MYCOPLASMAS IN FOOD ANIMALS

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The mycoplasmas (order Mycoplasmatales) are small organisms, bacterium-like but having no cell walls. Their range of metabolic ability is narrow and they require a moist, nutritious environment for growth; generally not surviving long in conditions in which they cannot multiply and therefore are sensitive to adverse chemical and physical agencies such as disinfectants and high temperatures. The bodies of warm-blooded animals suit them well, and the great majority of the group so far recognised are obligate or facultative parasites. If these, a much higher proportion can cause, or exacerbate, disease than in any other group of micro-organisms. They cause serious disease problems in all species of food animals, although the most severe diseases of the mammals are absent from Britain.

Diagnosis of infection by isolation of the organism is most certain, but demonstration of mycoplasmas in smears, e.g., of lung tissue or milk, with polychrome methylene blue, or by a fluorescent antibody method, can be useful. Serodiagnosis may be attempted by several methods, but this is more satisfactory at the herd than at the individual animal level.

Although there are antibiotics active against mycoplasmas, most diseases caused by them are refractory to treatment; it may be possible to eliminate the organisms but the condition will not necessarily improve.

However, treatment of poultry with various antibiotics has a high success rate, and it has proved possible to control outbreaks of Mycoplasma hyosynoviae synovitis in pigs by treatment of the herd with tetracycline immediately. Signs of the disease are noticed. Vaccination is used against the main killer diseases, and a sucrose bovine pulmonary agalactia of sheep is reduced. Control of most mycoplasmas diseases is therefore based on detection and culling, or segregation of infected animals; this is usually quite successful, if expensive, but is often difficult to prevent reinfection.

By far the most common and important mode of spread of infection is contact, and some infections spread only slowly even with close contact unless there are contributory factors, for example, M. gallicolica may take days to spread through a broiler house in the absence of other respiratory pathogens, but may spread as much as five times faster if a virus such as Newcastle Disease or Infectious Bronchitis Virus is present. Infections via the nose, udder or genital tract occur and many mycoplasmas can then spread from the local infection to other sites, presumably in the bloodstream; joint infections usually occur in this way. Udder fluid or dried secretions, and preferably eye to eye spread, usually take place through an intermediary, chiefly milker and fly respectively.

Attempts at control, or prevention of spread, will not take account of these factors, but it is often impossible to detect the source and mode of entry of a mycoplasma infection.

Isolation and cultivation

The basic requirements for survival are mentioned above; media are highly nutritious and usually contain 10-20% of serum. Formulate are legion, but are generally based on beef heart infusion or digest, or brain-heart infusion, and contain yeast autolysate or extract at the equivalent of 1% of autolysate. Horse or pig serum is usually used and most species are indifferent, but a few, notably M. suis and M. hyopneumoniae, will grow only with pig serum. Chicken or turkey serum, if adequate supplies are available, are also excellent. M. dispar seems to prefer bovine serum, usually supplied as foetal calf serum, whereas that for contagious bovine pleuro-pneumonia will grow only with pig serum. Chicken or turkey serum, if adequate supplies are available, are also excellent.

Such antibiotics can be added at a concentration of 1% rather than the usual 1.5%. This is because the colonies of most species penetrate the medium for optimum growth and a 1.5% agar does not permit this. Mycoplasmas generally are highly resistant to the penicillin types of antibiotic, hence for isolation and transport purposes such antibiotics can be added at high concentrations for control of possible contaminating organisms. The other widely used substance for this purpose is thallous acetate, which does inhibit the growth of mycoplasmas even at the usual concentration of 0.025% but much less than it does other micro-organisms, and it is most useful against fungal contaminates. A special case for the use of antibiotics is in the isolation of M. bovis from milk, as this can multiply in milk and at room temperature, and the addition of an antibiotic permits it to grow so that with the minimum of adverse alteration (especially acidification) of the milk by other organisms. We use ampicillin at 1 mg/ml, but 0.1 mg/ml has no adverse effect on the isolation rate. The milk sample thus acts as its own enrichment medium, and isolations have been made from samples left for one or two days on the bench when the fresh samples have failed to yield the organism.

The general purpose medium used at Weybridge contains 2% horse serum and 0.002% DNA in addition to the usual base, yeast extract and penicillin G. If used, ampicillin is at 0.1 mg/ml, and we try to avoid using thallous acetate. The energy source is 1% oxoid llin and at room temperature, and the addition of an antibiotic permits it to grow so that with the minimum of adverse alteration (especially acidification) of the milk by other organisms. We use ampicillin at 1 mg/ml, but 0.1 mg/ml has no adverse effect on the isolation rate. The milk sample thus acts as its own enrichment medium, and isolations have been made from samples left for one or two days on the bench when the fresh samples have failed to yield the organism.

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organisms, but can survive and multiply in the animal body. A lactic acid is frequently isolated from all bovine species. This frequent isolation from milk has led to claims that it can cause mastitis of cattle. Similar claims have been made for A. alcaligenes, A. gallinarum, and A. modicum, but experimental studies have failed to confirm these claims. A. alcaligenes, A. gallinarum, and A. modicum, when grown on peptone broth, produce a substance similar to the so-called "P" factor, which is believed to be responsible for certain urogenital conditions in cattle. However, recent studies have shown that these organisms do not cause disease, but can survive and multiply in the bovine udders and urogenital tracts of cattle.

**Genus Ureaplasma**

As the name suggests, members of this genus are urea-utilizing bacteria that inhabit the urogenital tracts of mammals. Another term for this group, still sometimes used, is T (for "tiny") strain mycoplasmas, because they are very small on ordinary mycoplasma media. Growth is very poor. It seems possible, but is not proven, that ureaplasmas can cause such urea-utilizing conditions as vaginitis and infertility in humans. In addition, they have been isolated from the respiratory tracts and udders of cattle, and there is experimental evidence that they can cause disease. Ureaplasmas have also been isolated from the air-sacs of chickens, where they were believed to be believed to be at least partly responsible for disease, and from the urogenital tracts of turkeys. Experimentally, these latter strains proved capable of causing disease. The subdivision and taxonomy of this genus is still in a primitive condition. It is not yet clear how much difference exists between the strains.

**Mycoplasmas**

Mycoplasmas will often grow in ordinary mycoplasma media, especially if urea is included, much better growth is obtained on media which are not yet acidified with urea. The ability to grow on media which are not yet acidified with urea is the basis of whether the organism can be classified as a mycoplasma. This is the only method available for differentiating mycoplasmas from other organisms which can grow on media not acidified with urea. The ability to grow on media not acidified with urea is the basis of whether the organism can be classified as a mycoplasma. This is the only method available for differentiating mycoplasmas from other organisms which can grow on media not acidified with urea. The ability to grow on media not acidified with urea is the basis of whether the organism can be classified as a mycoplasma. This is the only method available for differentiating mycoplasmas from other organisms which can grow on media not acidified with urea. The ability to grow on media not acidified with urea is the basis of whether the organism can be classified as a mycoplasma. This is the only method available for differentiating mycoplasmas from other organisms which can grow on media not acidified with urea. The ability to grow on media not acidified with urea is the basis of whether the organism can be classified as a mycoplasma. This is the only method available for differentiating mycoplasmas from other organisms which can grow on media not acidified with urea. The ability to grow on media not acidified with urea is the basis of whether the organism can be classified as a mycoplasma. This is the only method available for differentiating mycoplasmas from other organisms which can grow on media not acidified with urea.
The diagnosis of gonorrhoea

R.R. Willcox, M.D., F.R.C.P., Honorary Consulting Venereologist, St Mary's Hospital London, and Locum Consultant King Edward VIII Hospital, Windsor and Wycombe General Hospital

1. Importance of accurate diagnosis
Diagnosis is achieved by smear and culture of material from the potentially infected sites and suspected gonococci have to be distinguished from other organisms of the Neisseria group which have a similar morphology and staining characteristics.

2. Sampling sites
The diagnosis of gonorrhoea is achieved by repeat smear and cultures if necessary, from a number of sampling sites. These comprise the urethra of heterosexual males; urethra and/or rectum of homosexual males; urethra, cervix and rectum of females (ocassionally also the ducts of Bartholin's glands) and the oropharynx of both sexes.1

As with meningococcal infections, gonorrhoea may present in patients with septicaemic symptoms of rash, fever and/or arthritis (Fig. 1) and the responsible organism may be isolated from the blood stream, joints or (rarely) the cerebrospinal fluid. Ocular infections can occur in neonates and occasionally in adults.


Gourlay, R.N. and Howard, C.J.

FIGURE 1. So called 'benign gonococcal septicaemia' or disseminated infection.

3. Taking of specimens
Calcium alginate or cotton wool swabs on wooden sticks must be of low toxicity for bacteriological purposes. Urethral specimens may be obtained directly from the urethra or by "milking" it forward, and those from the cervix by first cleansing with a swab before gently squeezing it by the partial closure of the blades of the vaginal speculum. Smears from the rectum should be taken by proctoscopy and cultures taken through the latter can be made by inserting the collecting swab into the anus for half a minute before removal and plating.

4. Smear examination
Smears can be examined 'on site' and given a 'while you wait' presumptive diagnosis at the first visit which may influence the treatment, e.g. a penicillin for gonorrhoea or tetracycline for non-gonococcal urethritis. They are not of value in the detection of asymptomatic infections and cultures are therefore required for screening purposes. Neisseria gonorrhoeae conjugated with fluorescein isothiocyanate is added, can be more efficient than other methods including cultivation from skin lesions or joints in septicaemia.

5. Culture
5.1 Growth media
Media used for the culture of the gonococcus have a basic agar base with protein hydrolysates enriched by blood or haemoglobin and other supplements (e.g. cocarcboxylase, glutamine, dextrose, ferric ions, cysteine).

Bovine mycoplasmas, pp. 50-95.

Cottew, G.S. Caprine-Divine mycoplasmas, pp. 103-130.


TABLE 1. Media for primary culture of the gonococcus.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Appearance</th>
<th>Antizobiotics</th>
<th>Blood enrichment</th>
<th>Possible supplements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-selective</td>
<td>'Chocolate' Agar</td>
<td>Dark brown, opaque</td>
<td>Nil</td>
<td>Heated, defibrinated horse or rabbit blood</td>
</tr>
<tr>
<td>Selective</td>
<td>Modified Thayer-Martin (TM)</td>
<td>Dark brown, opaque</td>
<td>Vancomycin&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Heated haemoglobin</td>
</tr>
<tr>
<td>Modified New York City (MNWC)</td>
<td>Red, translucent</td>
<td>Lincomycin&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Colistin&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Saponin-lysed haemoglobin</td>
</tr>
<tr>
<td>Neisseria medium&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Clear</td>
<td>Vancomycin&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Colistin</td>
<td>Nil</td>
</tr>
</tbody>
</table>

<sup>a</sup> Transgrow has increased agar content
<sup>b</sup> Originally nitrocin
<sup>c</sup> Originally polymycin
<sup>d</sup> A later addition
<sup>e</sup> Vancomycin and pimaracin may be substituted

<sup>f</sup> Contains vitamin B<sub>12</sub>, glutamine, adenine, guanine, p-aminobenzoic acid, 1-cysteine, glucose, diphosphoglycerate, nucleotide, cocarcboxylase, ferric nitrate, thiamine and cysteine.

<sup>g</sup> As used at St. Mary's Hospital

Base Corn starch with protein hydrolysates (Oxoid special peptone includes meat, plant and yeast digests) and phosphate buffer.

Beta-Lactamase Detection Papers

CODE BR41

The ability to rapidly detect lactamase production by microorganisms is of great importance to microbiologists. Without waiting for the susceptibility test, the clinician can be warned about antibiotic treatment. Within 3-5 minutes of picking visible growth from the culture medium a positive result can be reported.

Campylobacter Growth Supplement

CODE SR84

Strains of Campylobacter species vary in their oxygen tolerance. Therefore, the probability of isolation depends on the atmospheric conditions immediately around the medium. The addition of the Oxoid Growth Supplement SR84 to the medium increases the tolerance of oxygen and thus increases the probability of isolation.

Product and package, positive lactamase reaction, culture dish with colonies of bacteria.

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Product and package, positive lactamase reaction, culture dish with colonies of bacteria.
which have been shown to stimulate growth from small inocula (Table 1). Selective media, e.g., Thayer Martin *4* or New York City *4* and their modifications contain antibiotics to suppress Gram-positive and Gram-negative contaminants. Trimethoprim is aimed specifically against Proteus species and an antifungal antibiotic against Candida spp so that virtually only Neisseria spp will grow. These are particularly necessary in sampling sites where secondary contamination is most likely (rectum, vagina, throat). However, a few gonococcal strains may be sensitive to the antibiotics (e.g., vancomycin) and non-selective plates (e.g., Chocolate Agar) are recommended to be used in parallel.

5.2 Seeding of plates

Swabs from more than one site can be seeded on the same selective plate. Seeded plates must be incubated as soon as possible at 35°C with at least 70% humidity and 3-7% carbon dioxide provided by the introduction of gas into the incubator, by the use of candle jars or carbon dioxide generating pellets in a sealed container.  

5.3 Transport media

Plates of media are best inoculated in the clinic but if there are no facilities for prompt incubation a non-nutrient holding transport medium can be used, e.g., Amies’ modification of Stuart’s medium, which is dispensed in small screw-capped bottles into which the ends of the swab sticks are broken. This gives satisfactory results in this country, up to 24 hours of transit time, but is less efficient in hot (or very cold) climates.  

5.4 Recognition of the gonococcus

The cultures are examined at 24 hours and if negative the plate is re-incubated and re-examined at 48 hours. Possible gonococcal colonies (Fig. 3) are examined by Gram stain and are subjected to the oxidase reagent (di-tetra methyl phenyl endiamine hydrochloride) which quickly produces a dark blue colour with Neisseria. For the certain diagnosis of N. gonorrhoeae a confirmation test is still required. The methods available are (a) fluorescent antibody (FA) test, (b) sugar utilization, (c) coagglutination procedures.  

Combined growth and transport media

Some “grow as you go” techniques which may permit longer transit times before incubation have been evolved using a selective medium and provision for the maintenance of an environment of carbon dioxide. Transgrow medium is similar to Thayer Martin medium but has an increased agar content and is presented as small screw-capped bottles containing carbon dioxide-free bottles. Variants of this technique exist.

Antibiotics will combine not only with antibody but also with cell protein A of dead staphylococci. A combination of the latter with antibody results in a visible coagglutination lattice. A drop of protein A-containing staphylococci combined with gonococcal antibody is added to smear of culture. Similar staphylococci attached to non-immunized rabbit’s will act as control.

Available methods include complement-fixation with various antigens: haemagglutination, immune-fluorescence, radio immune assay and enzyme-linked immunosorbent assay (ELISA). The ELISA test would appear to be likely to hold the most promise but at present these procedures have little place in the routine diagnosis of gonorrhoea. They are too insensitive (particularly in the male) to be sufficiently specific (false positive to antibodies to other Neisseria) and unable to distinguish between past infection and active disease.

5.5 Antibiotic susceptibility tests

Rapid carbohydrate utilization tests may be adapted by the inclusion of a tube containing ampicillin to screen for beta-lactamase activity. For more detailed tests subcultures on sensitivity test agar with antibiotic discs are required. Beta-lactamase activity can be confirmed by the use of clavulanic acid added to the iodine and starch test (in tube or on filter paper) or lactamase papers.

6. Serological tests

TABLE 2. Confirmatory tests for the gonococcus

<table>
<thead>
<tr>
<th>Test</th>
<th>Fluorescent antibody (FA)</th>
<th>Sugar utilization</th>
<th>Coagglutination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Principle</td>
<td>Neisseria have differing</td>
<td>Neisseria carry</td>
<td></td>
</tr>
<tr>
<td></td>
<td>patterns of gas</td>
<td>differing</td>
<td></td>
</tr>
<tr>
<td></td>
<td>fermentation giving acid</td>
<td>enzyme responsible</td>
<td></td>
</tr>
<tr>
<td>Method</td>
<td>Sub-cultures made in</td>
<td>for sugar</td>
<td></td>
</tr>
<tr>
<td></td>
<td>special media containing</td>
<td>fermentation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>glucose, maltose, or sucrose</td>
<td>results</td>
<td></td>
</tr>
<tr>
<td></td>
<td>and colour change noted</td>
<td>after incubation</td>
<td></td>
</tr>
<tr>
<td>Time for result from examination of</td>
<td>30-60 min</td>
<td>of all Neisseria</td>
<td></td>
</tr>
<tr>
<td>culture</td>
<td></td>
<td>spp*</td>
<td></td>
</tr>
<tr>
<td>Advantages</td>
<td>Restricted to large</td>
<td>Also permits rapid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>laboratories where</td>
<td>test for carriage</td>
<td></td>
</tr>
<tr>
<td></td>
<td>considerable saving in</td>
<td>of non-Neisseria</td>
<td></td>
</tr>
<tr>
<td></td>
<td>media and work time</td>
<td>with agglutination</td>
<td></td>
</tr>
<tr>
<td></td>
<td>compared with sugar tests</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Additional tubes with lactose and fructose occasionally required.

For further information contact:

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References


FIGURE 3. Selective culture medium—gonococcal colonies.

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- New low temperature catalyst
- More efficient Gas-Generating Kit
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FIGURE 4. Sugar fermentation.

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Rapid carbohydrate utilization tests can be adapted by the inclusion of a tube containing ampicillin to screen for beta-lactamase activity. For more detailed tests subcultures on sensitivity test agar with antibiotic discs are required. Beta-lactamase activity can be confirmed by the use of clavulanic acid added to the iodine and starch test (in tube or on filter paper) or lactamase papers.

Acknowledgements

Thanks are expressed to Professor A.A. Glynn, Wright Fleming Institute, St Mary’s Hospital, London W2, for kindly providing material which has been used in the preparation of this paper. Also to Dr Peter Carlaw of the Department of Audiovisual Communication, St Mary’s Hospital Medical School, for permission to use the pictures.

Culture is published as a service to microbiology by Oxoid Limited, Wade Road, Basingstoke, Hampshire RG24 0PW, England.

The opinions expressed in this publication are those of the authors concerned and do not necessarily represent the views of the publisher, Oxoid Limited.