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SERO-EPIDEMIOLOGICAL DIAGNOSIS IN THE LABORATORY AND IN ANIMAL DISEASE CONTROL

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REPORT
Individual S tudy Project

FOOT-AND-MOUTH DISEASE:
Diagnostic methods

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techniques developed to identify FMD replace animal experiments in the diagnosis. Three of the serotypes are endemic to sub-Saharan Africa, namely SAT1, SAT2, SAT3 (South African Territory) (Bastos, 1997). The European serotypes 0, A and C were historically exotic to southem Africa but have been identified in a number of Northern African countries . Although disease eradication has been achieved in North America and Western Europe, the chances of achieving this in sub-Saharan Africa are remote due to the role wildlife plays in the epidemiology of this disease and the greater complexity of antigenic types. The situation is further exacerbated by the presence of six of the seven serotypes and high levels of intratypic variation in the indigenous virus type making prevention and control problematic.

At present the disease is controlled in South Africa by the restriction of animal movement,and by vaccination.Early diagnosis and virus characterization is thus critical to minimize the potential detrimental economic effects of an outbreak . Presently this is achieved by virus isolation on tissue culture and/or serological techniques. The reported speed and sensitivity of virus detection by means of the polymerase chain reaction (PCR) prompted an investigation into the applicability and usefulness of the techniques in the African context. For the detection of FMD virus by PCR there are a number of primers sequences, but these primers do not readily amplify the SAT-types in particular (Vos100 et al., 1995). It is apparent that universal FMD detection can only be substantiated by testing a wide range of divergent viral subtypes or prototypes.

Materials and methods

Clinically typical cases of FMD are characterized by vesicular conditions of the feet, buccal mucosa, and , in female, the mammary glands. Clinical signs can vary from mild to severe and fatalities may occur, especially in young animals. The tissue preferred for diagnosis is epitheliae tissue from unruptured or freshly ruptured vesicles. Where this is not possible, blood and/or oesophageal-pharyngeal fluid samples taken (probang) in ruminants and throat swabs from pigs, provide an alternative source for virus isolation. From fatal cases, myocardial tissue or blood can be sampled, but vesicles are again preferable if present. It is vital that samples from suspected cases are transported under secure conditions and according to international regulations.

To screen for this disease, samples are taken from diseased and apparently non-diseased animals by random sampling method in a cross-sectional study. However, non-diseased animals in an FMD infected herd may be a

Introduction

During 1996, an epidemic of FMD disease was reported in West Africa (OIE/FAO Reference Laboratory for FMD Jan-March), (1996).
With the fast expansion of FMD disease, was of interest for me to familiarise myself with diagnostic methods for FMD.
FMD disease is a highly contagious, economically devastating disease of cloven-hoofed animals. There are seven serotypes of FMD virus, namely 0, A, C, SAT1, SAT2, SAT3 and ASIA1. Infection with one serotype does not confer immunity against another.Clinically, FMD cannot be differentiated from other vesicular diseases, like swine vesicular disease,vesicular stomatitis and vesicular exanthema. Laboratory diagnosis of any suspected FMD case is therefore a matter of urgency.
The World Reference Laboratory of FMD Disease makes the confirmatory diagnosis (Pirbright in Great Britain) for the International Office of Epizootics (OIE).
During my stay at the Federal Research Institut for Animal Virus Disease in Tiirbingen (Federal Republic of Germany) I had the opportunity to acquire some useful and relevant diagnostic techniques to investigate FMD disease.
Theses techniques include the neutralisation test, the FMD-plaque test and the Enzym Linked Immunosorbent Assay (ELISA) antibody detection. Some others techniques such as.:methods to inactivate FMD virus and to isolate the virus in cell culture (infected cell culture by FMD, or African swine Fever) were also demonstrated.

Statement of study objectives

The objectives of this project are to learn methods to diagnose Foot-and-Mouth Disease.

Literature review

Foot-and-Mouth Disease remains currently the most contagious disease with a great potential for causing heavy losses in susceptible cloven-hoofed animals. Most veterinarians and scientists in the world deal with the problematic disease and many publications were made. (OIE chapter 2.1.1. 1996)
The seven serotypes 0, A. C, SAT1, SAT2, Sat3 and ASIA1 originally were determined by cross-challenge experiments in cattle; now serological

problem due to the contagiosity of this disease; if necessary a neighbouring non- infected herd can be choosen to find non-diseased animals.
Some difficulties may also occur with some diagnostic methods based on serological responses in endemic areas due to the possibility of a previous infection. The detection of specific humoral antibodies requires also absence of any history of vaccination, as it is not always possible to differentiate a serological response due to natural infection from a response due to vaccination.

A/ Materials

ELISA antibody detection

1/ Phenol red solution (0,2%)
2/ Azide solution/1M)
3/ Coating buffer(0,05M carbonate-bicarbonate buffer ph 9,6)
4/ Phosphate buffered saline (Dulbeccoo PBS)
5/ ELISA diluent
6/ Blocking buffer
7/ Substrate (OPD in phosphate-citrate buffer, ph 5,0)
8/ Acid stopper solution
9/ Disinfectant
10/ PBS T

B/ Others materials

1/ Flat bottomed ELISA
2/ Tips multichannel pipette and one channel pipette
3/ Centrifuge
4/ Incubator
5/ Gloves and others protectives
6/ Orbital shaker, and magnetic stirrer
7/ Flat Nunc

C/ Others reagents

1/ ELIS A FMD- Antigen
2/ Serum
3/ Rabbit anti-FMD serum
4/ Guinea pigs anti-FMD serum

5/ Peroxidase conjugated rabbit immunoglobulins to Guinea Pigs immunoglobulins (conjugate)
6/ OPD-substrate

Method

1/ Coating with rabbit serum in coating buffer (0,05M NaCO3/NaHCO3, ph 9,6) the flat bottomed ELISA plates

2/Incubation over night at 4°C or freeze at -70°C 2H-3H
Add 50µl/well in all wells

2/ Prepare the mixture sample (serum) plus antigen (Serotypes: OMannish, A22, and C 10)

Serum dilution: 1/5 ,1/10, 1/20, 1/40 .
0 Mannish dilution: 1/1 20
A22 dilution: 1/1 00
C 10 dilution: 1/100
Hyper-immune serum against FMD dilution: 1/5, 1/10, 1/20, 1/40.
Control 50% positive dilution for A22: 1/3000, Omannish1/4400, c 10: 1/4400
Control 100% positive only with virus and buffer
Keep some wells as blank for each serotype

31 This plate of mixture is incubated over night at +4°C

4/ Flick off the solution of the coating rabbit serum

5/ From the mixture antigen/sample take 50µl/well at column 1 to column lof the flat bottomed test ELISA .Column 2 to column 2 etc until column 12 .

6/ Incubate 1 hour at 37°C

7/ Flick off the solution and wash 3 times with PBS-Tween 20 0,05%;dry the flat with filter paper

8/ Add the guinea pig anti-serum against FMD diluted in PBS-Tween20 0,1% + 5% skimmed milk powder of each serotype :50µl by well in all well

91 Repeat step 6

10/ Dilute the conjugate at 1/2000 and add 50µl by well in all wells
N B :Guinea Pig serum against these serotypes and the conjugate must be pre-diluted at ½ with normal rabbit serum before the proper dilution with PBS Tween 20 1%.

11/ Flick off the solution and wash 5 times and dry carefully the flat.

12/ Mix the OPD-substrat : 10ml OPD + lml H2O2(pre-dilute at 1/60) add 50 µl by well in all wells

13/ Incubate 15 minutes at room temperature in a dark box.

14/ Add the stopper solution: H2SO4 dilute at ¼ :amount 50µl by well in all well .

15/ Read the flat with a wavelenght of 492 nm by using the photometer ELISA reader.

For this test two sera were tested for demonstration .

Results

Both sera which were tested (for demonstration) by ELISA antibody detection were negative. The optical densities for serum 1 were 0.8 15 , 0.912 , 0.85 1 , 0.931 at the respective four dilutions 1/5 , 1/10 , 1/20 , 1/40 .
The optical densities for serum 2 were 0.90 1, 0.873, 0.824, 0.93 1 at the same dilutions like serum 1 .
The tut-off of this test was set at 1.5

In the neutralisation test the results indicate a certain cross-reaction between C 1N and A24 and further cross-reaction between Asia1 and A24. No crossing was observed with reaction A87, SAT1 and A24 .

With the plaque test we had also positive reactions which were indicated by the presence of virus forming plaques on the surface of the agarose gel medium. Observing theses plaques under the microscope with the 40* objective, beautiful ggregates, destroyed by the virus, could be seen .

With these results obtained during this study the major findings for me are that:

these test are relevant and useful and not very expensive for developing countries; they give satisfactory results during and after the viraemic period of FMD.

using these tests, two types of studies can be applied:a cross sectional study and a case control study.

for antibody detection using ELISA, I have learnt to adjust the reagent dilution (antigen, sera, conjugate, OPD-substrat) to fmally get the correct result as titer or in measured optical densities.

Discussion

The Virus Neutralisation Test as an accepted test for the quantification of antibodies against FMD has been used successfully. This test is considered sensitive, specific and relatively simple to perform but requires tissue culture cells which all too often vary in their sensitivity or fail to grow because of contamination, poor growth conditions or toxic substances in the test samples.

During my study a specific cell culture the so-called FMD Ct cells, from baby hamster kidneys which were treated to get the cell line were used. This neutralisation test requires also incubation at 37°C for three days before results can be read.

In the ELISA test using homologous reference serum antibody titers for each virus studied results were generally higher than those recorded in the virus neutralisation test. Cross reactions were recorded in both assays but the difference between the homologous and the heterologous antibody titers was greater using ELISA. The high intratypic variation observed for all FMD virus types make the disease difficult to handle. The cross-reactions in both tests with SAT1 and SAT3 reflect the true relationship between these two virus types. In such a case another test like the monoclonal ELISA or the polymerase chain reaction antibody should be performed.

So, for a first check for FMD virus the antibody detection ELISA which is rapid and relatively simple to perform should be applied. It is also economic in terms of reagents and results can be reported after one day .

It is also very important in case of a FMD epidemic to determine the strains (serotype) before making any proposa1 for control by vaccination.

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