

Lung Health



# A PRACTICAL GUDE TO GUDE TO DIAGOSOS DIAGOSOS Evine respiratory pathology



#### Bovine respiratory pathology

# A Practical Guide to Diagnosis

The economic losses associated with bovine respiratory disease are great, both for beef and dairy cattle. The administration of medical treatment or appropriate vaccination measures are initially based on identification, as accurately as possible, of the infectious causes, particularly if groups of animals are affected.

Clinical signs have a low predictive value of causal agent when considered on their own. However, a combination of epidemiological, clinical and pathological criteria produces leads to satisfactory diagnosis in a majority of cases. Even if not fully expedited, the post-mortem examination is an essential diagnostic procedure. The outcome of complementary tests depends on the initial orientation of sampling, guided by the clinical and necropsy phases, followed by quality of the samples and their condition at time of analysis.

This guide to diagnosis is intended to be practical in terms of presentation through the use of abundant illustrations. Its aim is to provide the practitioner with an effective tool at each stage of the diagnostic process with the objective of defining a few key principles and, at the same time, challenging a few common assumptions.

This project is the result of a fruitful partnership between

• A team of teachers from the **Ecole Nationale Veterinaire Toulouse (ENVT)**, who have been willing at all times to share their experiences of diagnosis in bovine pathology.

• The **Bovilis® technical team**, whose contribution to the prevention and treatment of bovine respiratory disease have made the Bovilis range a key player in the field of animal health in Europe and throughout the world.

Let us hope that this guide will increase operational knowledge of respiratory pathology, facilitate dialogue between practitioners and diagnosic laboratories and help veterinary surgeons keep abreast of the new diagnostic techniques.

**Professor François Schelcher, ENVT** 



# Preface



#### This work owes much to the pragmatic and scientific input of **Professor François Schelcher** and has also benefited from the considerable advice of colleagues working in various fields. We would like to thank, in particular:

- **Doctors Bertrand Guin** and **Hervé Ginestet**, practising veterinary surgeons, for their assistance and critical proof-reading,
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- Professor Paul Cabanié (ENVT), for his iconographic contribution,
- **Doctor Caroline Lacroux (ENVT)**, for her advice and contribution in terms of pathological anatomy and histopathology,
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- (Drs Olivier Bidaud, René Fournier, Philippe Houffschmitt and Yves Lagalisse).

#### Doctor Loïc Oliviero,

Technical Manager, Bovine Vaccines, Intervet/Schering-Plough, Co-ordinator Bovilis® Expert

#### Photo credits

- Paul Cabanié, François Schelcher, Caroline Lacroux ENVT (clinical, autopsy, pathological anatomy, histology)
- LDA 35, Rennes (analysis)
- Ed Van Der Meer, Pathology Dept., Intervet International bv (anatomy and lesions)
- Intervet SA, Angers (various)
- Nora Cesbron, ENVN (various)

The photos remain the property of their authors, any use is subject to their authorisation

#### Start with the basics



#### Caution

Example: acute and chronic refer to the duration of progression and not the severity of the condition



#### Do not confuse

Example: Do not confuse hypostasis with oedema

#### Tip

Example: Necrotic lesions typical of *M. haemolytica* make it possible to manage without a bacteriological examination



#### Never

Example: Pleurisy is never caused by a virus alone



#### WATCH OUT for pre-conceived ideas

Example: emphysema does not systematically indicate RSV

# There are three ways to use this guide

You can either

- read it from start to finish like a novel
- consult the INDEX and refer to the relevant sheet indicated
- go directly to the index tab that interests you, particularly in the **NECROPSY** and **ANALYSIS** sections

#### These two sections work in two ways

#### **NECROPSY**

**1** You identify a lesion (e.g.: emphysema) and are looking for the cause:

**2** You are considering a possible cause (e.g.: *Mannheimia* haemolytica) and searching for its lesional expression:

Pulmonary thromboembolism

# in the herd

Symptoms

Macroscopy

Microscopy



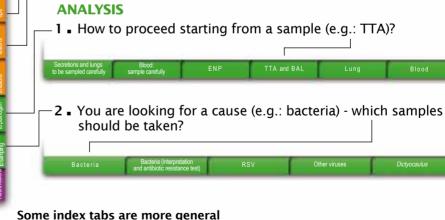


to pathogen

to sampling

bbreviations





**NECROPSY** • Practical tips

- ANALYSIS 

  From sampling to pathogen
  - Secretions, lung: Samples to be taken carefully.

## ANATOMY

Macroscopy

**Microscopy** 

## EPIDEMIOLOGY/CLINICAL MEDICINE

Disease progression in the herd

**Clinical signs** 

NECROPSY

**Practical tips** 

Upper respiratory tract – Pleurisy – Emphysema – "Congestion"/ Hepatisation - Necrosis - Bronchitis - Oedema - Atelectasis – Hypostasis and Autolysis

#### Note:

For pedagogical reasons, lesions resulting from mono-factorial infections are described in the chapter "from pathogen to lesions". These cases are relatively unusual in practice. In the index tab **"multiple infections"** the gross pathology more

frequently encountered in the field is described

From pathogen to lesions Mannheimia haemolytica - RSV - IBR - Dictyocaulus - Aspergillus - Thromboembolism – Multiple infections

#### **Duration of disease progression**



From sampling to pathogen

Secretions, lung: Sample carefully - Blood: sample carefully - Deep nasal swabbing - TTA (BAL) - Lung - Blood

#### From pathogen to sampling

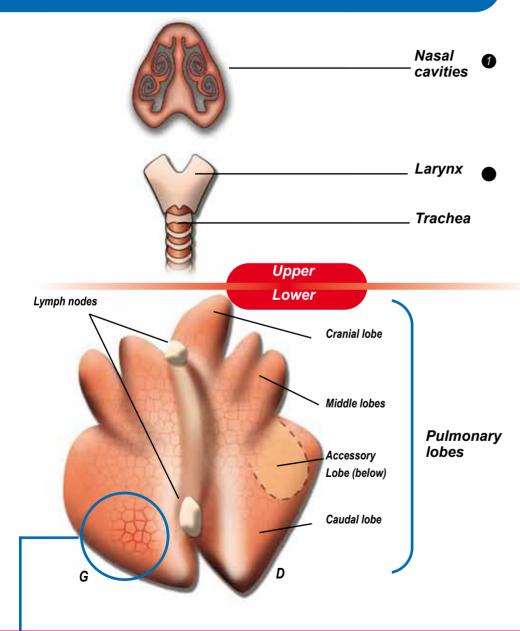
Bacteria - Bacteria (interpretation and antibiotics resistance test) - RSV - Other viruses - Dictyocaulus

INDEX

#### Index & abbreviations

# ANATOMY Macroscopy

## The respiratory system





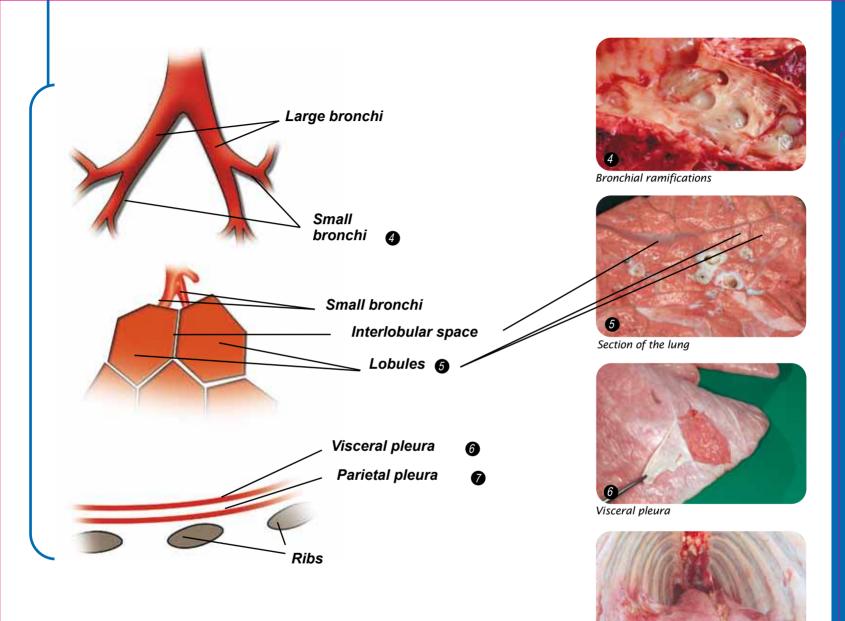
Cross-section



Larynx



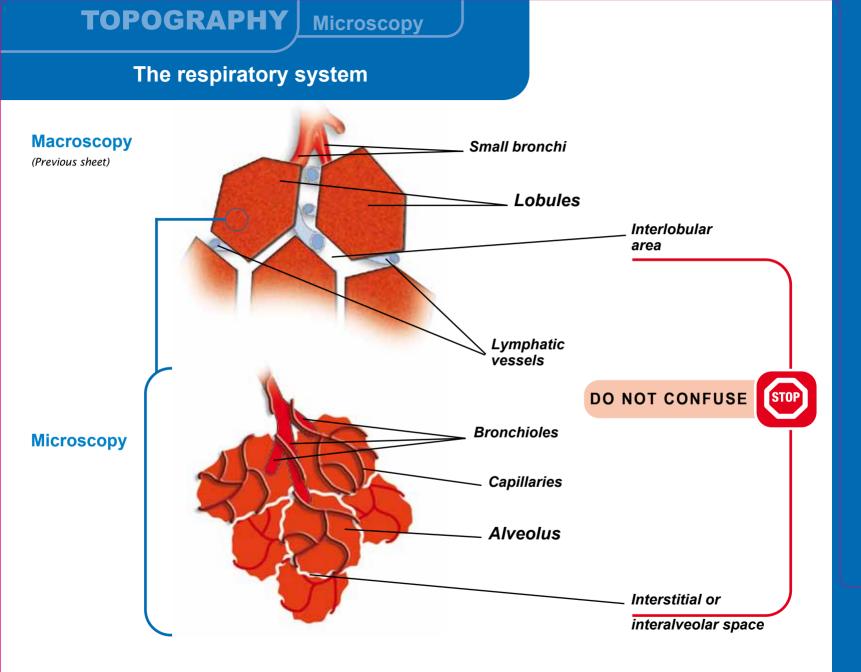
Pulmonary lobes

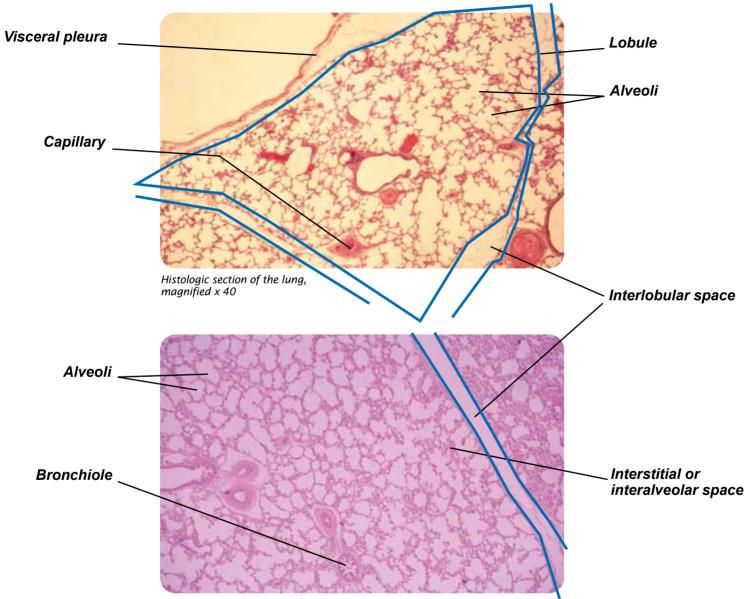


Parietal pleura, made conspicuous in this case by fibrous symphyseal pleurisy

(7

Sheet 1





Histologic section of the lung, magnified x 40

# **EPIDEMIOLOGICAL/CLINICAL MEDICINE**

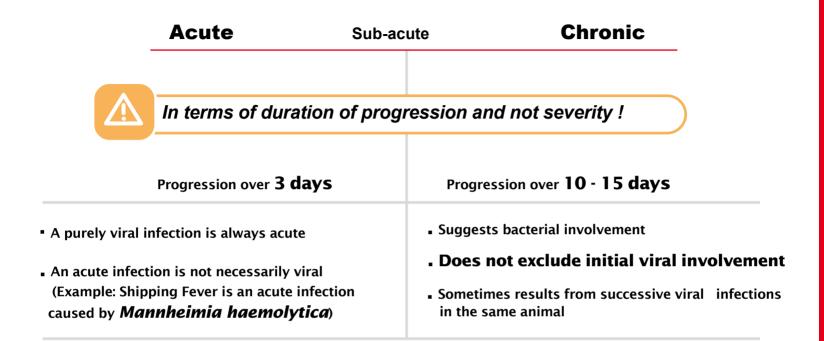
#### **Progression in the herd**

Epidemic	Endemic
"Epidemic outbreak"	"It's coughing all the time"
In terms of incidence = No	o. of new cases/unit of time
Definition: High incidence	Low incidence
Closed	herd
<ul> <li>Typically viral</li> </ul>	<ul> <li>Often bacterial</li> </ul>
<ul> <li>Frequently RSV</li> </ul>	Does not exclude viruses in synergy
<ul> <li>Does not exclude one particular bacterium: Mannheimia haemolytica</li> </ul>	with bacteria
Does not exclude non-infectious factors	Primary non-infectious factors

All in - all out fattening unit	
Frequent outbreaks	Endemics more rare
Viruses <b>and</b> bacteria	Bacteria
Infectious <b>and</b> non-infectious	Mainly non-infectious factors

#### **Continuous fattening**

Interpretation difficult





IMPORTANT No strict relationship between acute/chronic or epidemic/endemic and aetiology: an acute or epidemic episode can be bacterial (Mannheimia haemolytica)

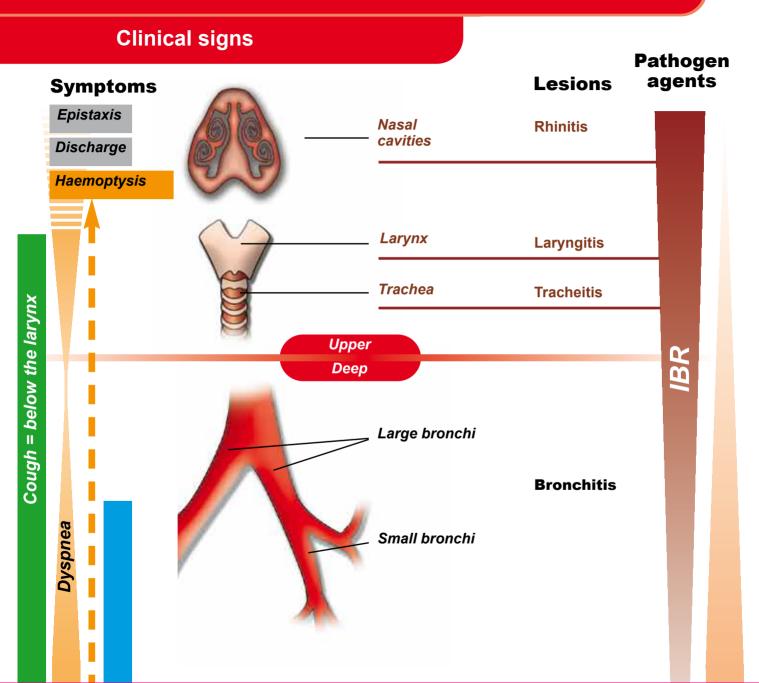
#### **Recurring problem in the herd**

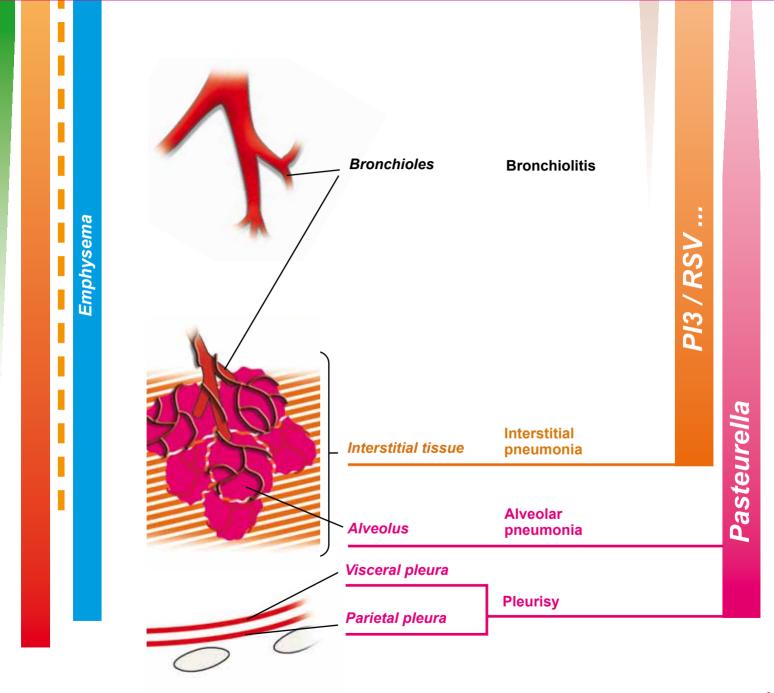
• In a closed herd: possible re-appearance of purely viral episodes; with RSV there is the question of chronic carriers (not demonstrated until now)

. In an open herd: possible involvement of several pathogens

Progression during preeding

# **EPIDEMIOLOGICAL/CLINICAL MEDICINE**





Symptoms

# **EPIDEMIOLOGICAL/CLINICAL MEDICINE**

### **Clinical signs**



- The appearance of the discharge can first be used to estimate the speed of progression
  - Serous: rapid progression 1
  - Mucous: prolonged progression ②
- Sometimes the discharge points to a cause
  - Yellowish: bacteria in the naval cavities 3
  - Abundant, erosions on external nares + pseudomembranes: 4 6
- Laryngitis can clinically mimic bronchopneumonia, though wheezing sound is specific

 Orthopnea\* (ARDS) is not specific to RSV (high predictive value however, for calves and young cattle)

- Acute bovine pulmonary oedema and emphysema (fog fever)
- Acute bacterial pneumonia
- If arthritis is present in a group of calves, consider *Mycoplasma bovis*. Possibility of IBP with *Mycoplasma bovis* without arthritis or arthritis without mycoplasma, accidentally associated with IBP
- "Intestinal flu" (diarrhoea outbreak affecting adults), occurring in the field, is an enteric syndrome with various causes, frequently including coronavirus.

\* Characteristic appearance: dyspnoea with extended members and stretched-out neck, which indicates acute respiratory distress syndrome (ARDS)



Serous discharge



Mucous discharge



Purulent discharge

erosion



Erosion: IBR



Discharge and pseudomembranes



Orthopnea

#### Epistaxis

Rare often **unilateral** 



Bleeding from the nostrils

## DO NOT CONFUSE

#### Haemoptysis

More frequent Often **bilateral** Blood in the mouth and nostrils Pulmonary origin: thromboembolism (sheet 22)



Epistaxis



Haemoptysis

# **NECROPSY**

#### **Practical tips**

• **Legal aspect** (EC Regulations No. 1774/2002 and 878/2004, rural code, book 2, articles L. 226-1 et seq., L.231-2, L.236-1, R226-1- R226-4 and R228-15; Code of Conduct, article R.242-33 and 242-83)

#### • Is it authorised on the farm?

Necropsies at the farm are **not permitted legally but are tolerated** by certain knackeries / rendering plants. Consult the latter with regard to the conditions for carcass recovery. In the absence of specific regulations or prohibition, respect the rules of good practice, particularly in terms of hygiene and safety. In the event of suspected ND, contact the SVS (State Veterinary Service).

#### • At the abattoir: Is this acceptable?

NO, this depends on the rendering plant. Necropsies conducted by an animal health expert are authorised subject to the conditions issued by the DDSV (Regional Veterinary Authority), provided the standards for installation and equipment are respected (Order of 06/08/2005).

#### • At the Regional Analysis Laboratory / National Veterinary College

- Ideal for taking useful samples
- Legal problem with transport (please refer to EC Regulation No. 1774/2002):
  - **In theory:** Vehicle suitable for the transport of substances in categories 1 and 2 and subject to authorisation, **accompanying document completed by the veterinary surgeon**
  - **In practice:** it is possible for the owner to provide transport. Transport can be organised by a small number of Regional Analysis Laboratories or National Veterinary Colleges

#### Complete autopsy necessary

- Do not limit the examination to the respiratory system e.g. lesions caused by ruminitis or liver abscess due to thromboembolism (young bulls) (*sheet 22*)
- Open the larynx, trachea, and bronchi and examine (section) the lymph nodes (tuberculosis etc.)

#### Timescale

- Following death
  - Summer: < 12 hours
  - Winter: < 48 hours
- Sampling for further analysis
  - Within 3 5 days after onset of infection (sheet 26)



#### Consider taking photos

- Ensure that the case being subject to necropsy is representative
- What you see is a photograph taken at a given moment: consider the aspect of progression
- Less important in case of "chronic" progression
- The predictive value of macroscopic lesions for pathogens is only appropriate for a small proportion of acute cases
- In the (frequent) absence of unambiguous lesions, the use of (laboratory) analysis is indispensable

#### Note:

For educational reasons, lesions caused by monofactorial infections are described in the chapter "from pathogen to lesions". These cases are relatively infrequent in practice.

In the index tab **"multiple infections"** the gross pathologies most frequently encountered in the field are presented (*sheet 23*)

#### **Upper respiratory tract**



#### Laryngitis

• Can clinically mimic bronchopneumonia

A minimal lesion can cause marked clinical signs ("croupous<sup>1</sup>" laryngitis)

• Consider IBR (sheet 19)

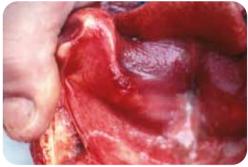
<sup>1</sup>: meaning necrotic laryngitis or diphtheria in the calf.

#### • Terminal tracheal haemorrhages

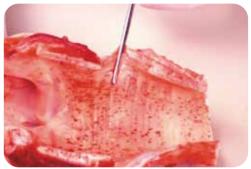
- These are petechiae
- These are terminal phase lesions

#### • Visual<sup>2</sup> dictyocaulosis

- Parasites present
- Mucus abundant
- <sup>2</sup>: Visual adult stage of the parasite



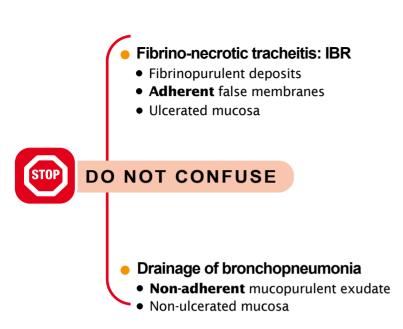
Laryngitis



Terminal tracheal haemorrhages

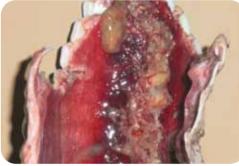


Visual dictyocaulosis





Fibrino-necrotic tracheitis



Exudate due to bronchopneumonia



Aspiration of regurgitated material (terminal)



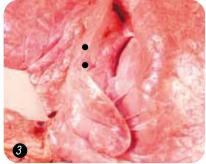
- Aspiration of regurgitated material (terminal)
  - "Non-lesion"
  - No inflammatory reaction in the mucosa

Sheet 7

#### Pleurisy

Attenuation of pulmonary sounds. Sounds "liquid" in case of liquid exudation. Possible confusion with pericarditis

- **Definition:** Inflammation of the pleura
  - On the lungs: visceral pleurisy
  - On the costal wall surface: parietal pleurisy ④
- Appearance and duration of progression
  - Fibrinous: Minimum 24 48 h
    - White yellowish
    - Slightly adherent
    - Elastic 🛛 🖉
  - Fibrous: 15 days 3 weeks 3 4
    - White
    - Very adherent
    - Non-elastic



Fibrous pleurisy



Localisation

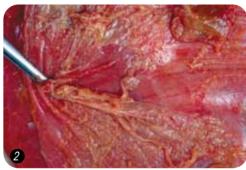
significance

without diagnostic

Fibrous pleurisy

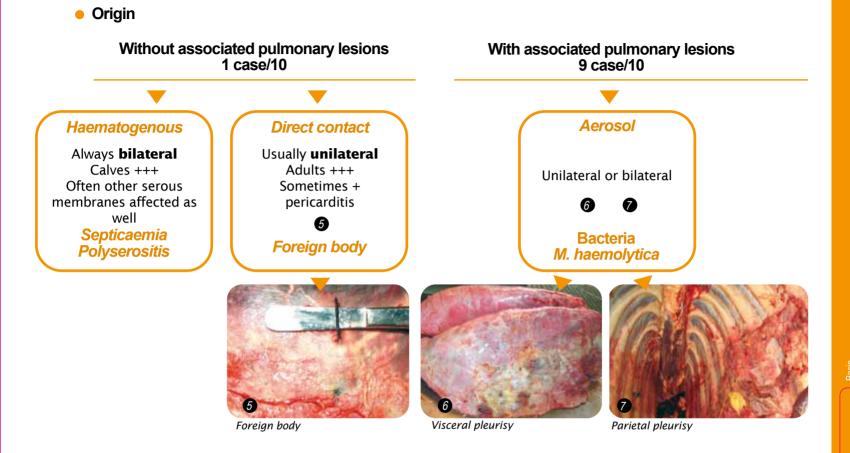


Fibrinous pleurisy



Fibrinous pleurisy







IMPORTANT Acute progression + pleurisy: Consider *M. haemolytica* 



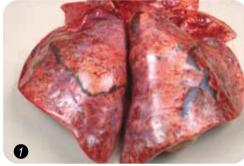
Pleurisy is **NEVER** caused by a virus alone



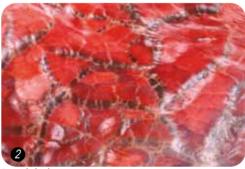
• **Mechanism:** alveolar distension due to air caused by a bronchial obstruction (whatever its cause) and likely to spread in the following areas:

4 5

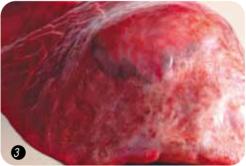
- Lobular
- Inter-lobular = swollen appearance
- Sub-pleural = bubbles on the surface
- Subcutaneous



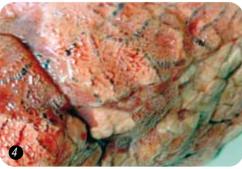
General appearance







Interlobular dissecting



Sub-pleural



Sub-pleural



#### Does not indicate the cause\*.

3 examples of emphysematous lungs - What is the cause?







V2A :Y9W2NA

Answer: IBR

Answer: DICTYOCAULOSIS

\* Most cases of bovine bronchopneumonia are "obstructive" and therefore promote emphysema. Emphysema alone: poor predictive value for the pathogen.



Look for lesions associated with emphysema:

- Section of the bronchi
  - Mucus and parasites: Dictyocaulosis (sheet 20)
  - Tracheitis: IBR (sheet 19)
  - Bronchial mucopus: RSV (sheet 18)
- Section of the lungs
  - Typical necrosis: Mannheimia haemolytica (sheet 17)

#### Terminal emphysema

Often without associated pulmonary lesion Associated with terminal frothy oedema (*sheet 17*)

• Acute bovine pulmonary oedema and emphysema (fog fever) Consider this possibility during the autumn grazing period

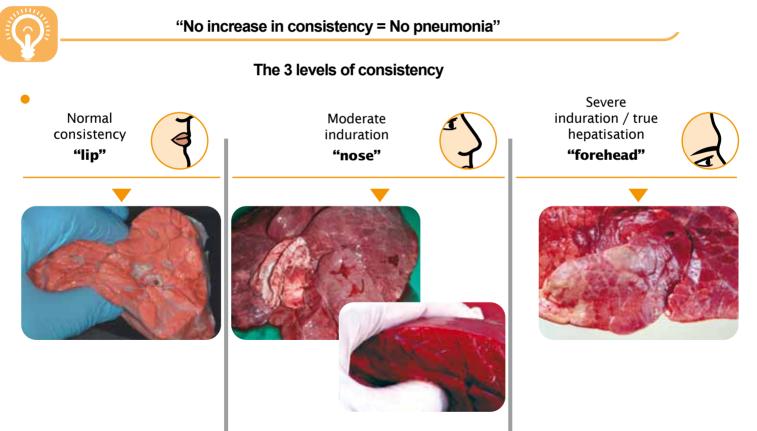
\* In the chronological sense

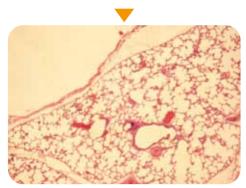
Sheet 9

#### "Congestion"/Hepatisation

#### Definition

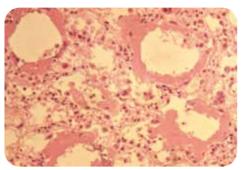
- Please note: In the field, the terms congestion, hepatisation or consolidation / induration are used interchangeably and incorrectly when the lungs are "red"
- The red colour indicates "congestion" while "hepatisation" or consolidation / induration indicate increased consistency



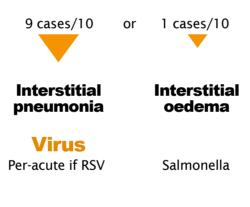


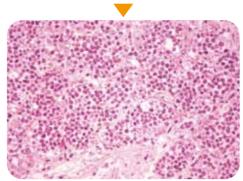
Histological section of the healthy lung





Interstitial" pneumonia





"Alveolar" pneumonia. Macrophages and polynuclear neutrophils invade the alveoli

Alveolar
pneumonia



(does not exclude an initial virus)

#### **Necrosis**

Definition: Death of tissues in the living animal

#### Either specifically due to *M. haemolytica*: FREQUENT

Can be seen on the surface 1 or more frequently in the section 2

- 1 2 cm diameter, irregular contours
- +/- grey, with characteristic whitish edge
- · On one or more lobes, especially cranial
- In the indurated areas
- Microscopic interpretation:
- Degeneration of neutrophils (action of the leucotoxins) 3
- "Oat grain" cells in the periphery (Oat cells)

# This lesion specific to *M. haemolytica* is not, however, systematic (sheet 17)

#### • Or aspergillosis: RARE

- Can be seen on the surface **5 6**
- 2 3 mm in diameter
- Blackish edge possible 🚺
- Multiple foci: all in the lobes, including the caudal lobes

#### Or necrotic-purulent focus: SPORADIC

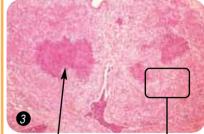
- Several centimetres
- Often old
- Progression of bronchopneumonia due to bacteria other than *M. haemolytica: A. pyogenes, E. coli, Pseudomonas*, necrobacillosis etc.
- Aspiration Pneumonia
- Foreign body



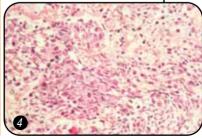
On the surface



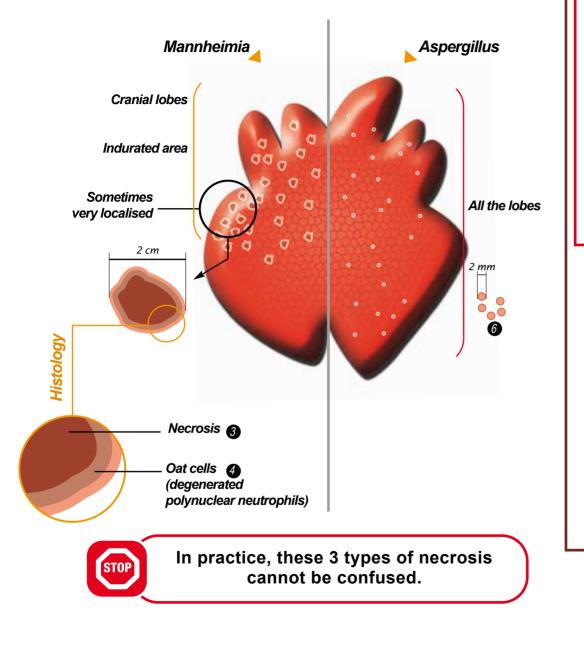
In the section



Necrotic focus

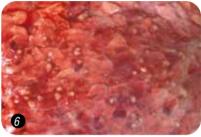


"Oat cells"

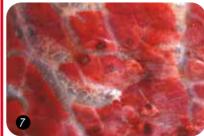




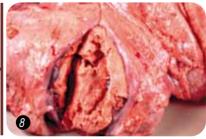
Aspergillosis



Aspergillosis



Blackish edge



Sheet 11

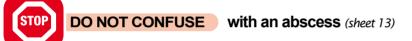
Necrotic-purulent focus

#### **Bronchitis**

**Definition:** macroscopic (bronchitis) and microscopic (bronchiolitis) lesions.

Following the **section** and when **the affected** lobes are subjected to pressure: pus or muco-pus flows.

#### • Or purulent bronchitis 🛛 🚺 🤇



#### Chronic progression

- 1- Purulent bronchitis
- 2- Bronchial abscess: bronchial wall clearly visible 3
- 3- Bronchiectasis: bronchial dilation with purulent
- content = chronic lesion ④



Often associated with true chronic hepatisation (alveolar pneumonia, *sheet 10*)



Purulent bronchitis



Purulent bronchitis (section)



Bronchial abscess



Bronchiectasis

#### • Or mucopurulent bronchitis 6

#### Secretions

- less thick
- less opaque
- rather white

#### Acute to sub-acute progression



Mucopurulent bronchitis

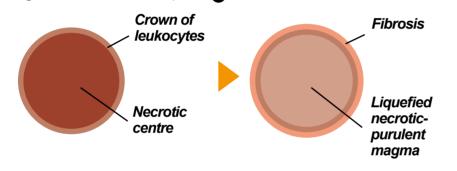


Often associated with moderate induration (acute interstitial pneumonia, *sheet 10*), suggestive of RSV (*sheet 18*)

#### Abscess

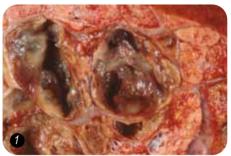
Definition: in practice, 3 different lesions are classified as abscesses

• **Sequestrum:** lesion resulting from the progression of necrotic foci (this progression does not occur frequently) (*sheet 11*) e.q.: *Mannheimia haemolytica* 

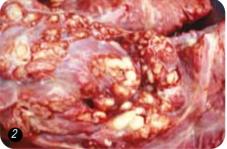


Purulent bronchitis
 2

Chronic progression (over 8 - 10 days) of bronchitis, sheet 12)



Sequestrum (Mannheimia haemolytica)



Purulent bronchitis



Purulent bronchitis

#### • "True" abscess 46

Definition: purulent lesion delimited by a shell



#### IMPORTANT

#### An abscess signifies

- chronic progression (more than 15 days)
- bacteria are always present

#### Certain bacteria are particularly pyogenic

A purely viral infection does not produce an abscess

- Arcanobacterium pyogenes
- Streptococci

IMPORTANT



Abscess



Abscess

#### Oedema

Distinguish clearly between ...

- Either terminal frothy oedema (tracheo-bronchial)
  - $\cdot$  Common terminal lesion 🛆
  - Is only seen a few hours after death: early autopsy
  - Small bubbles = frothy



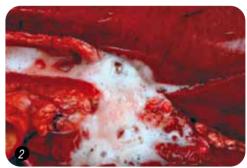
Not of diagnostic significance

Often associated with terminal emphysema, (sheet 9)





Terminal frothy oedema

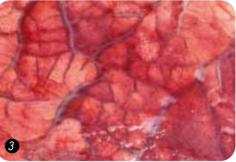


Terminal frothy oedema

#### Or, interstitial or perilobular oedema

34

- Lung not deflated
- Flows when dissected
- · Interlobular spaces vitreous and distended
- · Can even be observed several hours after death
- Origins:
  - Cardiogenic
  - Pulmonary inflammation of:
    - infectious origin
    - non-infectious origin > toxic: fog fever emphysema
      - (acute farmer's lung)



Perilobular oedema



Perilobular oedema



#### DO NOT CONFUSE

Oedema in the strict sense is a lesion. In the field, the term is sometimes used falsely when referring to acute respiratory distress syndrome or the terminal phase of progression that is likely to be acute.

Sheet 14

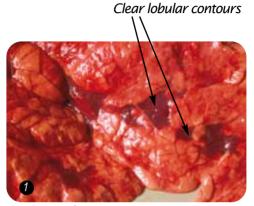
#### **Atelectasis**

**Definition:** Alveolar and therefore lobular collapse due to the absence of airflow

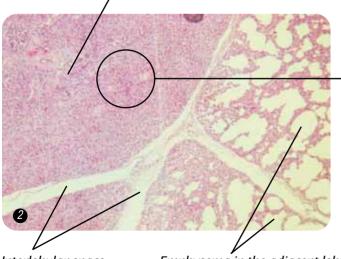
- Macroscopy: red tissue, slightly indurated,
   with clear lobular contours, depressed, associated with a bronchial lesion
- Microscopy: cellular "strata" 2 3

Atelectasis

• **Expression:** depression of one or more lobules in relation to the adjacent emphysematous lobules

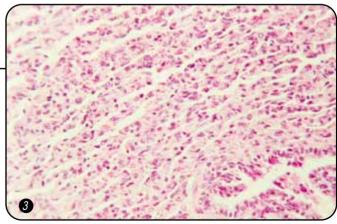


Atelectasis foci

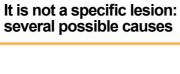


Interlobular space

Emphysema in the adjacent lobules



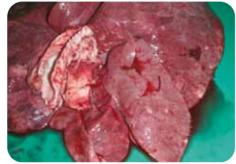
Cellular "strata"





#### DO NOT CONFUSE

In the field, a red and moderately indurated area (interstitial pneumonia, *sheet 10*) is often thought to be atelectasis, a less common lesion.



Interstitial pneumonia



Atelectasis, clear lobular contours



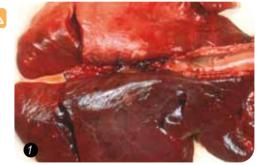
With newborn calves (up to a few days old), it is possible to observe "physiological" foetal atelectasis

#### Sheet 15

#### **Hypostasis and Autolysis**

#### Hypostasis

- Accumulation of blood in slanting position, occurring *post mortem*
- Cranial if the cadaver has been hung for several hours (rare in practice, sometimes at the abattoir)
- Unilateral if lateral recumbency 1
- No increase in consistency
- "flows" when cut 2
- Sometimes more visible in section (the pleura may mask the pathological aspect)



Right unilateral hypostasis



- Do not take samples at this location
- Hypostatic pneumonia is rare (lesion ante mortem
  - following prolonged recubency).



Hypostasis: unilateral, +/- associated with autolysis ②

#### DO NOT CONFUSE

 Oedema: often bilateral, dilation of the interlobular septa, appears shiny and moist when cut 3



#### Autolysis and putrefaction

Compared to other organs, such as the kidneys and liver: the lung is not the preferred location for the phenomena of autolysis and putrefaction

#### Sheet 16

Hypostasis and Autolysis

# **NECROPSY** From pathogen to lesions

## Mannheimia haemolytica

#### Always

• Marked lobar hepatisation (alveolar pneumonia, *sheet 10*)

#### Often

- Pleurisy (sheet 8) 2
- Typical necrosis (sheet 11) 3

#### Other pictures

- Hepatisation
  - without necrosis
  - or without pleurisy
- Hepatisation + emphysema (caudal lobes)
- Hepatisation + non-adherent bronchial exudate from drainage
- "Cavitation" following the elimination of necrotic elements (sometimes classified as an abscess, *sheet 13*)



The typical necrotic character of these lesions means that you do not have to carry out bacteriology **Typical picture** 



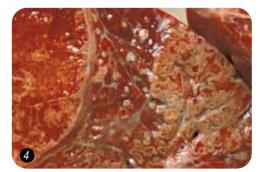
"Hepatisation"



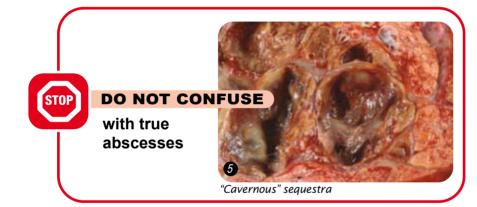
Fibrinous pleurisy



Necrosis seen on the surface



Necrosis seen in cross-section



### **NEVER**

any moderate consolidation (indicating interstitial pneumonia, *sheet 10*)



These lesions do not exclude a primary viral infection

Sheet 17

Mannheimia haemolytica

# **NECROPSY** From pathogen to lesions

## **Single RSV infection**

#### Always

- Interstitial pneumonia (sheet 10)
  - Cranial lobes 1
- Mucopurulent bronchitis (sheet 12) 2

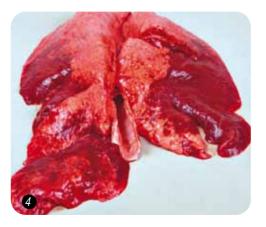
#### Often

#### • Emphysema

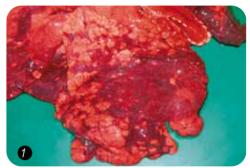
- Interlobular or subpleural
- Caudal lobes 3

#### Other pictures

 Interstitial pneumonia and mucopurulent bronchitis without emphysema



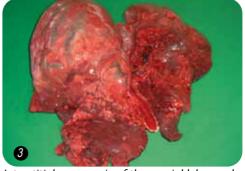
Typical picture



Interstitial pneumonia of the cranial lobes



Mucopurulent bronchitis



Interstitial pneumonia of the cranial lobes and emphysema of the caudal lobes

## NEVER

• pleurisy

• **true hepatisation** (alveolar pneumonia) (if only RSV)

### IMPORTANT

Possibility of RSV without emphysema Remember: emphysema does not necessarily mean RSV (sheet 9)

# **NECROPSY** From pathogen to lesions

## IBR

- Typical picture
  - Fibrino-necrotic rhino-tracheo bronchitis
     Highly suggestive lesions (1) (2) (3)
  - Yellowish coating adhering to the mucosa
- Other pictures
  - Emphysema frequent 🏼 🕘
  - Bronchopneumonia
  - Sometimes limited to laryngeal lesions



Severe emphysema



Fibrino-necrotic laryngitis







Hemorrhagic and fibrino-necrotic rhinitis



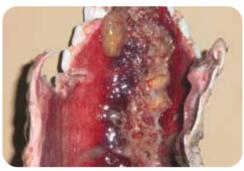
Necrotic-purulent laryngitis



Necrotic-purulent tracheitis



Adherent fibrino-necrotic coating



Non-adherent muco-purulent exudate

From pathogens to lesions

Adherent coating (yellowish) = IBR

draining of bronchopneumonia

Non-adherent muco-purulent exudate from

**DO NOT CONFUSE** 

ST0

# **NECROPSY** From pathogen to lesions

## **Dictyocaulus**

#### Bronchial wheezing







Abundant tracheal mucus



Numerous parasites

#### Always

- Tracheo-bronchitis
- Abundant traceal mucus 🛈
- Presence of parasites (5 6 cm), (2)
   (up to 36 hours after death)

#### Often

- Numerous parasites **2**
- Emphysema 🚯

#### Other pictures

- Few parasites
- Onset of infestation
- Chronic carrier
- First infestation, prepatent phase: mass emergence of larvae
- Reinfestation and allergy
- Complications





- In the case of emphysema, do not forget to open the bronchi (sheet 9)
- Verminous bronchitis can manifest itself during a housing period



Emphysema due to dictyocaulus

# **NECROPSY** From pathogen to lesions

## Aspergillus

#### Typical picture

- Multiple necrotic foci (sheet 11)
  - of several millimetres in diameter
  - without increased consistency in of the lung 1

#### Other pictures

#### Association with bronchopneumonia

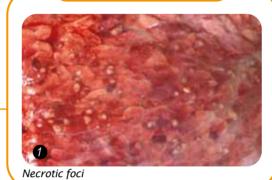
- Increased consistency 2
- Visceral pleurisy
  - Slight



Aspergillosis is most frequently a sporadic disease, sometimes anademic<sup>1</sup>, often secondary to immuno-depression

<sup>1</sup>: non-infectious but contamination of several animals from the same origin, such as mouldy straw (spread by a straw shredder, for example)

#### **Typical picture**





Bronchopneumonia + aspergillosis



Close-up view: blackish edge

## Sheet 21

pergillus

# **NECROPSY** From pathogen to lesions

## **Pulmonary thromboembolism**

#### • Upper respiratory system (symptoms)

- Heamoptysis, often bilateral (sheet 5) 1
- Sometimes without haemoptysis

#### Pulmonary lesions

• Embolism and pulmonary haemorrhages

3

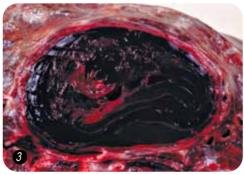
• Progression to abscessation



Haemoptysis



Pulmonary haemorrhage



Pulmonary thrombosis lesion



#### Other lesions (complete autopsy required, sheet 6)

#### Young bulls

- Thrombosis of the CVC (caudal vena cava)
- Hepatic abscess
- Ruminitis 🗿

#### Dairy cow

- Thrombosis of the CVC with hepatic abscess (a) caused by:
  - foreign body
  - ruminitis
- Mastitis
- Jugular phlebitis



Ruminitis



Thrombosis of the CVC

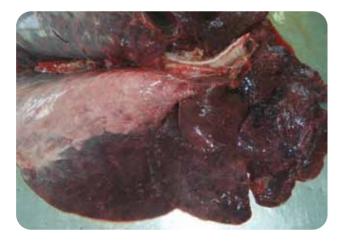


Lesions caused by ruminitis or acidosis are often no longer detectable at necropsy, when thromboembolism is observed.

# **NECROPSY** From pathogen to lesions

## **Multiple infections**

- In practice, most infectious bronchopneumonias (whether enzootic IBP or not) are consecutive to simultaneous or, more frequently, **sequential** mixed infections.
- They have in common:
  - a preferentially cranioventral localisation
  - the absence of any lesions of a specific nature



#### **Necropsy report**

- Gross pathological diagnosis: heavily extended cranioventral bronchopneumonia, acute/sub-acute character.
- Description of the lesion and aetiological orientation: Cranial and middle lobe, as well as anterior third of the right caudal lobe, with severe suffering from severe induration and congestion, without increase in volume. The degree of induration points primarily to alveolar rather than interstitial pneumonia. However, the gross characteristics identified exclusively during macroscopic examination do not suggest a specific bacterial or viral infection and indicate instead the intervention of several pathogens.



#### **Necropsy report**

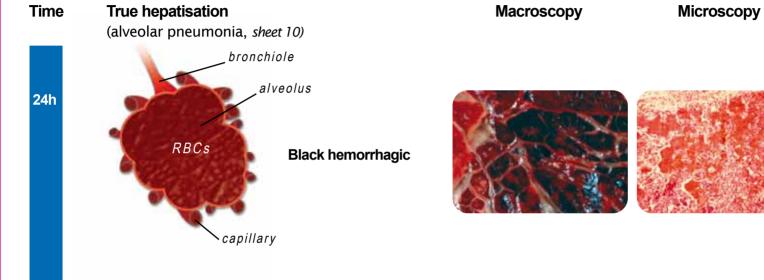
- **Comment:** Bovine lung: moderately extended cranioventral bronchopneumonia of sub-acute character.
- Description of the lesion and aetiological orientation: Cranial, middle and apical third of caudal lobes show moderate **induration** and **congestion**, with a slight increase in volume. In the absence of complementary examinations, the purely macroscopic characteristics lead one to suspect viral **pneumonia** without being able to exclude the intervention of bacteria.



In this case, laboratory examination (See **ANALYSES**) is indispensable in order to identify the pathogens.

# NECROPSY

# **Duration of progression**

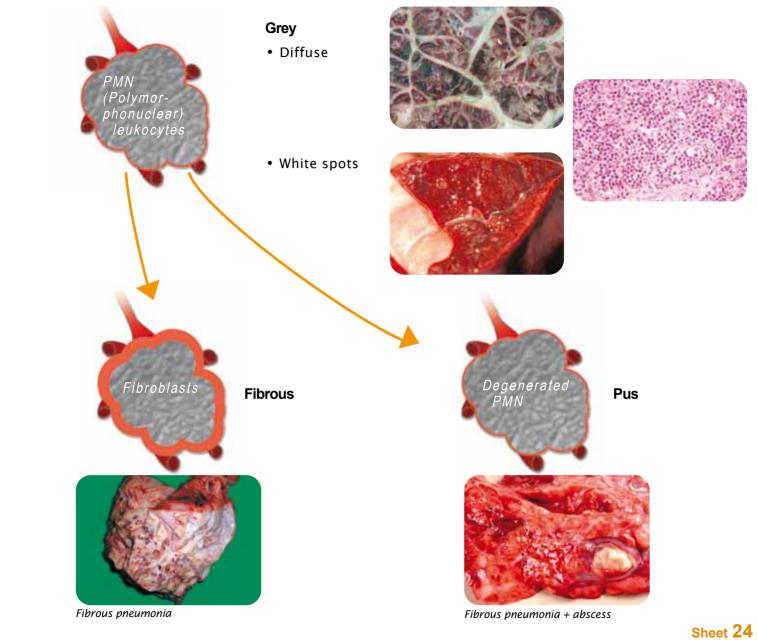


RBCs Fibrin

48h

Red





3 days

15 days

-3 wks.

Duration of evolution

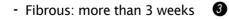
# **NECROPSY**

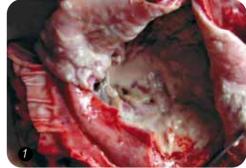
# **Duration of progression**

- Other criteria
  - **True abscess:** more than 3 weeks (*sheet 13*)

- Pleurisy (sheet 8)
  - Fibrinous: after 24 48 h

2





Abscess



Fibrinous pleurisy



Fibrous pleurisy

- Bronchiectasis (sheet 12)
  - Bronchial dilation with or without purulent content: more than 3 weeks



Bronchiectasis



# • Microscopy provides more precise information than macroscopy

- Margin of uncertainty:
  - greater if the evolution is chronic
  - for an acute phenomenon, it is counted in days



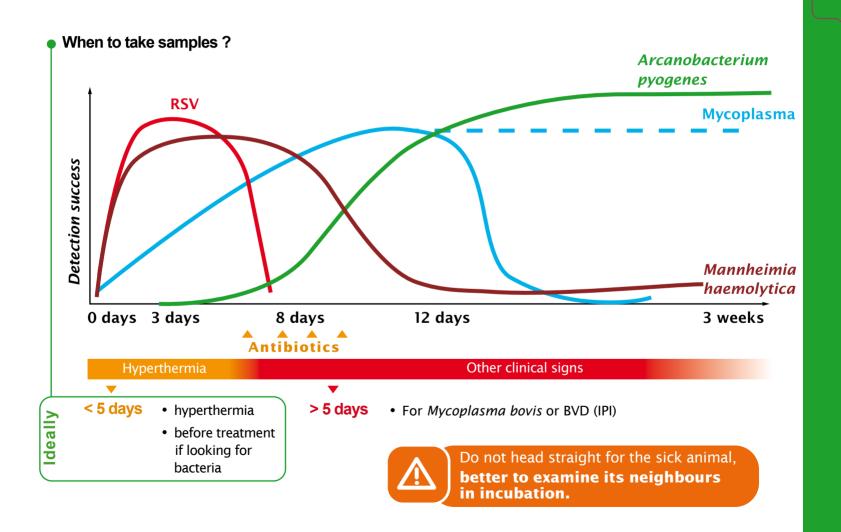
- The result of the progression by means of histology clearly depends on the **location where samples are taken**
- If the pneumonia is heterogeneous, the stage of the lesion is valid for one lobule only: Look at the oldest lesion, to date the onset of the process



Lesions at different stages in the same lung.

# **ANALYSES** From sampling to pathogen

# Secretions, lungs: sample carefully



#### • What to sample ?

### TTA/BAL Trans-tracheal Aspiration / Bronchio-alveolar Lavage

Virus +/- (BAL>>TTA)

Bacteria +++

#### Lung

Virus +++ Bacteria +++

#### • Focus on DNS for RSV\*

**Deep Nasal Swab** 

Virus +++

Bacteria +

- Focus on TTA or BAL for bacteria\*
- Avoid sampling from the posterior pulmonary lobes
- \* depending upon the working conditions, transportation, and techniques for analysis (sheets 29 and 30)



- Sample 10% of animals (with a minimum of 3)
- Taking samples at the analysis laboratory limits the risk of contamination: transport the carcass *(sheet 6)* or a half-lung

# **ANALYSES** From sampling to pathogen

# Secretions, lungs: sample carefully

# Transportation

- Timing (sampling analysis)
  - Ideal < 12 h (TTA +++)
  - Limit < 24 H
  - Caution: for the lungs, count the timescale from the time of death
  - To be adjusted according to:
    - the pathogens:
      - RSV very fragile
      - Mycoplasmas and Pasteurallae less labile than RSV
    - **sampling techniques:** for TTA < 24h is essential
    - **analysis techniques:** cell culture: for RSV, 3 h. at ambient t°, 6 h maximum at 4° C (other viruses less labile)
      - detection of viral Ag or PCR: < 24 h

#### Conditions

- Cool but above freezing point (0 4° C):
  - Histophilus: stable for 2-4 hours
  - Pasteurellae: stable for 2 days
  - Mycoplasmas: stable for 4 days
- Freezing (-20° C):

#### - Ensure that this condition is maintained until the laboratory is reached

- Freezing diminishes the titre
- Risk of false negative results
- Variable effect:
  - according to the initial titre
  - according to the pathogens
- > Pasteurellaceae: major reduction (Histophilus in particular)

(continued)

Viruses and mycoplasmas are more stable

#### • Transport medium

• Not necessary if the timing and temperature are adhered to.

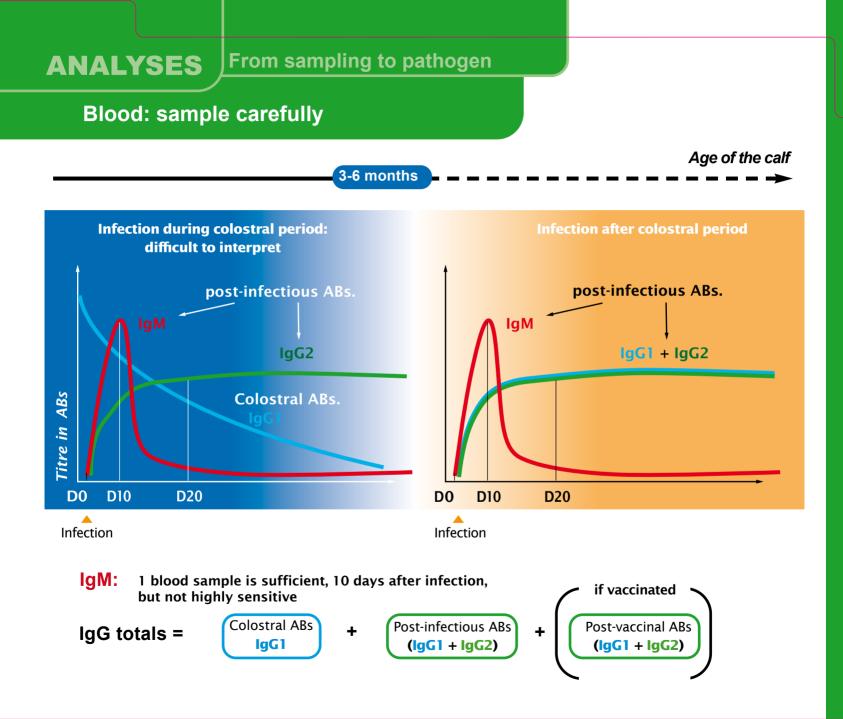
In p (mo it is

In practice: **if samples are taken on Friday** (more than 48h between sampling and analysis), **it is better to freeze them.** 

# **Timescale for results**

Destaria	Respo		ng to diagnostic m	nethod
Bacteria	Virus			
Culture identification	Cell culture	IF	ELISA Ag	PCR
V	▼	$\bullet$	$\checkmark$	•
3 - 15	5 - 30	A few	A few	8 - 24
days	days	hours	hours	hours
Mycoplasmas: 10 days				

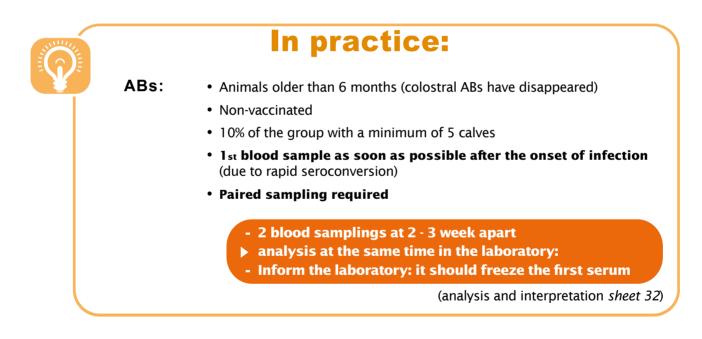
From sampling to pathogen



#### The colostral ABs. (up to 3 - 6 months)

- > are found at analysis after the first blood sample
- block the detection of post-infectious ABs of type IgG1

RSV, PI3 or IBR vaccinations lead to a positive test result



# **ANALYSES** From sampling to pathogen

# Deep nasal swabbing

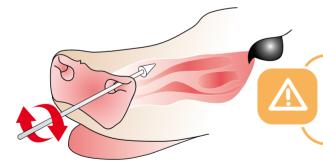
## Materials

- Ideal swab:
  - with brush, plastic stem, protected

### Procedure: schematic (section)

- Nasal meatus (cf. sagittal section below)
- Vigorous "brushing" of the mucosa





RSV is closely linked to the cells: vigorous brushing is indispensable in order to obtain a sample rich in cells.

### Analyses

#### Pathogens

- Virus: Good predictive value
  - RSV: in most laboratories
  - Other viruses: only certain laboratories
- Bacteria:

Some animals are healthy carriers of bacteria in their nasal cavities (false positive results), taking samples from several animals (> 5) makes it possible to interpret the results (good agreement on a collective scale with BAL).

- Techniques:
  - Bacteriology (sheet 33)
  - Virology according to the laboratory
    - Immunochromatography
    - Immunofluorescence (IF, *sheet 35*)
    - ELISA capture of Ag
    - Cell culture

- test for viral Ag
- cytopathogenic effect and identification (by IPX, for example, *sheet 36*)

- PCR

test for nucleic acids from the virus



### Rapid qualitative analysis kit

- Immunochromatography or Ag capture ELISA
- Relatively sensitive
- Specific
- For RSV only
- Pooling possible (3 samples)

# **ANALYSES** From sampling to pathogen

## **TTA** (Transtracheal Aspiration)

### Materials

• Probe: Centracath Vigon®, ref. 137/20, 75 cm 2

#### 

- Good retention is vital
- Limit 1/3 inf 1/3 centre of neck
- Catheter inserted 40-50 cm
- 50 ml of physiological saline solution injected then reaspirated immediately (only 2 10 ml are recaptured)

## Analyses

- Pathogens
  - Bacteria
  - Virus: RSV, IBR, PI3, adenovirus
- Analysis techniques (sheets 29 and 33 36)

## Interpretation

- Presence of virus: implication certain
- Presence of bacteria: see "bacteriology" (sheet 34)



- 2 ml per animal is sufficient
- Possibility of pooling up to 3 animals without reducing sensitivity\*
- The advantage of the technique (in comparison with deep nasal swabbing) is when searching for bacteria: there are no healthy carriers in the lung, in contrast to the nose.

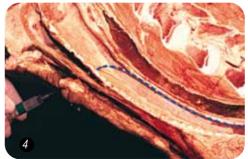




Catheter for TTA



Tracheal puncture



Sagittal section of the neck: position of the catheter in the trachea

## "Easy" BAL (Bronchoalveolar Lavage)

#### In practice

- "Academic" BAL is performed with a fibroscope and under anaesthetic. Under these conditions, its routine application is limited.
- However, with an adapted probe, BAL without a fibroscope can be conducted and produces good results
   Image: Image of the second sec

### Advantages

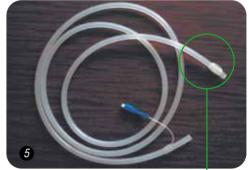
- Lower respiratory system
- Sample rich in cells (RSV is linked to cells)
- High volume collected: for 60 200 ml injected, one can recover 30 100 (useful for RSV, fragile and difficult to cultivate)

#### Disadvantages

- · Possible contamination in the upper airways
- More complex and expensive than TTA (price of the probe)

#### Procedure

- Several techniques
- With or without fibroscope **6**
- Probe diameter/age
  - Adults: 8 10 mm X 2.45 m (Bivona, Cook, Genia, with reference to horses)
  - Calves: human gastric probe (Vygon)



Probe for BAL





BAL without fibroscope

# **ANALYSES** From sampling to pathogen

# Lung

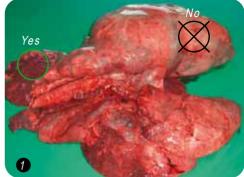
- Where should samples be taken? 0
  - Cranial area, if possible
  - Indurated area
  - Junction between healthy/affected tissue > Histology



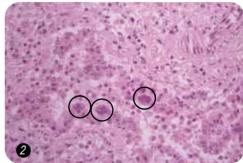
Virology and bacteriology

### Analyses

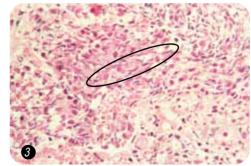
- Virology: RSV, PI3, adenovirus, BVD (ELISA Ag or IF from frozen section)
- Bacteriology
- Histology: lesion orientation for a "hesitant" macroscopic analysis
  - Physiopathological orientation (sheet 10):
    - Interstitial pneumonia
    - Alveolar pneumonia
  - Aetiological orientation in relation to the clinical examination and the picture of lesions
    - Syncytia: RSV or PI3 possible 2
    - Necrotic foci and oat cells:
      - *M. haemolytica* possible (*sheet 11*) **3**
    - Mycelial elements (PAS coloration): Aspergillosis



Where to take samples from?



Syncytia: RSV or PI3



Oat cells: M. Haemolytica

#### • For histology:

- Contact a specialist veterinary pathology laboratory
- Interpret the results in the context of the clinical signs and lesions

# **ANALYSES** From sampling to pathogen

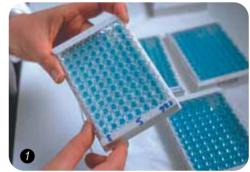
## Blood

### Routine serology

- Virus: RSV, PI3, IBR, adenovirus, BVD
- Mycoplasma bovis (according to laboratories)
- No serology in routine practice for pasteurellae

## Techniques

• ELISA in routine practice in all laboratories, semi-quantitative 🜒



ELISA plate



### What about seroneutralisation?

- It is only used for viruses
- It can only be conducted in a small number of laboratories equipped for cell culture

**Seroneutralisation** is a quantitative test mostly performed in microplates. Virus and the serum to be tested are pre-incubated for a certain time prior to the addition of cells.

In case of cytopathogenic viruses, the neutralisation will be judged after reading for cytopathogenic effects. Detection of non-cytopathic viruses requires immunostaining (fluorescence or enzymes like peroxidase).

The result is quantitative due to the serial dilutions of the serum to be tested.

### Interpretation

- Compare with the maternal seroprevalence
- Useful for germs with low individual prevalence
- For germs with **high individual prevalence** (RSV, PI3, adenovirus), seropositivity of animals is very frequent and makes the 2nd blood sampling compulsory.

The higher the initial seropositivity, the less chance one has of identifying an increase

- · Compare with the maternal seroprevalence
  - If seroconversion: 0 -------+
  - If there is an increase:
    - of 2 dilutions (titer X4) with seroneutralisation
    - of **2** + in ELISA, to be adjusted according to the kits



- The phenomenon of heterospecific increase of ABs can explain an isolated increase of ABs in 1 animal in a batch
- No virus isolation in the blood for RSV, IBR and PI3
- Cannot be a diagnostic method for emergency use: the sero-conversion require a minimum of 3 weeks



### In the future ...

The amount of IgG2 (even during the colostral period), would enable interpretation using a single blood sampling, without paired samples (sensitivity to be specified)



Cell culture: uninfected bovine foetus kidney cells



Cell culture: CPE due to PI3 virus

Sheet 32

# ANALYSES

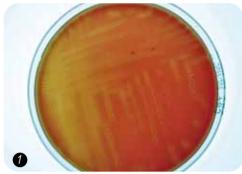
## From pathogen to sampling

# Bacteria

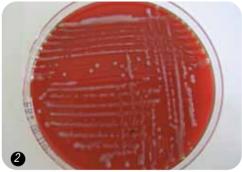
## Techniques

- Serology:
  - Conducted for Mycoplasma bovis
  - Not routinely used for pasteurellae
- Culture:
  - In routine practice: Pasteurellae (*Mannheimia haemolytica* **1**, *Pasteurella multocida* **2**), *Arcanobacterium pyogenes*
  - Specific request from the practitioner for:
    - Mycoplasma bovis and Mycoplasma dispar
    - Histophilus 3
    - Salmonellae

Identification of species is obligatory for the mycoplasmas, most laboratories restrict themselves to diagnosing the Mycoplasma type *(sheet 34)* 



Area affected by hemolysis: Mannheimia haemolytica



Pasteurella multocida

Histophilus somni

### Samples

- TTA, BAL, lung : +++ (sheets 30 and 31)
- Deep nasal swabbing (sheet 29):
  - Healthy carriage of pathogenic bacteria in the upper airways
  - Contaminations due to commensal bacteria or fungi, which are not relevant, compromise isolation

### • Transport (sheet 27)

- Within 24 h
- Transport media unnecessary if delay and temperature respected
- Store cool but above freezing point (2 4°C)
- Freezing if sampling analysis interval > 48 h



Some bacteria are very fragile (false negative results possible): *Histophilus somni* 

#### Bacteria

## From pathogen to sampling

# Bacteria (Interpretation)

#### The result should be interpreted according to the pathogenicity recorded and the problems observed.

Major pathogens

ANALYSES

imon	Unco	ommon
a multocida lus somni rium pyogenes	Salmon	r typhimurium ella dublin rgillus sp.
imal role • Cont	aminants	
<ul> <li>Klebs</li> <li>Stapl</li> <li>Aspe</li> <li>Protestate</li> </ul>	siella hylococcus ergillus sp. eus etc.	
e sample: no specific reque ne attribution of respirator	est is necessary <b>1</b>	
	a haemolytica a multocida ilus somni rium pyogenes sma bovis <b>nimal role • Cont</b> • Esch • Kleb • Stapl e • Stapl e • Aspe • Prote erate septicemic pathovar in the lungs, in ws in conventional media ( e sample: no specific reque	a haemolytica a multocida ilus somni rium pyogenes sma bovis himal role • Contaminants • Escherichia coli <sup>(2)</sup> • Klebsiella • Staphylococcus • Aspergillus sp. • Proteus etc.



### Serotyping of Mannheimia haemolytica?

- Unnecessary in practice, serotypes A1 and A6 are the most prevalent
- Some recent vaccines provide cross protection



## Is the antibiogram useful ?

• NO, if no common resistance is known (*P. multocida, H. somni, A. pyogenes*) or, *A. fortiori,* if pathogenicity has not been identified or suggested (*E. coli, Pseudomonas*)

• YES, in case of frequent antibioresistance (M. haemolytica, Salmonella spp., etc.)

## Technique

- The disc method is most frequently used in routine practice **2!!**
- This method is not applied to mycoplasmas

### Interpretation

### Ensure that the sample is representative

If the sample is taken from a treated animal, the isolated bacterium may have been produced by selection pressure due to the antibiotic treatment and **is not representative of the bacterium that caused the problem.** 





In practice

Samples taken from an **untreated living** animal are **preferable and even obligatory**.

Bacteria (interpretation and antibiotic resistance test)

# **ANALYSES**

## From pathogen to sampling

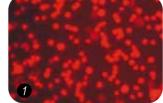
# **RSV**

## Particular characteristics of RSV

- Virus closely associated with the cells
- Highly labile virus: very rapid transport required if the cell culture technique is used

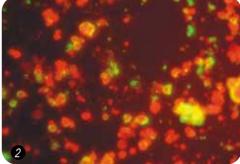
## Technique

- Serology
  - Obligatory kinetics (high seroprevalence: an isolated blood sampling is difficult to interpret - *sheet 32*)
- Virology
  - Cell culture: method unsuitable in practice <sup>(1)</sup>
  - Detection of Aq: good sensitivity
    - Immunofluorescence (IF):
    - at the analysis laboratory only 1 2
    - Immunoenzymology (IPX, ELISA Ag, rapid tests) 3
  - PCR<sup>•</sup> small number of laboratories **6**

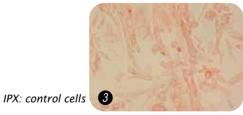


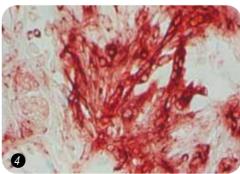


IF: control



Positive immunofluorescence in RSV





Cell culture infected by RSV: positive in IPX

### **Miraculous PCR?**

In practice, epidemiological sensitivity, which takes into account the sampling and transport phases, is often lower than that of common methods for the detection of Ag

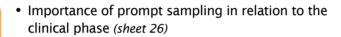
- Histology: sheet 31
- (1) Because the virus is very labile. However, following rapid transport (3h or 6h max. at 4°C) of good samples, this technique produces excellent results.

## Samples

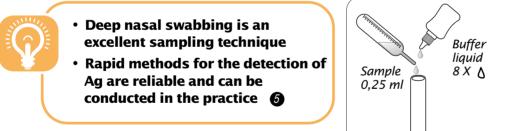
- Deep nasal swabbing, BAL, lung : +++
- TTA: centrifugation can make it possible to recover a sediment rich in cells

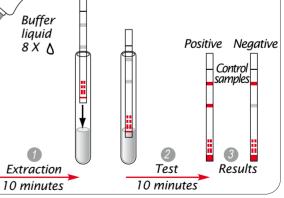
## Transport

- Store cool but above freezing point: 2 4°C
- Freezing possible if the timescale for sampling analysis > 24 h (and no thawing)



• Importance of transport, for the cell culture technique (very labile RS virus)





5

# **ANALYSES** From pathogen to sampling

## **Other viruses**

## • Particular characteristics

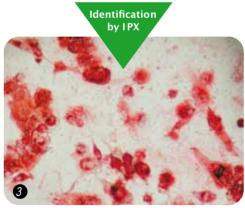
- Viruses less labile than RSV
- High prevalence of **PI3** and **adenovirus**
- Clinical IBR is rare
- The **BVD** virus contributes to respiratory problems, particularly due to immuno-supression

## Techniques

- Serology
  - Less useful for PI3 (high prevalence)
  - More useful for IBR (see status of the herd)
  - Kinetics obligatory, 2 blood samples analysed at the same time *(sheet 28)*
- Virology
  - Cell culture: much more sensitive for IBR (1) and PI3 (2) than for RSV
  - Ag detection methods (IF, ELISA, IPX) 3
  - No approved rapid test can be conducted in the surgery
  - PCR: not in routine practice except for BVD



Cell culture. ECP due to BoHV1 (IBR)



Cell culture infected by BoHV1 (IBR): positive in IPX

### Samples

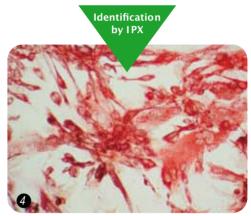
- Deep nasal swabbing, BAL, lung : +++
- TTA: centrifugation will enable to recover a pellet rich in cells
- Blood: for virology, only of BVD (Transient or Persistent Viraemia)
  - no viremia for strictly respiratory viruses

## Transport

- Store cool but above freezing point: 2 4°C
- Freezing of the lung if delay for sampling
  - Freeze if timing from sampling to analysis is >24h (do not thaw)



Cell culture. CPE: PI3



Cell culture infected by the PI3 virus: positive in IPX

# ANALYSES From pat

## From pathogen to sampling

## Dictyocaulus

### Particular characteristics

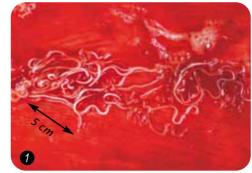
- Adult Dictyocaulus
  - visible to the naked eye (5 6 cm)
    - at the opening of bronchi (sheet 7)
    - if the necropsy is conducted without delay (parasite lysis after 48 h)
  - are abundant except for:
    - onset of infestation
    - chronic carrier
    - first infestation in prepatent phase: arrival of larvae en mass
    - reinfestation and allergy
- The larvae are identified using a specific coproscopic technique

## Techniques

- Dead animal: Necropsy and opening of trachea and bronchi 2
- Live animal: Analysis of faeces, Baermann technique (Baermann device + microscope)



The Baermann technique can be used in the surgery



Adult Dictyocaulus



Abundant mucus + parasites



Baermann device

## Samples

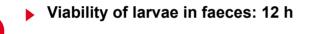
- Faeces
- 5 10% of animals
- Sample from the rectum

## Transport

• rapid (within 12 h), kept cool but above freezing point (lability of larvae)



Dictyocaulus larva



#### DO NOT CONFUSE

Viability of adults in the bronchi: it takes several days before adults are found in bronchi



- A single negative Baermann test does not necessarily exclude verminous bronchitis: the larval excretion is variable (time of sampling, immunity etc.)technique
- Several samples are therefore necessary to exclude Dictyocaulosis

Sheet 37

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U	Upper Respiratory Tract 1, 7
V	Verminous bronchitis

## Abbreviations

ABs	Antibodies
Ag	Antigen
ARDS	Acute Respiratory Disease Syndrome
BAL	Bronchoalveolar lavage
BVD	Bovine Viral Diarrhoea
BS	Blood Sample
CPE	Cytopathic Effect
CVC	Caudal Vena Cava
DNS	Deep Nasal Swabbing
EIBP	Enzootic Infectious Bronchopneumonia
ELISA	Enzyme Linked Immunosorbent Assay
ENV	Ecole Nationales Vétérinaire
ENVT	Ecole Nationales Vétérinaire Toulouse
FB	Foreign Body
IBP	Infectious Bronchopneumonia
IBR	Infectious Bovine Rhinotracheitis
IF	Immunofluorescence
lg	Immunoglobulin
lg G	Immunoglobulin G
lg M	Immunoglobulin M
IPI	Immunotolerant Persistently Infected
IPX	Immunoperoxidase
ND	Notifable Disease
PMN	Polynuclear Neutrophil
PAS	Periodic Acid of Schiff
PCR	Polymerase Chain Reaction
Pi3	Parainfluenza 3
RBC	Red Blood Cells
RSV	Respiratory Syncytial Virus
SVS	State Veterinary Services
TTA	Transtracheal Aspiration

