STUDIES ON THE TYING OF *ERYSIPLOTHRIX RHUSIOPATHIAE*

IV. EPIZOOTIOLOGICAL SIGNIFICANCE OF *ERYSIPLOTHRIX RHUSIOPATHIAE* HARBOURED IN THE TONSILS OF APPARENTLY HEALTHY PIGS

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(Received for Publication July 17, 1959)

In our previous papers\(^{13,14,15}\), descriptions were given of the findings made in the course of the investigations and experiments carried out in an attempt to clarify the relationship between the sero-groups of *Erysipelothrix rhusiopathiae* (*E.r.*) recovered from sick pigs, and their clinical manifestations. Besides, observations were also made on the fact that strains of several sero-groups, not belonging to the pathogenic Groups A nor B, were isolated rather frequently from the tonsils of apparently healthy pigs.

In the past, little consideration was paid to the epizootiological significance of *E.r.* harboured in the tonsils of healthy pigs, not to speak of the distribution or seasonal changes in their sero-groups.

In this connection, the authors' studies were directed on the following subjects:

1. Investigation of the epizootiological significance of the correlation between the seasonal changes in the distribution of the sero-groups of *E.r.* harboured in the tonsils of apparently healthy pigs, and that of the causative agents of swine erysipelas in the field.

2. By increasing the number of pigs to be examined we made further inquiries into the presence of subgroups among the strains belonging to Group Fr together with a more detailed antigenic analysis, and then—

3. Studies were undertaken to clarify the essential difference between the septicemic form of erysipelas, caused by Group A organisms, and the urticarial form of erysipelas, caused by Group B organisms.

Stated in the following are the findings made during the investigations on the isolation of *E.r.* organisms from the tonsils of a total of 1,045 apparently healthy pigs, and the making of epizootiological comparisons with the strains isolated from pigs suffering from erysipelas in the field.

Moreover, as a routine measure for the classification of sero-groups, the slide agglutination test was recommended in place of the precipitation test which required highly complicated techniques in the preparation of its antigen.

1. Slide agglutination test for differentiation of *E.r.*

MATERIALS AND METHODS

Strains: In the present experiment, 24 strains classified as Groups A, B, C, D, E, and F by the precipitation test, were used. (Two groups designated as Group E and Group F were newly differentiated from Group Fr during further investigations made on the tonsils of healthy pigs, the details of which will be reported later.)
Antigens were prepared from all of the 24 strains, while antisera were prepared only for the representative 12 strains (two strains each of the six groups).

Antigens: Organisms were seeded in broth containing 1% of glucose and incubated for 48 hours at 37°C; 200 ml of this broth culture was then centrifuged at 3,000 r.p.m. for 30 minutes, washed twice in saline and resuspended to give a density of McFarland No. 10.

Antiserum: The method of the preparation of the antiserum was exactly the same as that for the precipitation test.

Slide agglutination reaction: A drop of the antigen to be tested was added to each 0.05 ml of a series of diluted antiserum (1:5—1:160).

As a routine procedure, in this study, 12 series of diluted antisera of the 6 groups (2 antisera for each group) were placed on a glass plate and a drop of the antigen to be tested was added to each of them. Each mixture was then stirred well with a glass-bar, and the plate was tilted back and forth for 30 seconds. Reaction was observed against a black background, and the reading was made with the naked eyes within three minutes.

RESULTS

Antigens of all the six groups showed their characteristic reaction patterns, against the 12 series of diluted antisera, as shown in Table 1. They reacted strongly, and specifically, to a pair of the corresponding antisera of the strain differentiated by the precipitation test, while negatively or weakly to those of the other groups.

Consequently, the results, which were readily obtained by this slide agglutination test, were exactly the same serologically as those obtained after troublesome manipulation by the precipitation test.

2. Seasonal changes in the isolation rates of the 6 groups of E.r. harboured in the tonsils of apparently healthy pigs.

| Table 1. Reaction Patterns of Cross Slide Agglutination Test Drawn by the 6 Groups of E.r. Organisms Differentiated by Precipitation |
|---|---|---|
| **Serum Dilution** | **Antigen “Kuniyasu”** | **Group B “P-1”** | **Group C “P-190”** |
| **Antiserum** | 5 | 10 | 20 | 40 | 80 | 160 | 5 | 10 | 20 | 40 | 80 | 160 | 5 | 10 | 20 | 40 | 80 | 160 |
| Gr. A Kuniyasu | | | | | | | | | | | | | | | | | | | |
| Gr. B P-1, P-31 | | | | | | | | | | | | | | | | | | | |
| Gr. C P-190, P-206 | | | | | | | | | | | | | | | | | | | |
| Gr. D P-23, P-32 | | | | | | | | | | | | | | | | | | | |
| Gr. E P-43, P-99 | | | | | | | | | | | | | | | | | | | |
| Gr. F P-34, P-100 | | | | | | | | | | | | | | | | | | | |
In the previous report, an inference was made on the significance of the organisms, especially, those of Groups A and B found in the tonsils of apparently healthy pigs.

If the organisms harboured in the tonsils have some significance in the occurrence of swine erysipelas in the field, some seasonal fluctuation in their distribution or isolation rates should also be observed in connection with the frequency of the disease. Tonsils, investigated in this study, were from 1,045 pigs raised in the Towada district and slaughtered, twice per week, at the Towada Slaughter House during a period of one year, from Sept. 1956 to Aug. 1957.

Bacteriological examinations were done by the mouse inoculation tests as follow:
About 1.0 g each of the parenchym taken aseptically from a pair of tonsils of a pig, was emulsified in a mortar with 1.0 ml saline, and 0.5 ml of the emulsion was then put into a broth tube. The tube was incubated at 37°C for 18-20 hours, and then the culture was inoculated on the scarified surface of the ear-lobes of a mouse.

Gram positive organisms resembling E.r. were isolated on a solid medium from the heart blood of mice which had died within 3-5 days after inoculation.

The organisms, isolated, were identified as E.r., on the basis of the biological behaviours described in Bergey's manual.

Their serological behaviours, were further examined by the procedures mentioned in the previous chapter.

Consequently, 362 strains of E.r. were isolated from the 1,045 pigs investigated, and they were differentiated, serologically, into 6 groups by their pattern on slide agglutination reaction.

It was a matter of great surprise that 19 of the Group A strain, and 175 of Group B strain, both of which are considered to be pathogenic to pigs, were found among those 362 strains. And the results, shown in Table 2, were almost identical with those of the former investigation performed on the 129 pigs from the Shibaura Slaughter House, in Tokyo.

Thus, in Japan, there is no denying the fact that the E.r. of all six groups, including the pathogenic strains of Groups A and B, are harboured in the tonsils of apparently
Table 2. Monthly Isolation of E.r. from the Tonsils of Apparently Healthy Pigs

<table>
<thead>
<tr>
<th>Period</th>
<th>No. of Strains isolated and differentiated</th>
<th>Isolation rate (averaged by three months)</th>
<th>Cases examined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>Sept., 1956</td>
<td>2</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>Oct.,</td>
<td>0</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Nov.,</td>
<td>2</td>
<td>19</td>
<td>4</td>
</tr>
<tr>
<td>Dec.,</td>
<td>2</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>Jan.,</td>
<td>0</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Feb.,</td>
<td>1</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>Mar.,</td>
<td>2</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Apr.,</td>
<td>2</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>May,</td>
<td>1</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>June,</td>
<td>3</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>July,</td>
<td>3</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Aug.,</td>
<td>1</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Total (Average)</td>
<td>19</td>
<td>175</td>
<td>20</td>
</tr>
</tbody>
</table>

Seasonal changes in the isolation rate of the six groups of E.r. are shown in the figure by the curves drawn as the mean of three months. The curve of Group B showed a significant seasonal fluctuation, ascending in summer and descending in winter, opposite to the frequency of swine erysipelas cases in the field. A high isolation rate of the organisms of Group B was seen in autumn (Oct.~Dec.) and the rate was low in spring (Mar.~June), while that of Group A was found to be high in summer (June~July) and low in winter (Nov.~Feb.) with a little slip from that of Group B. Curves of Groups D and F were, more or less, similar to the curves of Groups A and B.

3. Isolation of E.r. by mouse inoculation and direct cultivation.

Whether E.r. organisms harboured in the tonsils are of a single sero-group, or, the mixture of several groups, is of great importance for the consideration of their etiological significance in the case of swine erysipelas.

As an attempt to clarify this point, bacteriological examinations of the organisms harboured in the tonsils of 215 pigs, were made by both mouse inoculation and direct cultivation.

Results obtained were compared with each other and discussed in the following: Medium used for cultivation was agar-agar (pH 7.8) containing sodium-azide (0.05%),
crystal-violet (0.001%), horse-serum (5.0%), and bacto-tryptose (2.0%).

The results obtained were as follows:

1. Negative cases by both methods ........................................ 142
2. Positive
   
   25
3. Positive cases only by mouse inoculation ...................... 40
4. " direct cultivation ........................................ 8
Total ........................................................................ 215

As shown above, although mouse inoculation is by far the most efficient for the isolation of *E.r.* as compared with direct cultivation, the fact that there were 8 positive cases determined only by direct culture, is quite important for the consideration of the distribution rate of *E.r.* organisms harboured in tonsils.

Strains isolated from the 25 cases, on which both mouse inoculation and direct cultivation were positive, were confirmed in their sero-groups.

On comparison, it was found that the sero-groups agreed in 19 pairs out of the 25 (13 pairs of Group B, 2 pairs each of Groups C, D and F), while the sero-groups were discrepant in 5 pairs (E:F, B:A, D:B, B:A, and C:B—mouse inoculation: direct cultivation).

In the remaining pair, sero-group of the mouse inoculation strain was not determined on account of the development of a severe nonspecific agglutination.

4. Serum agglutinin titres of slaughtered pigs.

A number of the pigs undergoing the present investigation were examined as to their serum agglutinin titres on 2 occasions, once in autumn and once in spring. Results obtained are shown in Table 3.

<table>
<thead>
<tr>
<th>Investigation</th>
<th>Period</th>
<th>Case</th>
<th>1:64 or more</th>
<th>1:16 &amp; 1:32</th>
<th>1:8 or less</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oct., 1956</td>
<td>80</td>
<td>11 (4)</td>
<td>9 (2)</td>
<td>60 (14)</td>
</tr>
<tr>
<td>2</td>
<td>June, 1957</td>
<td>97</td>
<td>5 (1)</td>
<td>12 (4)</td>
<td>80 (3)</td>
</tr>
</tbody>
</table>

( ) . . . Positive Isolation of the Group B Organisms from Tonsils

5. Frequency of the occurrence of swine erysipelas, in the Tohoku district.

Occurrence of swine erysipelas, in the Tohoku district, was more frequent in spring (April~June) than in autumn or winter.

For comparison with the findings of bacterial isolation, a detailed investigation was made on sick pigs discovered in the same area during the same period as the 1,045 pigs employed in the present study. As shown in Table 4, findings of the field investigation generally coincided with those of the bacterial isolation from tonsils.

CONSIDERATION

As the result of comparative studies made on the serological differentiation of *Erysipelothrix rhusiopathiae*, it was made clear that slide agglutination reaction with concentrated antigen is superior to the precipitation reaction used hitherto, requiring much less time and trouble in its antigen preparation.
Table 4. Sick Pigs Discovered in the Same Area and at the Same Period as Those of 1,045 Pigs Investigated by the Authors

<table>
<thead>
<tr>
<th>Period</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
<th>Jun</th>
<th>Jul</th>
<th>Aug</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swine erysipelas</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>7</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>31</td>
</tr>
<tr>
<td>Other diseases</td>
<td>99</td>
<td>70</td>
<td>38</td>
<td>4</td>
<td>4</td>
<td>9</td>
<td>20</td>
<td>30</td>
<td>28</td>
<td>48</td>
<td>62</td>
<td>47</td>
<td>458</td>
</tr>
<tr>
<td>Total</td>
<td>101</td>
<td>71</td>
<td>40</td>
<td>5</td>
<td>4</td>
<td>10</td>
<td>22</td>
<td>37</td>
<td>33</td>
<td>51</td>
<td>65</td>
<td>50</td>
<td>489</td>
</tr>
</tbody>
</table>

(From the note of a local veterinarian)

The 6 kinds of reaction patterns, moreover, demonstrated by the antigens tested against 6 groups of antisera, easily enabled the differentiation of E.r. isolated from the pig tonsils.

In addition, they suggested the existence of some sub-antigenic correlations prior to their antigen analysis carried out parallel to the present study (a separate report will be made in the near future).

Rate of the isolation of each E.r. group, harboured in the tonsils of apparently healthy pigs, was investigated for a period extending over one full year, and the total number of pigs slaughtered during this period, was 1,045.

Consequently, the organisms, biologically identified as E.r., were isolated at the rate of 34.6%, including 1.82% of Group A, and 16.74% of Group B. And the isolation rate of E.r. was observed to be high in autumn, and low in spring. Moreover, pigs investigated in autumn showed higher serum titres than those investigated in spring.

On the other hand, incidences of swine erysipelas in the field, were most frequent in spring, and rare, or almost negligible, in autumn and winter, contrary to the tendency observed in the isolation rate of E.r. from the tonsils of healthy pigs.

E.r. organisms, harboured in the tonsils of healthy pigs, were of a single sero-group, suggesting that the E.r. organisms which had invaded into the tonsils were multiplying there, regardless of whether the pigs showed clinical symptoms, or not.

As for the seasonal discrepancy found between the distribution of E.r. in the tonsils of apparently healthy pigs, and that of the swine erysipelas in the field, it might partly be due to the method of swine raising in this district, and the ages of the pigs investigated. Namely, in this district the pigs are raised mostly as a side job by the farmers, and the piglings are habitually produced twice in a year (in early spring and in autumn), because of the unfavourable circumstances of a stimulative climate, and the feed supply. And, nearly all of the pigs were investigated 6–8 months after their birth.

Since E.r. organisms are widely distributed in these surroundings, they come in contact with the animals as they get older, and the isolation rate from the slaughtered pigs may not be directly related to that of the sows and youngsters.

The piglings which were farrowed in autumn continue to grow until spring under few pathogenic and immunogenic influences of E.r. on account of their inherent immunity, as well as an inactive period on the side of the E.r. organisms themselves, though their distribution is yet high among adult pigs. As spring comes round, the piglets which were farrowed in autumn, having been less exposed to the organisms since their birth, become easily affected because the activities of the E.r. harboured in the adult pigs are now recovered, though their distribution is not yet high.

On the contrary, piglings which were farrowed in spring grow into adults showing
few clinical symptoms on account of their inherent immunity and rather, acquiring some immunity through the invisible pathogenic influence of the *E.r.* organisms which have regained their activities and have invaded into their tonsils, or other parts. The above inference is based on the results of the present investigation and the findings of the experiment which showed that the pigs which had previously dealt with the live virulent organisms of Group B showed an obvious resistances to the challenge, not merely to the same organisms but also to those of Group A (a separate report will be made in near future), and also on the common tendency observed in all the microorganisms to be viable in the summer season, and not so in winter.

The significance of the organisms harboured in the tonsils would be increased with the use of more than two mice for each isolation.

As the result of the present investigation, the distribution of *E.r.*, especially of Group B organisms, in the tonsils of apparently healthy pigs, was found to be much wider than hitherto imagined. Nevertheless, only a few of them develop the clinical symptoms of swine erysipelas in the field.

Further, the causative agents of swine erysipelas are the same as the organisms of Group A and B found in the tonsils as apparently normal flora. It may be considered, therefore, that the swine erysipelas is a disease generally caused, not merely by the organisms, but that it also requires some predisposing factors which allow active multiplication and further invasion of these organisms. These above mentioned considerations suggest that it is a disease which may be included in the category of the autogenous infectious diseases of Prof. Ochiai.

On the other hand, there were evidences to lead one to believe that the pigs, other than those which had developed obvious swine erysipelas, were also receiving, in some pathogenic and immunogenic manner, influence through the organisms harboured in their tonsils and other parts.

Further, tonsils harbouring the organisms of other groups which might possess an antigen in common to that of Group A and B, e.g., Group F organisms, which will be discussed in the next paper, are supposed to have some immunogenic significance against the infection of Group A and B organisms, though they were not pathogenic by themselves.

The above consideration on the discrepancy between the distribution of *E.r.* harboured in tonsils, and that of the swine erysipelas cases in the field, may also give some suggestions as to the selection of the pigs, and the time suitable for experimental infection.

**SUMMARY**

1. Slide agglutination pattern drawn by the six group immune sera, and concentrated antigen prepared from the objective strain, is recommended as the routine test for the differentiation of *E.r.* as a substitute of precipitation test.

2. Antigenic correlation among the six groups of *E.r.* was determined from the patterns drawn by slide agglutination prior to the antigen analyses (which will be reported in the next paper of this series).

3. Isolation rate, or distribution rate, of *E.r.* from the tonsils of apparently healthy pigs, in the Tohoku district, was low in spring, and high in autumn.

4. The rate of swine erysipelas incidence in the field, in the same district, was high in spring, and low in autumn and winter, contrary to the distribution rate of *E.r.* in the tonsils of healthy pigs.

5. The discrepancy found between the incidence rate of swine erysipelas in the field, and the isolation rate of *E.r.* from the tonsils of apparently healthy pigs, was explained as
due to the method of swine raising in this district, and the age of the pigs employed in the present investigation.

6. Generally, the E.r. organisms harboured in the tonsils of apparently healthy pigs, are of a single sero-group, but while undergoing multiplication they give some pathogenic and immunogenic influence on the animal, though they may not manifest any clinical symptoms.

7. Swine erysipelas is a disease which may be included in the category of the autogenous infectious diseases of Prof. Ochi.

8. Particular care should be taken as to age in the selection of the animal, and the time of the experiment, when attempting artificial infection of swine erysipelas.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Director Dr. S. Ishii and the former Director, Dr. M. Kobayashi, respects to Prof. Y. Ochi for his valuable advice, and sincere thanks to K. Ajiki for his kind help in this study.

REFERENCES

豚丹毒菌の分類に関する研究

IV. 里掛上健康な豚の扁桃腺に保有される Erysipelothrix rhusiopathiae の疫学的意義について

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農林省家畜衛生試験場
(昭和34年7月17日受付)

1. 1, 2, 3 報では、各種の病変（豚・鳥類・牛・人）に由来した E.r. の血清学的分類とそれらの由来した動物の病型との関係、海産魚類の体表粘液から分離されたいわゆる E.r. の血清学的分類とその感染状態に見るような E.r. の疫学的検討及びそれらの豚に対する疫学的な意義について、また、一部健康な豚の扁桃腺からマウス接種で分離される E.r. と同定されたものについても検討され、それらの間に、血清学的には、急性敗血症型から分離される Group A 又は主として脳症型の Group B と異なるが、生物学的には E.r. と同定させるを得ない数群のものが、かなり高率に見出される事実についても触れた。

本報告では青森県十和田市市営屠場において、1年間に屠殺された1,045頭の健康な豚の扁桃腺を対象に E.r. の分離を試み、それらの関係について、沈降反応のほかにセガラス法凝集反応を併用して、先に推論した Group A～D 以外の群を探求、また、その結果と野外の豚丹毒発生の実態とを比較しながら、これを一見健康な豚の扁桃腺などに保有される E.r. の疫学的意義を検討するとともに、本病の病性の本質について考察したところを述べた。それらを要約すると次のとおりである。

1. 一見健康な豚の扁桃腺からマウス接種によって分離され、生物学的性状から E.r. と同定されるもののなかには、Group A, B, C, D 以外に Group E 及び F が独立して存在することが知られた。

2. これら6群（A～F）の菌の抗血清に対しての、被検 E.r. 菌株の濃厚粘液を抗原とするのセガラス法凝集反応による抗原検査は、沈降反応に代わる簡易な方法として、E.r. の血清学的分類に充分慣用されることもあり得る。しかも、これら6群の群が識別する可能性のある抗原検査は、E.r. 6 群間の抗原的相互関係が、次報で述べる抗原分析の結果を得たときに予測された。

3. 十和田地方で生産され、販売用として摘き取られた一見健康な豚の扁桃腺から分離された E.r., 特に Group B の分離率高くて分布度は、明らかに豚に低く高い。

4. 里掛上健康な豚の扁桃腺に保有される E.r. は、部別に単位である傾向が強く、臨床症状の有無にかかわらず、そこで発症度が異なる。何らかの病原学的・免疫学的影響を、その動物に与えるものがあるかある。

5. 十和田地方における豚丹毒の発生は 3. に述べた一見健康な豚の扁桃腺から E.r. の分布率の季節的変動と反対に、明らかに豚に多く秋から冬にかけては少し少しと見られない。

6. 扁桃腺からの E.r. 特に Group B の分布率（これは分布度）の季節的変動と豚の豚丹毒の発生の幅とときに注目すべき事実を、このごとく示明した。すなわち、この地方の仔豚の生産が春秋2期に大別され、しかも、調査対象の大半の豚が 6～8 であったこと、本畜産との間には生産免疫の成立が容易であること、刺激的状態のこの地方では、当然、この種の豚の活動は春から夏にかけて盛んになると考えられること、又その高い分布度を考えると、考察の項で詳しく述べたと同く、概して仔豚の成長するに伴って種々の程度の菌の影響小さく、いわばは種々の程度の免疫を獲得するにいたるとみる根拠があるが、その実態、いわゆる春仔と秋仔ではかなり異なり、秋仔の場合仔豚の E.r. の分布度の高い時期に生産されながら、その低感受性と季節的な関係で豚の影響をうけることと成長をついにつづき、豚の分布度が中や低いこともかかわず、その活動力の旺盛である春に至って感染発症するものが差、これに反し、春仔は、この種の豚に対する感受性の低い時からその汚染影響
をうけること多く、自然に免疫を獲得してゆき、秋から冬にかけてはそれらに発症をみるとことが少ないものと考えられる。

7. 上述の調査試験の結果並びに考察から、この豚丹毒も本質的には越智の雲の自発性感染病の1であり、その発症には種々の前提条件を必要とすると考えられるが、豚を用いての感染試験に当っては、著者らの調査試験の結果を考慮して、対象豚と時期を慎重に選択すれば、従来に比べてはるかに整った結果が得られることを知られた。（この見解に立っての豚の感染試験の成績は稿を改めて報告する）