Diagnosis and control of contagious caprine pleuropneumonia

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Summary: The diagnosis of contagious caprine pleuropneumonia (CCPP) has often been considered difficult. This is because of the confusion that can arise with other mycoplasmoses of small ruminants. Symptoms and lesions can be similar and the isolation of M. capricolum subsp. capripneumoniae (MccF38) requires skilled technicians.

Once MccF38 strains are isolated, their identification should not be difficult. New techniques, such as polymerase chain reaction, now offer the possibility of identifying MccF38 directly from dried samples. However, the isolation of MccF38 strains is always required for an official declaration of infection. Until now, the official serological test has been the complement fixation test; the main drawbacks being lack of sensitivity and specificity and also the short persistence of antibodies detected by this technique. The specific competition enzyme-linked immunosorbent assay has now been developed and should enable wide serological enquiries to determine the real prevalence of the disease. Antibiotic treatments are effective but may not prevent persistence in latent carriers. An inactivated vaccine with saponin as an adjuvant has been produced in Kenya, which protects goats for approximately one year.

KEYWORDS: Competition enzyme-linked immunosorbent assay – Contagious caprine pleuropneumonia – Diagnosis – Disease control – Goats – Mycoplasma capricolum subsp. capripneumoniae – Mycoplasmoses – Polymerase chain reaction – Ruminants.

INTRODUCTION

The procedures for the diagnosis of contagious caprine pleuropneumonia (CCPP) have already been described in comprehensive papers (11, 16). The aim of this article is to give some additional information on the classic methods of diagnosis but also to introduce some new techniques that should greatly enhance knowledge on CCPP:

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polymerase chain reaction (PCR) for the detection of *Mycoplasma* deoxyribonucleic acid (DNA) in samples and the blocking enzyme-linked immunosorbent assay (ELISA) for the specific detection of antibodies to *M. capricolum* subsp. *capripneumoniae* (Mcc).

**DIAGNOSIS**

**Clinical and post-mortem diagnosis**

Clinical diagnosis of CCPP may be confounded by the existence of intercurrent diseases, such as heartwater, peste des petits ruminants (PPR), coccidiosis, etc. In the case of an acute outbreak, however, the clinical signs and the lesions observed may give rise to strong suspicions.

Morbidity is very high, and the disease spreads inevitably to the whole herd. In the absence of treatment, mortality is also very high and may reach 80%. Pathological changes are confined to the chest cavity and consist of pleuropneumonia: unilateral hepatisation, an accumulation of straw-coloured pleural fluid (Fig. 1) and acute pleuritis of the pleura adjacent to the affected lung (Fig. 2). The affected lung is enlarged, firm and oedematous, varying in colour from grey to red, these colours forming a mosaic. Sections appear ‘granular’ with various colours (Fig. 3), but without any thickening of the interlobular septa. Evolving lesions are characterised by round foci of hepatisation with a grey pinpoint centre of necrotisation and dark red hyperaemic margins, which contrast markedly with the pink unaffected lung (Fig. 4).

It may be difficult to distinguish CCPP from an infection by *M. mycoides* subsp. *mycoides* LC (*MmmLC*), which has a pulmonary localisation. In the case of an *MmmLC* infection, thickening of the interlobular septa may be evident. These lesions are similar to those observed in the case of contagious bovine pleuropneumonia (CBPP). Sometimes this thickening is absent or inconspicuous and laboratory confirmation is needed.

In a region where CCPP is enzootic, the clinical picture of the disease may be less clear and confirmation always requires laboratory examination.

From a clinical point of view, CCPP must be distinguished from the following:

- peste des petits ruminants, to which sheep are also susceptible, although to a lesser extent than goats. Typical lesions may be observed on the gingiva and diarrhoea follows the onset of respiratory disorders
- pasteurellosis. Lung lesions are usually bilateral and affect the cranial lobes of the lungs. The colour of the lesion is uniform
- other mycoplasmal infections, which have been described in a previous paper as comprising a ‘MAKePS’ syndrome (31). In these cases, the pulmonary localisation is concomitant with other prominent lesions of mastitis, arthritis and keratitis. Confusion is not likely unless the strain has an unusual pulmonary tropism.

Some difficulties in diagnosis should be outlined.

It is sometimes difficult to verify that only goats are affected when there are no sheep in the herd. Even when sheep are present and do not show any respiratory disorder, it should be remembered that sheep are usually less susceptible to respiratory disorders than goats. Conversely, if sheep are also apparently affected, it may be by
other pathogens, such as *Pasteurella*, and the disease may have evolved in parallel because of common factors favouring infection.

The localisation solely in the lungs, which characterises CCPP, may be masked by other infections. Diarrhoea may co-exist because of intestinal parasitism, a common feature in herds in developing countries. Pox viruses may also co-exist and induce skin lesions, as well as favouring the development of CCPP, while caprine arthritis and encephalomyelitis virus may induce immunosuppression and cause arthritis. Finally, other mycoplasmas may co-exist with *MccF38* in herds affected with CCPP. This may explain the frequent isolation of *M. mycoides* subsp. *capri* (*Mmc*) from CCPP-affected herds in both Kenya (18) and Turkey (12) before the F38 strain was identified.

**Laboratory confirmation**

*Isolation*

Until recently, isolation was the only way to confirm the presence of CCPP. The success of this isolation depends primarily on the attention that is given to sample collection.

The isolation of *MccF38* may be a long and difficult process. The attention of the microbiologist must be drawn by complete and precise reports from the field diagnostician.

*Samples*

One of the best samples is pleural fluid. Ten millilitres should be harvested aseptically from an animal that is in the acute phase of the disease. Dead animals should be avoided. The best method is to sacrifice at least one animal of the herd that did not receive any antibiotic treatment. Other samples may be gathered from live animals by lower intrathoracic puncture with broad diameter needles.

Samples of hepatised lungs are also desirable. Square 3 cm sections should be taken from an area that borders normal lung tissue.

There is no need to multiply the number of samples and all efforts should be directed towards obtaining samples of good quality.

Samples can be kept at +4°C if transport to the laboratory does not take more than one or two days. Otherwise, they can be deep frozen at −20°C. In the latter case, samples can be stored for months without loss of mycoplasma viability. For storage longer than 10 months, it is recommended to keep the samples at −70°C. In the absence of cold chain, penicillin or ampicillin should be added to the sample to limit contaminant growth.

A precise description of the isolation and identification process for these mycoplasmas is outside the scope of this paper, and can be found in recent comprehensive papers (11, 16, 28). However, it is important to point out some of the difficulties that can be encountered.

*Difficulties in isolating MccF38*

Two main difficulties can be described. First, *MccF38* grows very poorly *in vitro* and, secondly, samples are often contaminated by other mycoplasmas.
Gross lesions observed in an acute case of contagious caprine pleuropneumonia in an adult goat in Ethiopia, 1991

The opening of the chest cavity reveals an accumulation of straw-coloured pleural fluid (bottom), an accumulation of coagulated fibrin that covers the lower part of the lungs (right), and the presence of (dark red) hepatised lung, which contrasts with the normal (pink) lung.

Gross lesions observed in an acute case of contagious caprine pleuropneumonia in a kid in Ethiopia, 1991

The opening of the chest cavity reveals a right lobe which is completely hepatised, with a mosaic of colours from grey to dark red. The lung is covered with a well-developed layer of fibrin which sticks to the lung and the opposite costal pleura.
**FIG. 3**

Gross lesions observed in an acute case of contagious caprine pleuropneumonia in an adult goat in Ethiopia, 1991

Cross-section of an affected lung. The section appears 'granular', with colours varying from grey to dark red. There is no thickening of the interlobular septa.

**FIG. 4**

Gross lesions observed in an acute case of contagious caprine pleuropneumonia in an adult goat in Ethiopia, 1991

Three active foci of hepatisation. Their centre is already necrotised (grey) and the lesions are extending at the periphery, which is marked with (dark red) hyperaemia, contrasting with the normal (pink) lung.
The growth of *MccF38* usually takes four to five days, when first isolated, and the diameter of the colonies may be only 0.1 mm. These colonies can only be seen by close observation with a binocular microscope. In a liquid medium, the turbidity is very faint and should be compared with uninoculated medium.

The composition of the medium is very important and determines the rate of success as well as the size of the colonies. An example of medium composition is given in Appendix I. Some authors have recommended using fresh meat infusion to obtain good growth (13), though this may not be necessary and commercial components may ensure more regular results. Horse serum permits good growth of *MccF38*. The serum should be added at a concentration of 20% to 30% by volume. Once again, the quality of this component must be tested, by comparing different batches from different manufacturers with dilutions of freeze-dried reference mycoplasma strains. Some authors have recommended the use of foetal calf serum (12) or donkey serum (P. Perreau, personal communication). This latter serum may be interesting for developing countries, as it is very easy and cheap to obtain locally. Fresh yeast extract is also an important component of the medium and the yeast extracts that are available commercially are not of the same quality.

The other major difficulty lies in the presence of other bacterial contaminants in the samples. Usually, the classic bacteria are inhibited by the antibiotics, chiefly ampicillin, that are incorporated into the medium used for the primary culture. However, other mycoplasmas, such as *M. arginini* or *M. ovipneumoniae*, are often isolated. These two mycoplasma species are natural colonisers of the upper respiratory tract of goats and can be regularly isolated from healthy lungs (3), as well as from diseased lungs (6, 23). There is some indication that *M. ovipneumoniae* may have pathogenic potential (5), but this is probably secondary. *M. ovipneumoniae* may play a role in lung lesions that have a multifactorial origin, particularly in association with viruses or other bacteria, such as the *Pasteurella* species. In the case of CCPP lesions, *M. ovipneumoniae* is often isolated (12, 17). However, where *MccF38* and *M. ovipneumoniae* co-exist, the former is present in higher quantities, as has been demonstrated with a quantitative method, such as sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (28). The isolation of *M. ovipneumoniae* from CCPP lesions should therefore be interpreted with great caution and its aetiological role should be questioned. A highly pathogenic bacterium would presumably disseminate rapidly to numerous animals, hence all isolates from the same herd would be very homogeneous. That is not the case with *M. ovipneumoniae*, as numerous distinguishable strains of *M. ovipneumoniae* can be isolated from a single herd or even from a single animal (9, 32).

**Difficulties in identifying MccF38**

There should be no difficulty in identifying *MccF38* once it has been obtained in pure culture. The rate of growth of this organism can be compared only with that of *M. ovipneumoniae*. All other members of the mycoides ‘cluster’ grow within 24 to 48 hours, producing bigger colonies (1 to 3 mm in diameter). *M. ovipneumoniae* can be suspected when the colonies lack the classic ‘fried-egg’ appearance and do not stick to the agar surface. Finally, there is no antigenic relatedness between these two mycoplasmas.

Some serological cross-reactions can occur between two subspecies of the mycoides ‘cluster’: *M. capricolum* subsp. *capricolum* and *Mycoplasma* sp. group 7. However,
the reactions with the homologous sera are always stronger. A precise identification always requires testing with a panel of antisera from the whole *mycoides* ‘cluster’. In regard to the subspecies *capricolum*, it is advisable to use a mixture of antisera which have been prepared with different strains, as there are many variations within this subspecies and some strains might not react with an antiserum prepared against a single strain. This may be particularly the case with sera prepared from ‘California Kid’, the reference strain of the *capricolum* subspecies, which seems to be quite different from the other strains.

*Polymerase chain reaction*

This relatively new diagnostic method has radically improved the detection and identification of micro-organisms which do not grow easily *in vitro*. PCR is based on the amplification of specific DNA sequences with thermostable enzymes and nucleotidic primers that must be judiciously chosen (19).

This novel technique might improve dramatically the laboratory diagnosis of mycoplasma disease in developing countries, as PCR can be performed with dried material, hence removing the need for a constant ‘cool chain’ for samples. In the case of CCPP, the best sample may be pleural fluid that has been dried onto filter paper. The dried filter paper can then be wrapped in a plastic bag and sent to a reference laboratory (4).

The presence of antibiotics in the sample should not interfere with the reaction. However, it does jeopardise the success of isolation. Drying the sample reduces the size, thus facilitating transport and inactivating many pathogenic organisms.

This technique has some limitations.

PCR is not a reference technique as the reliability of the process may sometimes be questioned. It is still necessary to isolate *MccF38* to confirm, without doubt, the presence of CCPP in a country where it has not been isolated before. The isolation of strains is always needed to confirm periodically that the PCR technique used is specific. In addition, the isolation of strains gives access to the whole genome of the bacteria, whereas PCR amplifies only a portion. Obtaining the whole genome of a strain might be the only way to design improved diagnostic tools, based on different gene locations.

Paradoxically, the high sensitivity of the method may sometimes prove to be a limitation, as the manipulation of amplified products may contaminate some reactions and could result in false positive results. The prevention of this risk should be a continual concern for technicians working with PCR. As a result, multiple negative controls must be included in all reaction sets.

The PCR method which has been described for the detection of *MccF38* (1) is based on the amplification of a segment of the gene that codes for the 16S ribosomal RNA (rRNA). This gene is well conserved in bacteria but also possesses regions that are variable enough to ensure a distinction between species or subspecies. These variations are used to construct phylogenetic trees (34, 35) and diagnostic tools (10). In the case of CCPP, the primers were chosen specifically to amplify a fragment of this gene for the *mycoides* ‘cluster’. The identification of *MccF38* is made in a second step, by digestion of the amplified products with an endonuclease: *pst*I. Three fragments are obtained with *MccF38* while only two are seen with other members of the *mycoides* ‘cluster’. This difference indicates a single nucleotide substitution in one of the two
genes that code for the 16S rRNA in the mycoplasmas; a difference, however, which is typical of MccF38. The suitability of PCR for the detection of CCPP has already been demonstrated in Uganda and Kenya (4), and also more recently in Chad, where it enabled the diagnosis of CCPP in remote areas such as the Borku region in the northern part of the country.

Serology

Different techniques have been used. Such techniques usually imitated the procedures used for CBPP (22), such as the following:
- the complement fixation test (CFT)
- the passive haemagglutination test (20)
- agglutination of latex beads sensitised with a polysaccharide (25, 26)
- indirect ELISA (33).

The interpretation of serological results is often difficult as these tests were not comprehensively validated. Precise information on sensitivity, specificity, precocity and persistence of antibody detection is lacking. Specificity is usually poor as these techniques detect antibodies to whole mycoplasma antigens, many of which are shared by the different species of the mycoides ‘cluster’. The fact that different mycoplasma species may co-exist within a goat population will confuse the picture even further.

As a consequence of all these limitations, there is no actual test which is applicable for individual animal testing and all tests are therefore only suitable for a herd diagnosis. The diagnosis is then based on the testing of a representative number of sera against a panel of antigens of the mycoides ‘cluster’ and M. agalactiae (11). Results are sometimes difficult to analyse in spite of these precautions. For example, Jones and Wood reported conflicting results in Oman (12). After a CCPP outbreak, a higher proportion of animals had antibodies to M. mycoides subsp. capri than to M. capricolum subsp. capripneumoniae, when this latter strain was the only one isolated from affected lungs. Antibodies to M. mycoides subsp. capri might have originated from a previous infection, as this strain was later shown to exist in Oman (15).

Serological tests can be used to follow a rise in antibody after vaccination (14, 33). The evolution of the serum antibody level can be monitored but it is difficult to establish a correlation between this level and protection. Measurement of the secretory immunoglobulin A (IgA) level in lung secretions might give a better indication. This could possibly be done indirectly by measuring antibodies in saliva, as has been proposed for M. pulmonis in rats (27). Protection, however, may depend on a cellular immune response and not solely on humoral or mucosal responses.

All serological results that are obtained with a single antigen are prone to a lack of specificity and the presence of CCPP cannot be established in this way. The presence of antibodies to MccF38 in camels (21) or in alpaca (7) is not sufficient to confirm the existence of CCPP in these species. Isolation of the mycoplasma is needed, as has been performed in Niger recently. Complement-fixing antibodies were detected in the Zinder region in 1991 (2) and isolation was successful in 1995 (Y. Maïkano and F. Thiaucourt, personal communication), hence confirming the presence of CCPP.

Competition enzyme-linked immunosorbent assay

Competition enzyme-linked immunosorbent assay (c-ELISA) is a newly developed test which permits the specific detection of antibodies in animals which have been
affected by CCPP (29, 30). This test is based on the use of a monoclonal antibody (MAb), which is competing with goat antibodies to bind to the antigen that is coated on the plates. The specificity of the test depends on the epitope which is recognised by the MAb. A quantitative result is obtained with a single dilution of serum. This assay method permits the testing of numerous sera in large serological enquiries.

Antibodies detected by c-ELISA persist longer than those detected by the CFT. The latter vanish completely three months after the infection, while the former persist for more than six months. The exact persistence period is not yet established but may last much longer. Analysis of sera from field cases has shown that seroconversion did not occur in all animals, whatever test was used. In the case of c-ELISA, the percentage of positive animals in affected herds varies between 30% and 60%. As a consequence, it is clear that none of the tests gives an indication for individual testing and that the control of imported animals cannot be based on such tests.

The introduction of the c-ELISA for CCPP will permit the implementation of serological enquiries on a large scale for the first time. This test combines the well-known advantages of the ELISA format with the specificity provided by the use of a MAb.

**CONTROL**

**Treatment**

The earliness of the treatment is a key to its efficacy. It is therefore not necessary to wait for laboratory confirmation before beginning such treatment. Samples must, however, be taken first.

The most active antibiotics belong to the tetracycline group and macrolide family (such as spiramycin, lincomycin and erythromycin). Some new compounds belonging to the fluoroquinolones may also be active (such as enrofloxacin). As for any other antibiotic treatment, the duration and the dosages prescribed by the manufacturer must be followed exactly. A duration of five days is deemed necessary, and may be achieved by the injection of long-acting formulations. This kind of treatment may be the only way to ensure that all animals have been treated correctly, especially in nomadic herds in developing countries. Such treatments are more expensive than traditional methods and it is the responsibility of the technician or the veterinarian to convince the owner of the necessity for this treatment.

Antibiotics belonging to the penicillin or aminoside families must be strictly prohibited. The first, because they are inactive against mycoplasmas; the second, because they favour the development of resistant mycoplasmas, which may be causing relapses in the same herds or in neighbouring ones.

**Prophylaxis**

*Medical prophylaxis*

An inactivated vaccine with saponin as an adjuvant which protects goats for approximately a year has been produced in Kenya (24). Vaccination is followed by a rise in antibody titre. These antibodies can be detected by CFT but titres do not reach high levels. In kids, maternal antibodies persist for eight weeks and vaccination is
more efficient if it is performed when kids are older than ten weeks (14). Conversely, antibodies detected by c-ELISA may be detected as early as in the CFT, and persist at very high levels for more than a year (30). However, more studies are needed to establish if there is a correlation between an antibody level and the level of protection that is afforded. Attenuated vaccines are under development now but their efficacy has not yet been proved. The advantage would be a lower production cost plus the ability to couple such vaccines in a combined vaccine against PPR. PPR is another disease which is a major infectious constraint in goat raising.

**Animal health control**

This type of prophylaxis is the only way for a country or region to remain free of CCPP. Animal health controls can be implemented if there is strict control on animal movements and a prohibition on the importation of live animals from infected regions. The list of infected countries can be obtained from the Office International des Epizooties (OIE) and the development of new diagnostic tools should permit workers to determine exactly where CCPP is present.

Slaughtering infected animals is to be recommended for countries which are newly infected. Swift and energetic action may then prevent dramatic losses and eradication can be achieved. This was demonstrated by Hutcheon in the Cape Province after the introduction of the disease in 1881 (8), before anything was known of its real aetiology.

**CONCLUSION**

Advances in molecular biology have led to the development of new tools which now permit confirmation of clinical suspicion of CCPP to be obtained, whatever the location. A precise geographic distribution of the disease should be established. This will make it possible to determine what the true impact of the disease is in countries which are infected. This impact is not solely economic, but should also be evaluated at the socio-economic level, since goats, as well as camels, survive in difficult environments where no other such species would live. These animals play a major role in the feeding of families and contribute to the maintenance of the population in rural areas, thus reducing migration towards urban areas. The limitation of urban migration is one of the major goals for developing countries in the short term. Thus, control of CCPP contributes to regional socio-economics.

When the real impact of CCPP is known, it will be possible to design strategies to control the disease. These strategies may combine treatments, vaccinations and/or slaughter, according to the local situation.

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- further developing the laboratory confirmation of CCPP by using the new diagnostic tools provided by PCR and c-ELISA
– evaluating the vaccines currently available
– developing new tools for the diagnosis or prophylaxis of the disease.

Such approaches should allow a dramatic improvement in our knowledge of CCPP and control of this infection within the next few years.

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Résumé : Le diagnostic de la pleuropneumonie contagieuse caprine a souvent été jugé difficile, cette maladie pouvant être confondue avec d’autres mycoplasmoses des petits ruminants. Les symptômes et lésions peuvent être similaires et l’isolement de Mycoplasma capricolum subsp. capripneumoniae (MccF38) nécessite une bonne compétence technique.

Une fois les souches MccF38 isolées, leur identification ne devrait pas poser de problème. De nouvelles techniques, telles que l’amplification en chaîne par polymérase, offrent désormais la possibilité d’identifier MccF38 directement à partir de prélèvements lyophilisés. Toutefois, l’isolement de souches MccF38 reste obligatoire pour une déclaration officielle d’infection. Jusqu’à présent, le test sérologique de référence était l’épreuve de fixation du complément. Les principaux inconvénients étaient l’absence de sensibilité et de spécificité, ainsi que la brève persistance des anticorps décelés au moyen de cette technique. L’épreuve immuno-enzymatique (enzyme-linked immunosorbent assay : ELISA) de compétition, récemment mise au point, devrait désormais permettre de déterminer, à l’occasion de larges enquêtes sérologiques, la prévalence réelle de la maladie. Les traitements antibiotiques sont efficaces, mais ils ne peuvent prévenir la persistance d’un portage latent du mycoplasme. Un vaccin à mycoplasmes tués, adjuvé à la saponine, a été mis au point au Kenya ; il confère aux caprins une immunité d’environ un an.


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Resumen: El diagnóstico de la pleuroneumonía contagiosa caprina (PPCC) es comúnmente considerado difícil de lograr. El motivo de ello estriba en la posible confusión de esta enfermedad con otras micoplasmosis que afectan a los pequeños ruminantes. Los síntomas y lesiones que todas ellas causan pueden resultar similares, y el aislamiento de Mycoplasma capricolum subsp. capripneumoniae (MccF38) requiere el concurso de profesionales especialmente adiestrados.
Una vez que se han aislado las cepas MccF38, su identificación no presenta mayor dificultad. Hoy en día, la existencia de nuevas técnicas, como la de reacción en cadena de la polimerasa, ofrece la posibilidad de identificar directamente a MccF38 a partir de muestras secadas. No obstante, el aislamiento previo de MccF38 sigue siendo obligatorio para toda declaración oficial de la infección. Hasta ahora la prueba serológica de referencia ha sido la de fijación del complemento, cuyos principales inconvenientes radican en su falta de sensibilidad y especificidad, así como en la corta persistencia de los anticuerpos que esta técnica detecta. Ahora se ha desarrollado una prueba inmunoenzimática (enzyme-linked immunosorbent assay: ELISA) de competición específica para el diagnóstico de esta enfermedad, lo que debería permitir la realización de encuestas serológicas de gran escala para determinar la prevalencia real de la misma. Los tratamientos a base de antibióticos resultan eficaces, aunque tal vez no impidan la persistencia del patógeno en portadores asintomáticos. En Kenia se ha elaborado una vacuna inactivada con saponina como adyuvante, que confiere protección a las cabras durante aproximadamente un año.


Appendix I

An example of liquid medium (modified Hayflick’s medium) (28)

Part A

Bacto-pleuroneumonia-like organisms (PPLO) broth without crystal violet 21 g
High-quality demineralised water 700 ml

Part B

Inactivated horse serum 200 ml
Fresh yeast extract 100 ml
Glucose (sterile solution 0.5 g/ml) 2 ml
Sodium pyruvate (sterile solution 0.5 g/ml) 8 ml

Part A is sterilised by autoclaving at 121°C for 30 min. Part B is then added aseptically to part A. For primary isolation, ampicillin (0.1 g/l) and thallium acetate (250 mg/l) can be added to prevent contamination. The final pH should be 7.4-7.6.
REFERENCES


