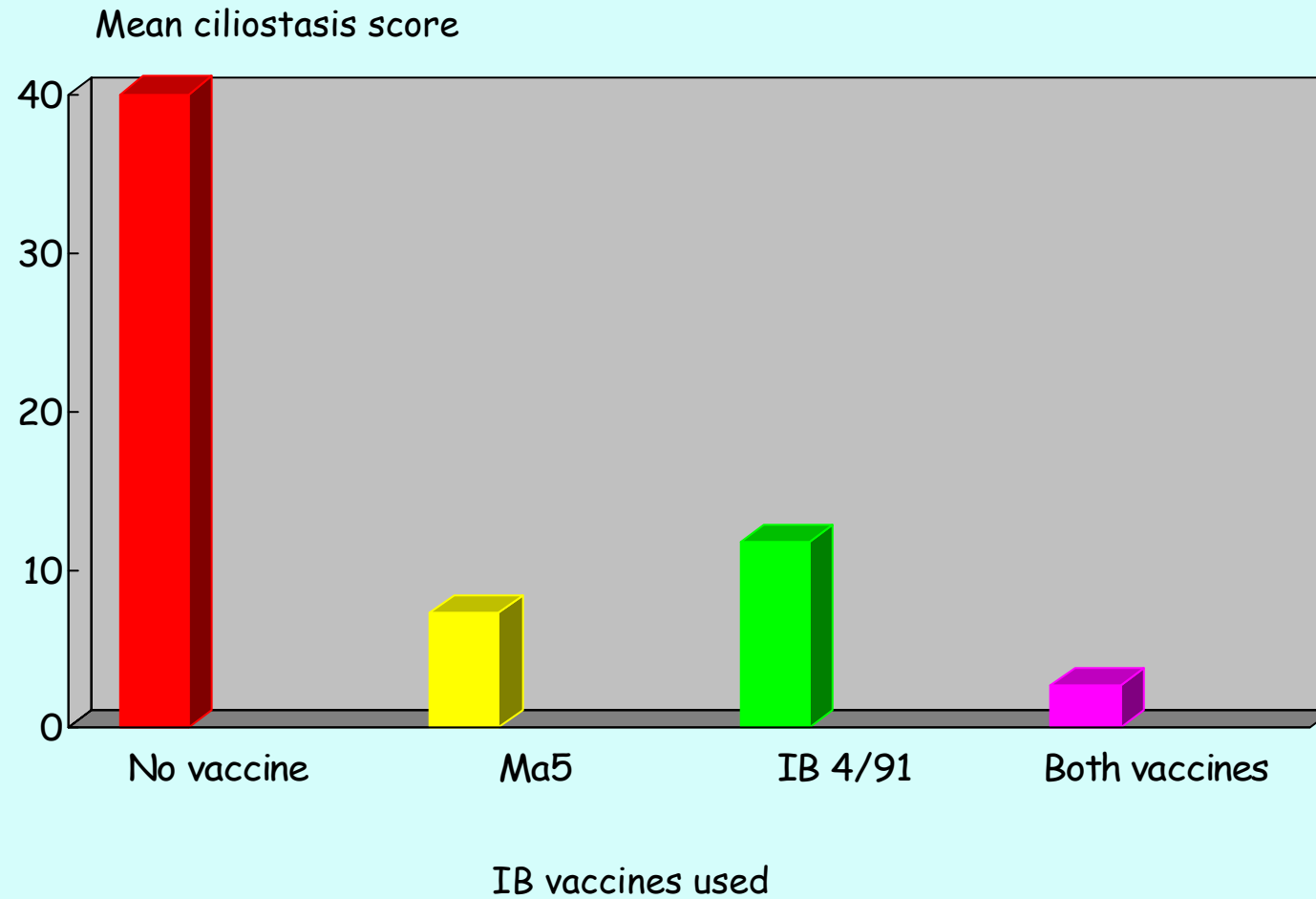
# IB isolate from Honduras



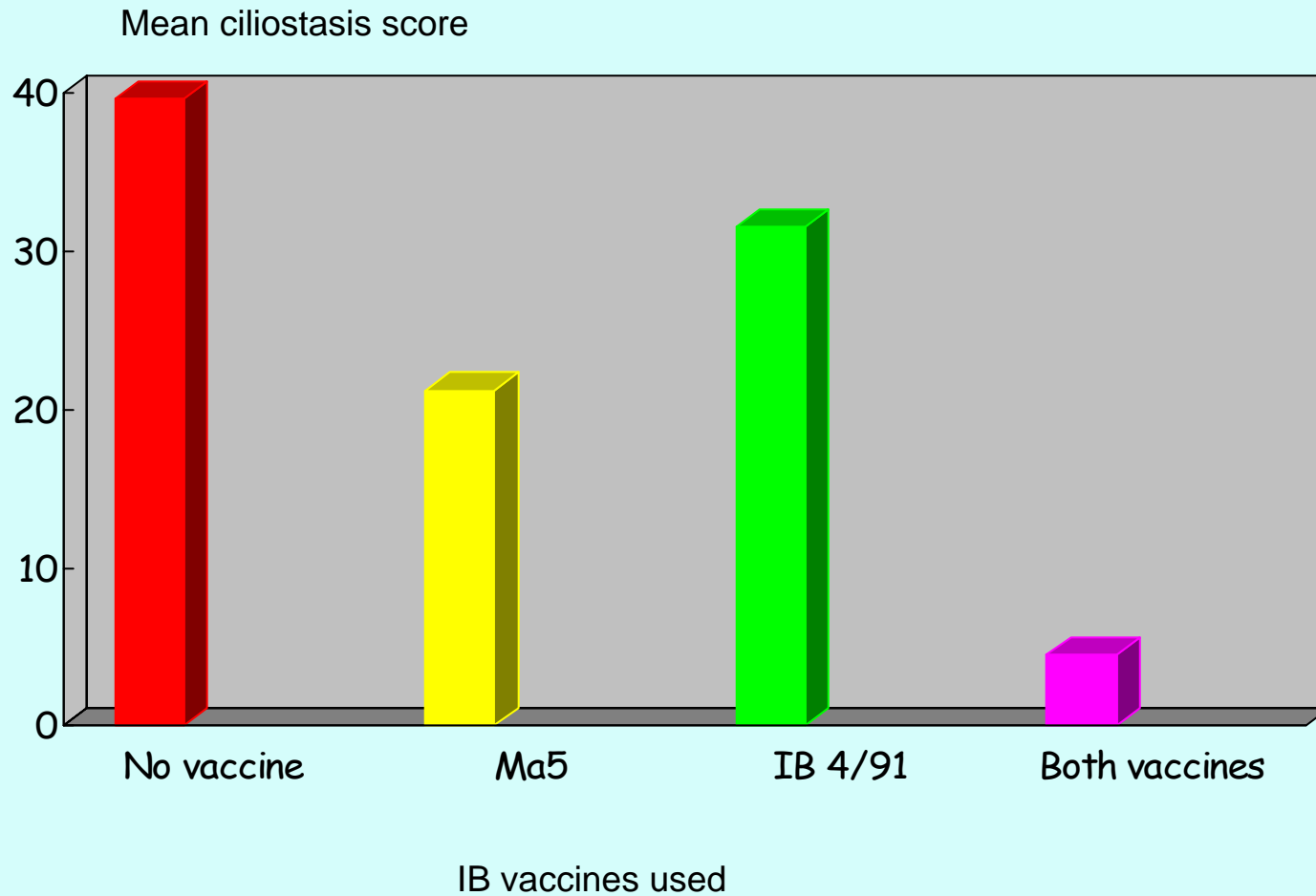
# IB isolate from Taiwan



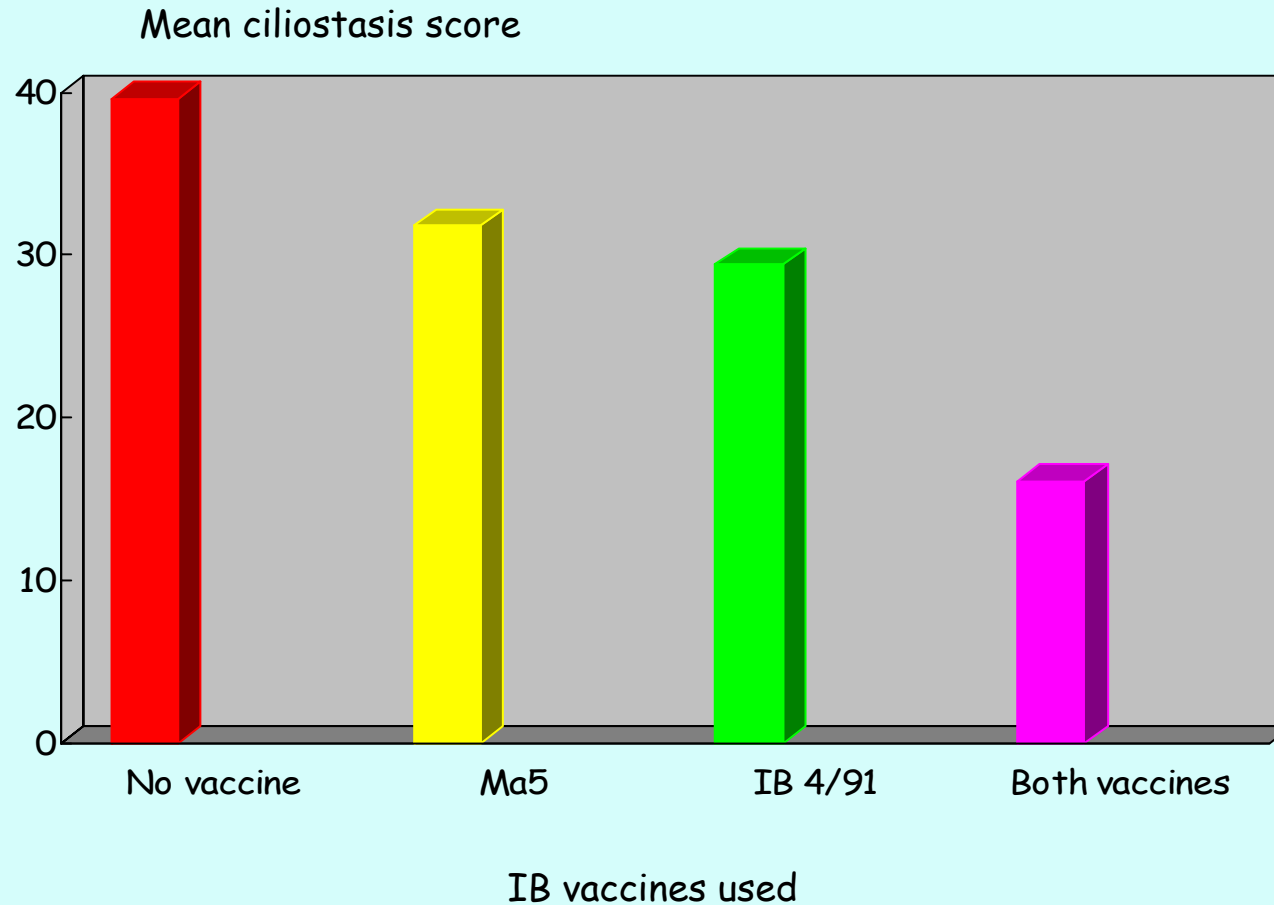
# IB isolate from S. E. Asia



# IB isolate from South Africa



# This programme will not protect against every IB variant!



# Studies in broilers- experimental design

**Vaccination -**  
(spray)

Day old	2 weeks
Ma5+4/91	none
Ma5	4/91
none	none

**Challenge -** Mass at 6 & 8 weeks of age

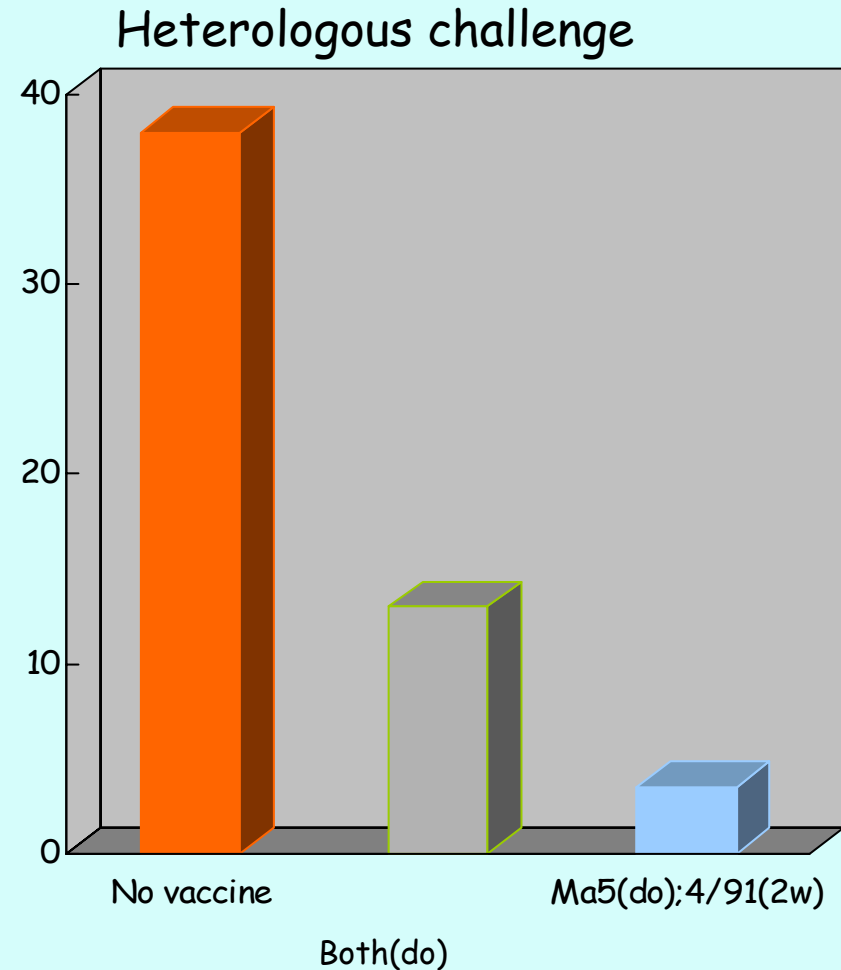
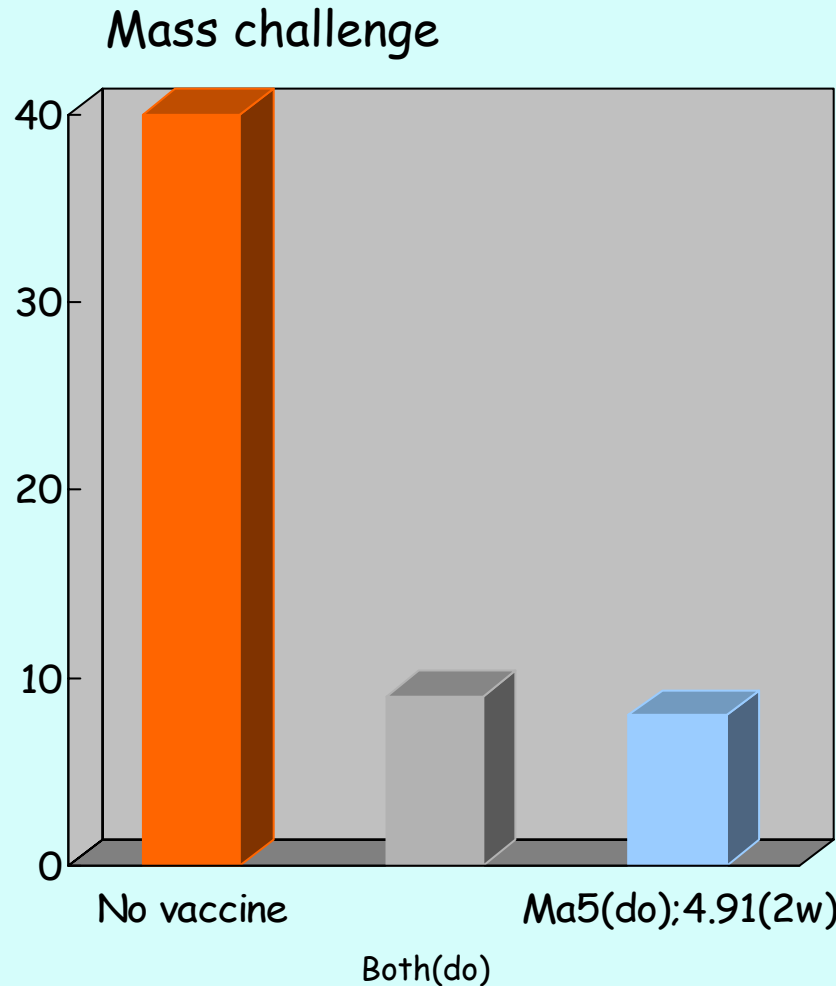
**Protection assessed -** virus isolation 5 days later

# Studies in broilers- results

Vaccination		Challenge	
Day old	2 weeks	6 weeks	8 weeks
Ma5+4/91	none	protected	protected
Ma5	4/91	protected	protected
none	none	not protected	not protected

Use of both vaccines at day-old protects against homologous challenge

# Timing of vaccinations



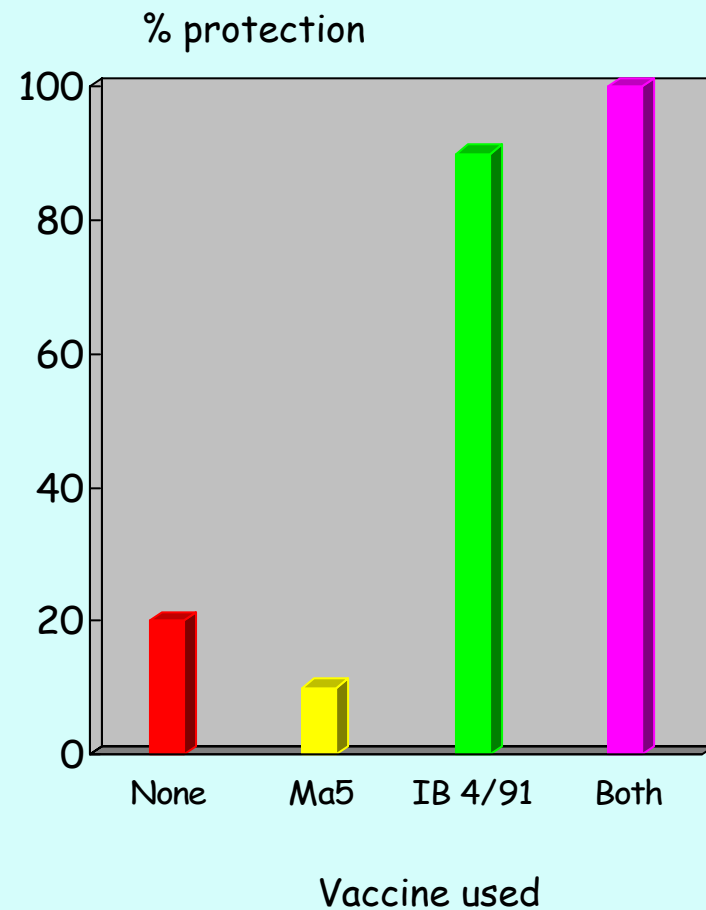
Heterologous challenge; benefit from delaying 2<sup>nd</sup> vaccination

# Protection of kidneys

*Broadening of protection  
using Mass & 4/91  
vaccines*

## *Parameters:*

- Mortality
- Gross & microscopic lesions
- Viral detection by PCR



# Protection against variant IB challenge in breeders & layers

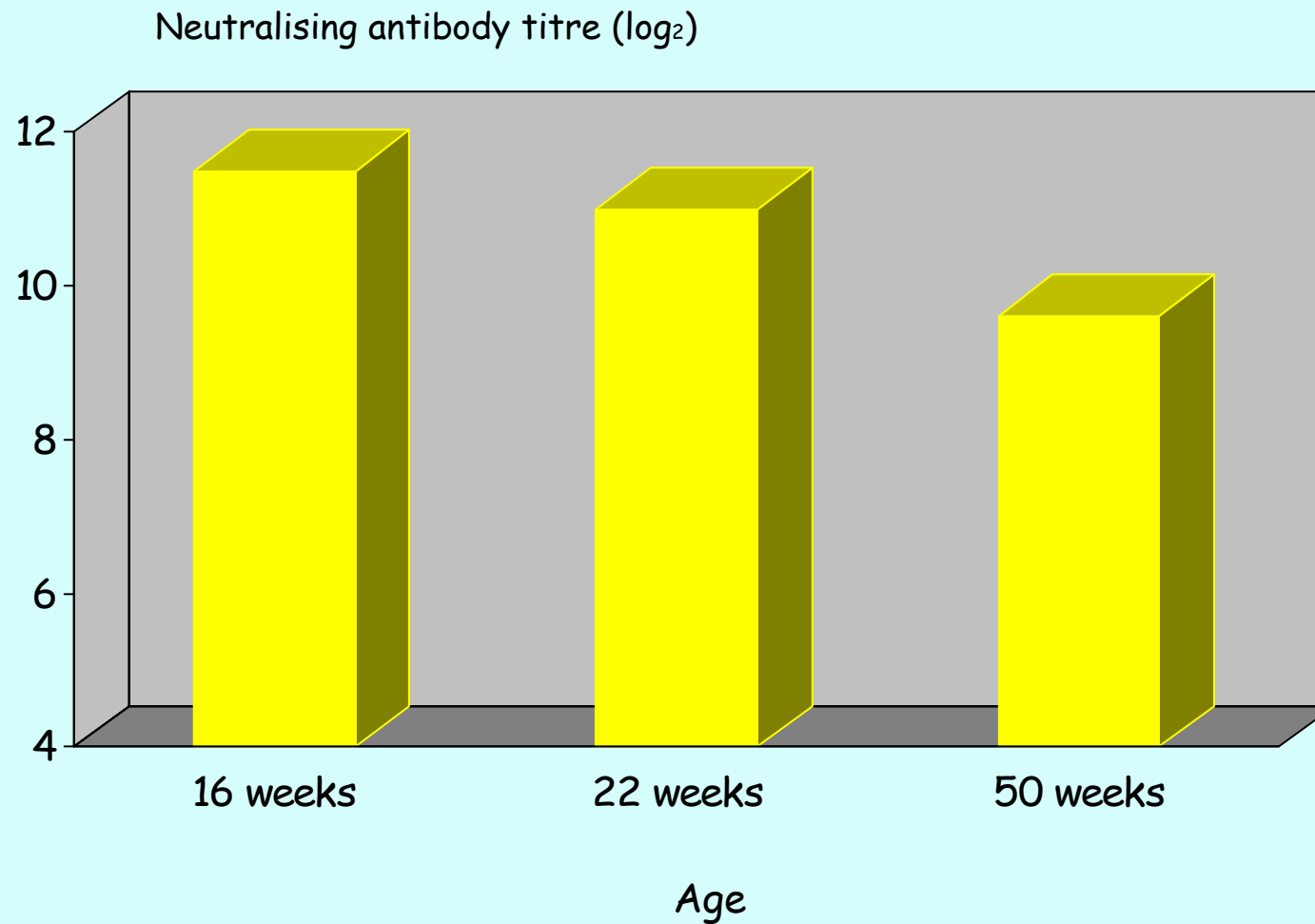
- Massachusetts 4/91 priming, followed by Massachusetts or IB multi inactivated vaccine protects against 4/91 challenge at the end of the laying cycle
  - Without the need for homologous inactivated vaccine

# Cross protection studies - inactivated vaccines

## *Experimental design*

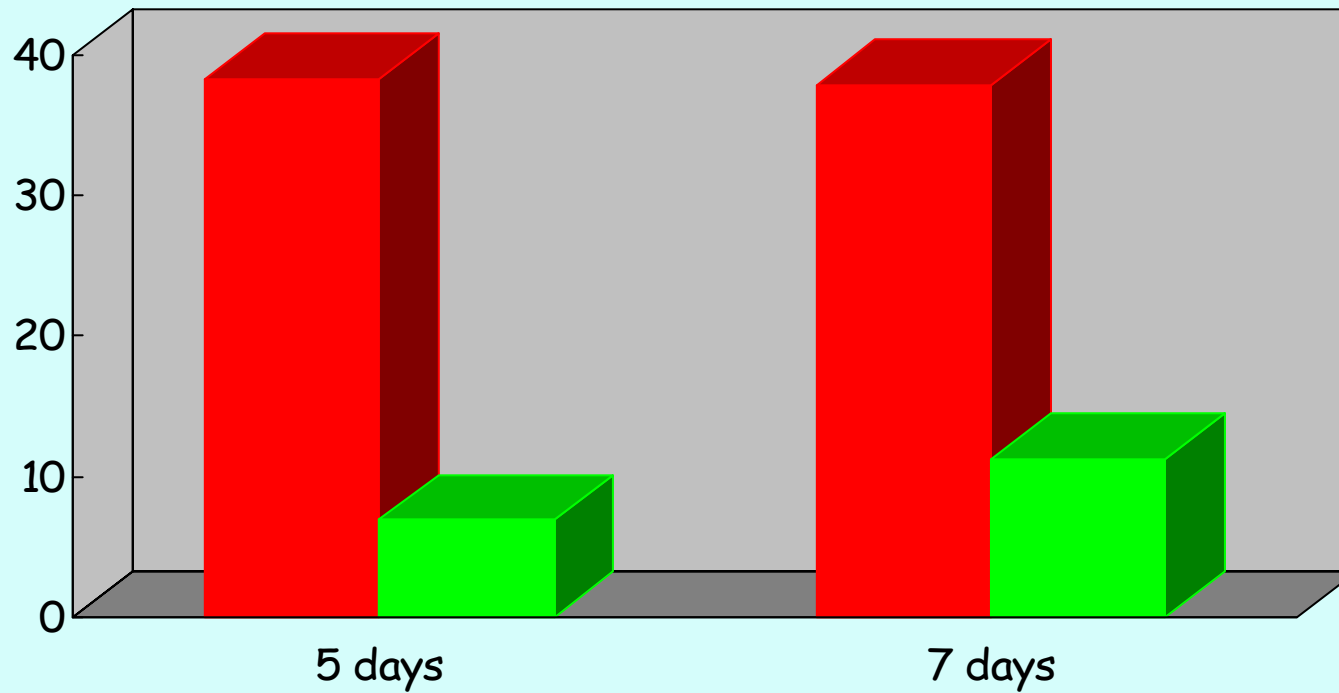
Group	Vaccination			Challenge
	1 day	6 weeks	16 weeks	
Vaccinated	Ma5	4/91	Inactivated	56 weeks
Control	-	-	-	56 weeks

# Serology- 4/91 antibody titres



# Challenge at 56 weeks

Mean ciliostasis score



■ not vaccinated ■ vaccinated

# Conclusions

## Broadening protection - broilers

- The combination of a Massachusetts vaccine and 4/91 protects against *many* (but not all) IB variants
- Therefore it is a worthwhile programme to use
- For **heterologous protection**, better to use Massachusetts first, then 4/91, rather than both together

# Conclusions

## Broadening protection - layers

- Priming with a Massachusetts vaccine and 4/91 can help broaden protection during lay
- Careful administration of inactivated vaccine is very important
- Use of IB multi may help to broaden protection against **heterologous** challenge

# Improved Control of IB

## *Broilers*

- Careful vaccination
- Revaccinate
- Use a different 2<sup>nd</sup> vaccine
- **Only use vaccines to IBVs present in the area**

## *Breeders/layers*

- Careful priming
- Same or different strain
- 6 weeks between priming & inactivated
- Bivalent inactivated
- Use H120 during lay

# Careful vaccination

- **Store vaccine correctly**
  - refrigerate
  - not in sunlight
  - use within date
- **Apply carefully**
  - all birds exposed
  - ensure birds are healthy
  - *spray*: control droplet size
  - *water*: clean drinkers (no chlorine)

# Conclusions 1

- The increasing prevalence of new IB serotypes creates difficulties in the design of adequate vaccination programs
- It is neither desirable or necessary to develop new live vaccines for each new serotype
- The concept of protectotypes is highly relevant from a practical point of view

## Conclusions 2

- The use of IB vaccines of different serotypes broadens protection against many variants
- **In broilers**, vaccination at day-old with Ma5 and at 14 days with 4/91 gives very broad protection against many variants (*"Golden" program*)
- **In layers and breeders**, priming with Ma5 at day 1 and 4/91 at 6 weeks, then boosting with IB multi also induces broad protection

# Conclusions 3

## Live attenuated IB vaccine Nobilis 4/91:

- Proven safety
- Applied via spray, eye-drop or drinking water
- An effective primer for Nobilis IB inactivated vaccines
- Gives broad protection against many IB variants when used in combination with Nobilis IB Ma5

A scenic landscape photograph featuring a river or lake in the center, surrounded by dense green trees and foliage. In the foreground, a wooden fence runs across the bottom right. The sky is bright with some clouds. The text "Thank you for your attention" is overlaid in a green, stylized font.

Thank you for your attention