SEROLOGICAL DIAGNOSIS OF ERYSIPELOTHRIX RHUSIOPATHIAE: A COMPARATIVE STUDY BETWEEN THE GROWTH INHIBITION TEST AND THE COMPLEMENT FIXATION TEST.

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Erysipelothrix rhusiopathiae (ER) outbreaks in pigs are common, and can lead to economic losses and form a source of human infection (5.11). In pigs the disease can cause abortion (4) as well as chronic polyarthritis (2).
Serological diagnosis of ER infections can be performed by means of the Agglutination test (9), Growth Inhibition test (GIT) (7,9) or Immunofluorescence test (7).
Studies on more than 3000 pig and sheep sera showed that many of the examined animals and particularly the sows had GIT titres against ER, but as the majority of the sows were vaccinated against ER it seemed very unlikely that all these sows should abort due to ER infection. Positive GIT titre was therefore not conclusive, as the titres found could have been due to a subclinical infection or a vaccination.
In order to be able to differentiate between infection titres and vaccination titres, a Complement Fixation Test (CFT) was developed and the results were compared with those of GIT.

Material and methods

Sera: Sera were taken from sheep suffering from polyarthritis, vaccinated breeding sows with an abortion history, normal vaccinated sows and apparently healthy pigs.
Antigen: For the CFT and the GIT the Marienfeld strain was used. The strain was propagated in the following medium:

Na_2HOP_4, $12H_2O$ (analytical grade)	a.g.	18 g
Glucose	a.g.	6 g
Brünengräber peptone		5 g
Yeast extract (Merck)		5 g
L. arginine HCl		0.5 g
Tween 80		0.5 g
Distilled water to		1000 ml
pH 7.8 - 8.0		

After 24 hrs incubation at 37°C a Merthiolate solution was added to the culture to obtain a final concentration of 1:10,000. The culture was kept 24 hrs at room temperature and then centrifuged at 2000 g for 80 mins.
The sediment was washed twice with saline and resuspended in 1:5,000 Merthiolate solution to give 1/10 of the original culture volume. The suspension was then freeze-dried in 1 ml vials.
The antigen concentration for the CFT was determined by means of chessboard titration. The dilution which gave a complete fixation of complement with the smallest quantity of serum was used in the routine test.

The Complement Fixation Test. The haemolytic amboceptor was used in a dilution of 1:350. The sheep red blood cells were standardized to 50% haematocrit. For the haemolytic system a 2% suspension was used. The complement concentration was determined by using 1:350 diluted haemolytic amboceptor and 2% erythrocytes suspension.
The CF test was carried out in V microtitre plates. The sera were diluted 1:5 with saline and inactivated for 55 mins at 60°C. Rows A and H were filled with 0.05 ml diluted serum and the others with 0.025 ml diluted veronal buffer pH 7.2 (veronal buffer was diluted 1:4 with a gelatine solution containing 400 mg bactogelatine in 1000 ml distilled water). The serum from row A was further diluted by transfer of 0.025 ml from one row to the next. Before transferring to row H the diluters were dried off on blotting paper; then transferred to row H taking out 0.025 ml diluted serum and used as an anticomplementary serum control. After adding 0.025 ml of both antigen and complement the plates were shaken for 3 mins and incubated for 30 mins at 37°C. The haemolytic system was also kept at 37°C for 30 mins. After adding 0.025 ml of haemolytic system the plates were shaken again and incubated for 30 mins at 37°C. Then the plates were centrifuged for 1 min at 2000 rpm and the results were recorded.

Results and discussion

Only 63% of 200 apparently healthy pigs were found to be negative in the GIT, compared to 98.5% when examined in the CFT.
Of 200 animals vaccinated against ER only 8.5% were negative, while the rest had titres from 1:10 to 1:320 when the GIT was used; 93.5% of the animals were negative when the CFT was used.
Of the 200 animals with polyarthritic symptoms which were examined with the GIT and the CFT, 21% had titres in the GIT, compared to 57.5% in the CFT.
When animals with an abortion history were examined there was no significant difference in titre height and distribution between the GIT and the CFT. As the bacteria are carried by many species (6, 8, 3, 10) it is not surprising that many of the apparently healthy animals have titres against ER, when examined with the GIT.
The GIT is a very reliable test for diagnosing ER, but the high number of positively reacting animals makes the interpretation of the results very difficult. The titres obtained are usually not high enough to indicate an infection, but sometimes they are not so low as to be considered non-specific and therefore irrelevant. The CFT gives more reliable results compared with the GIT when healthy or vaccinated animals are examined. The CFT can be very useful to determine chronic infections, because 57.5% of the examined animals had titres in comparison with only 21% when the GIT was used.

In conclusion it may be said that the CFT is a very useful, reliable, and accurate test which has many advantages in comparison with the GIT. There is no need to sterilise the serum and the results can be recorded within a few hours. The antigen is very stable and will keep for years without losing its activity, but the most important advantage is the fact that there is no hazard to the laboratory personnel.

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