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D E C I S I O N
of 6 February 2002

Case Number: T 0465/98 - 3.3.4

Application Number: 89202080.1

Publication Number: 0354628

IPC: A61K 39/102

Language of the proceedings: EN

Title of invention:
Vaccine suitable for prophylaxis and control, respectively, of the pig disease caused by Haemophilus pleuropneumoniae as well as a method for its production

Patentee:
CENTRAAL DIERGENEESKUNDIG INSTITUUT

Opponent:
Akzo Nobel N.V.

Headword:
Haemophilus pleuropneumoniae/CENTRAAL DIERGENEESKUNDIG INSTITUUT

Relevant legal provisions:
EPC Art. 56

Keyword:
"Inventive step (no)"

Decisions cited:
T 0939/92

Catchword:
-

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Case Number: T 0465/98 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 6 February 2002

Appellant: (Opponent)
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Decision under appeal: **Interlocutory decision of the Opposition Division of the European Patent Office posted 17. Februar 1998 concerning maintenance of European patent No. 0 354 628 in amended form.**

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: A. L. L. Marie
S. U. Hoffmann

Summary of Facts and Submissions

I. The appeal lies from the decision of the opposition division to maintain the patent in suit in amended form.

II. This decision was based on a set of 6 claims, claims 1 and 6 of which read:

"1. Universal vaccine suitable for prophylaxis and control of Haemophilus pleuropneumoniae in pigs, characterized by an effective content of a mixture of an extracellular proteinaceous material derived from the culture medium of at least one H. pleuropneumniae strain selected from the group of serotypes 1, 5, 9 and 11 on the one hand and extracellular proteinaceous material derived from the culture medium of at least one H. pleuropneumoniae strain selected from the group of serotypes 2, 3, 4 and 8 on the other hand."

"6. Vaccine suitable for prophylaxis and control of H. pleuropneumoniae in pigs, characterized by an effective content of a mixture of proteinaceous material derived from the culture medium of at least one H. pleuropneumoniae strain selected from the group of serotypes 1, 5, 9 and 11 on the one hand and of at least one H. pleuropneumoniae strain selected from the group of serotypes 2, 3, 4 and 8 on the other hand, which proteinaceous materials are obtained according to one or more of claims 2-5."

Claims 2 to 5 referred to methods for preparing said vaccine.

III. Oral proceedings were held on 6 February 2002.

IV. The following documents are cited in the present decision:

(2) P.J. Fedorka-Cray and G.A. Anderson, Ann. Meeting Am. Soc. Microbiol., 1987, page 40, abstract B-91

(3) P.J. Fedorka-Cray et al., Ann. Meeting Am. Soc. Microbiol., May 8-13, 1988, page 3, abstract B-37

(4) S. Rosendahl et al., Am. J. Vet. Res., July 1988, Vol. 49, pages 1053-1058

(5) J. Perrin et al., Poster presented at the Meeting of "Schwezerische Gesellschaft für Mikrobiologie (SGM)", June 1988, St-Gallen, Switzerland

(10) W. Goebel and H. Schrempf, Journal of Bacteriology, 1971, Vol. 106, No. 2, pages 311-317

(11) US 4,136,181

(12) W. Goebel et al., Journal of Bacteriology, 1974, Vol. 118, No. 3, pages 964-973

(13) "The Virulence of *Escherichia coli*", M. Sussman editor, Academic Press, 1985, pages 47-77

(14) M.E. Himmel et al., Am. J. Vet. Res., 1982, Vol. 43, No. 5, pages 764-767

(15) J.F. van den Bosch et al., J. Med. Microbiol., 1981, Vol. 14, pages 321-331

(17) F.A. Udeze and S. Kadis, Conference of Research Workers in Animal Disease, 16-17 November 1987, abstract 18

(22) M. Beck et al., J. Clin. Microbiol., 1994, Vol. 32, pages 2749-2754

(23) J.M. DiRienzo et al, Infection and Immunity, 1985, Vol. 47, No. 1, pages 31-36

(27) E.M. Kamp et L.A.M.G. van Leengoed, Journal of Clinical Microbiology, 1989, Vol. 27, No. 6, pages 1187-1191

(28) E.M.Kamp et al., Infection and Immunity, 1994, Vol. 62, No. 9, pages 4063-4065

V. The appellant, arguing in view of lack of inventive step under Article 56 EPC, submitted that document (3), the closest prior art, identified the hemolysin of *H. pleuropneumoniae* serotype 1 as a major immunogen which may be used in a subunit vaccine. The technical problem to be solved was to provide a vaccine against *H. pleuropneumoniae* with improved properties as compared to that of document (3). This improvement was defined as being either the extension of the protection to other selected *H. pleuropneumoniae* serotypes responsible for the disease in a given country (this amounting to a reformulation of the technical problem underlying the patent in suit) or to all the existing serotypes, so that a universal vaccine against *H. pleuropneumoniae* was produced. The appellant submitted that both forms of improvement were rendered obvious by the prior art. For instance, as far as the first form was concerned, *H. pleuropneumoniae* disease outbreaks were known, in the Netherlands, to be due to serotypes 1 and 2. In order to protect pigs from these two serotypes, the skilled person, taking Table 8 of document (4) into consideration, came to the conclusion that, since these serotypes differed in their virulence factors (hemolysin for serotype 1 and cytotoxin for serotype 2), both virulence factors had to be associated in the vaccine composition. The skilled person following an obvious need and using the information contained in prior art in a straightforward manner would thus arrive at an embodiment encompassed by the claims of the patent in suit. The second form of improvement (ie the universal vaccine) could be deduced from Table 8 of document (4), if attention was only

focused on the reference strains. Then, serotypes 2, 3, 7 and 8 defined the group containing cytotoxin, whereas serotypes 1, 5, 9 and 10 represented the group with hemolysin/cytotoxin.

Furthermore, if attention was also drawn to the non-reference strains of Table 8 in document (4), then some embodiments covered by the claims of the patent in suit did not solve the technical problem. For instance, serotype 3 strain EM4431 contained hemolysin/cytotoxin activity as did serotype 1 strain Shope 4074. Their association, although falling within the scope of claim 1 of the patent in suit, did not result in a universal vaccine, since none of these strains showed a cytotoxin activity not associated to hemolysin as requested by claim 1 of the patent in suit. Reference was made in this context to decision T 939/92 (OJ EPO 1996, 309).

The alleged "confusing character" of document (4) in view of the number, nature and distribution of the molecules carrying the hemolysin and cytotoxin activities was denied. Indeed, as far as the number of molecules was concerned, document (17) showed that the hemolysin and cytotoxin activities were carried on a single molecule. Further, the presence of various serotypes did not imply that the hemolysin/cytotoxin and/or the cytotoxin were different from serotype to serotype. Indeed, the serotype classification was based on the LPS (lipopolysaccharides) present on the surface of the bacteria and not on the nature of the toxins, so that the differences in the LPS were not to be taken as necessarily reflecting a difference at the level of the toxins. Confirmation thereof was seen in documents (10)-(15) and (23), which did not show the existence of

serotype-dependent toxins in other organisms. Thus, Table 8 of document (4) only referred to two molecules: the first one carrying the hemolysin/cytotoxin activity and the second one the cytotoxin activity.

The combination of documents (3) and (5) also rendered the subject-matter of the claims of the patent in suit obvious, since document (5) disclosed two types of hemolysins, one being induced by Ca^{2+} ions and the other not being Ca^{2+}-inducible, but using Ca^{2+} as a co-factor. The first hemolysin was present in serotypes 1, 5, 9-12, whereas the second one was found in serotypes 2, 4, 5, 7-12.

In view of the fact that the sole example of the patent in suit concerned with a vaccine was only directed to serotype 9 and hence did not at all reflect the claimed subject-matter, it was argued that the claimed invention had not been made and hence the technical problem as defined in the patent in suit not solved.

VI. The respondent submitted that the argumentation of the appellant was primarily based on hindsight.

Further, the respondent, also considering document (3) as the closest prior art, defined the technical problem to be solved as the provision of a universal vaccine protecting pigs against disease caused by any of the known serotypes of *H. pleuropneumoniae*. The universal character of the vaccine was mandatory, since said disease and its prevention had to be considered on a worldwide basis and not only in view of a given country, where only some of the serotypes occurred.

The respondent considered that prior to making a

vaccine, the skilled person had to know the number, the nature and the distribution of the toxins involved and, in this context, put the accent on the confusing character of document (4), in particular, and of the prior art cited in relation to the claimed subject-matter, in general. First of all, document (4) was only concerned with activities, from which no information about the immunological relatedness between the serotypes could be deduced. Moreover, Figure 2, Figure 3 and Table 2 of document (4) seemed to indicate that the hemolysin and cytotoxin activities of *H. pleuropneumoniae* serotype 1 strain CM5 were not carried on the same molecule. Confirmation of this was also seen in the fact that the hemolysin/cytotoxin activity ratio as shown in Table 8 of document (4), which should be the same in all the serotypes exhibiting both activities, if said activities were carried on the same molecule, was actually different from serotype to serotype. Another explanation for this result of Table 8, which did not reduce the confusion, was that, if both activities were really carried on the same molecule, the hemolysin/cytotoxin was then serotype-dependent and each of them had to be introduced in the vaccine composition. This was not only confusing, but also stood in contradiction to the teaching of documents (10)-(15), (17) and (23), as far as they were assumed to show that toxins were not serotype-dependent. Further, document (2) showed that the cytotoxin activity of serotype 1 was lethal in mice, whereas that of serotype 5 was not and thus suggested the existence of a serotype-dependent cytotoxin. This teaching was again in contradiction to that of documents (10)-(15) and (23). The respondent also interpreted document (17) differently from the appellant: the serotype 1 hemolysin was said to elute

as a single protein peak and also to exhibit a cytotoxic activity. This did not necessarily imply that both activities were carried on the same molecule, but only established that, under the conditions used, ion exchange chromatography was not able to separate the two possibly different molecules from each other .

Further, the respondent argued that serotypes were defined according to LPS and not to the produced toxins. Therefore, a reference strain for a given serotype was not necessarily representative for all the strains of said serotype as far as a molecule other than LPS was concerned. In other words, two strains belonging to the same serotype did not need to have the same hemolysin and/or cytotoxin pattern. This resulted in the necessity for the skilled person to consider each strain on its own for hemolysin and cytotoxin.

Moreover, in the preparation of a vaccine, the field strains had to be considered, since they were potentially responsible for infection. If reference and field strains were taken into consideration, then Table 8 of document (4) was confusing, because of the differences in hemolysin and/or cytotoxin activities between and within the serotypes.

Further, the combination of documents (3) and (4) did not lead the skilled person to a universal vaccine, because Table 8 of document (4), apart from being confusing, was silent about serotypes 11 and 12.

The respondent argued that the combination of documents (3) and (5) did not lead the skilled person to the claimed subject-matter of the patent in suit for reasons similar to that already mentioned for document

(4). Indeed, document (5) only referred to hemolysin and was silent about cytotoxin, did not lead to the groups defined in the claims of the patent in suit and showed that universality was not possible, since serotypes 3 and 8 were deprived of hemolysin.

As far as possibly inoperable embodiments covered by the claims of the patent in suit were concerned, the respondent submitted that the appellant had the burden of proof. However, post published documents (22), (27) and (28) demonstrated that less than 1% of the tested strains did not behave according to the pattern defined in the claims of the patent in suit.

The respondent considered that the skilled person, being in fact a team composed of at least an immunologist and a biochemist, facing the confusion of the cited prior art documents, in particular of documents (4) and/or (5), would not have begun an immunological study of the 12 *H. pleuropneumoniae* serotypes, but would have tried to modify the hemolysin/cytotoxin pattern of the serotypes by changing the culture medium or would have carried on the biochemical characterization of the hemolysin and cytotoxin of the 12 serotypes and, if necessary, would have included in the universal vaccine the extracellular proteinaceous material of the 12 serotypes. A reason for the reluctance to begin an immunological study was in the activity differences between and within the serotypes disclosed in Table 8 of document (4), which could have implied a total absence of immunological relatedness between the serotypes and even among the strains, so that each strain should have been considered on its own.

VII. The appellant requested that the decision under appeal be set aside and that the European patent No. 0 354 628 be revoked.

VIII. The respondent requested that the appeal be dismissed and that the patent be maintained.

Reasons for the Decision

Article 56 EPC

1. Document (3) is considered by the Board as the closest prior art, since it refers to a vaccine against *H. pleuropneumoniae*, as do the claims of the patent in suit. Document (3) describes the use of a crude preparation of the hemolysin obtained from an unidentified *H. pleuropneumoniae* serotype 1 strain, which has been precipitated from clarified supernatant and then dialysed. This preparation is used to successfully immunize pigs. The hemolysin is said to be "*...a major immunogen... and may be necessary to include during development of future subunit(s) vaccines...*". By referring to serotype 1, document (3) draws the attention of the skilled person to the existence of other *H. pleuropneumoniae* serotypes.

2. The technical problem can be defined in view of document (3) as the provision of a vaccine suitable for protecting pigs against disease caused by any serotype of *H. pleuropneumoniae*. This amounts to the provision of a universal vaccine efficient against all the known *H. pleuropneumoniae* serotypes.

3. The solution is given by the vaccine of the claims of the patent in suit, in which the extracellular proteinaceous material of at least a member of the group defined by serotypes 1, 5, 9 and 11 is added to the extracellular proteinaceous material of at least one member of the group defined by serotypes 2, 3, 4 and 8.

4. The Board considers that the main focus under Article 56 EPC is on the question whether the solution proposed in the claims of the patent in suit may be derived in an obvious manner from the cited prior art and in view of the conclusion reached below (cf infra, point 18) the questions raised by the appellant whether some embodiments falling within the claims do not solve the technical problem or whether the claimed invention has been performed may be left unanswered.

5. First of all, it should be noted that document (3), besides hemolysin, ie a virulence factor, also suggests for the preparation of a vaccine the use of whole-cell bacterins, OMPs ("outer membrane proteins"), capsules and LPS.

6. The Board however considers that the skilled person, even if he could in theory have used these elements for making a vaccine, would have in fact disregarded them, because document (3) itself does not sound very promising as far as they are concerned, since it indicates that these elements only confer a **partial** protection (without even defining how the adjective "*partial*" has to be understood). Furthermore the use of these elements would result in the Board's opinion in a cumbersome, expensive and unsuitable solution for the provision of a vaccine on an industrial scale.

7. Indeed, LPS are identifying and characterizing factors of the serotypes carried on the surface of the bacteria. They must therefore be structurally different from each other. As a consequence, the skilled person would not have expected them to be immunologically cross-reactive, but would have, on the contrary, assumed that each of them has to be included in the vaccine formulation.

8. The skilled person would have had the same cautious assumption as far as OMPs and capsules are concerned, since no information can be drawn from the cited prior art about a possible cross-reaction between the various serotypes on the basis of OMPs and capsules.

9. Under these circumstances, in order to prepare a vaccine composition, the skilled man would have had two possibilities: he could have either isolated each of the 12 LPS, OMPs and/or capsules or have used killed whole-cell bacteria carrying said LPS and OMPs and/or capsules.

The first possibility would have resulted in a cumbersome, time-consuming and expensive purification process unsuitable for the preparation of a vaccine composition on an industrial scale. Furthermore, LPS, OMPs and capsules, being on the surface of the bacteria, may naturally interact with the bacterial membrane. This interaction may have an influence on their spatial structure and hence on the presence of epitope(s), which could possibly be destroyed during the purification. Furthermore, the necessity to include an efficient amount of the LPS, OMPs or capsules of the 12 serotypes in the vaccine composition would be expected to result in solubility and/or viscosity

problems, if the volume of the composition has to be maintained reasonably low for use as a vaccine in pigs.

On the contrary, if, according to the second solution, these elements are not purified, but introduced into the vaccine composition in the form of killed whole-cell bacteria for the 12 serotypes, then viscosity problems would have to be expected even more. The same consideration applies *mutatis mutandis* to whole-cell bacterins.

10. Therefore, the skilled person would not consider the use of whole-cell bacterins, LPS, OMPs and/or capsules as a suitable way to solve the technical problem mentioned above, but would concentrate his efforts on the other suggestion of document (3), ie the virulence factors, such as hemolysin.

11. Virulence factors, being extracellular, have *per se* two important advantages for the skilled person: they do not need to be purified from the bacteria, but can be obtained from the culture medium. The number and the structural complexity of the molecules secreted into the culture medium being limited, the purification of extracellular components can be expected to be much easier than that of intracellular molecules. Furthermore, the producing bacteria do not need to be lysed to obtain the desired protein, but can be continuously used, this resulting in lower costs.

12. In this context, the Board is of the opinion that the skilled person in his search for information concerning *H. pleuropneumoniae* serotypes other than the seroytpe 1 mentioned in document (3) and their virulence factors would have taken document (4) into consideration, since

it concerns *H. pleuropneumoniae* and is particularly dedicated to the virulence factors of the various serotypes.

13. Document (4) identifies two proteinaceous virulence factors in the various serotypes: the hemolysin and the cytotoxin. The Board agrees with the respondent's position that document (4) is only concerned with the activity of these two virulence factors and not with the immune response they may induce in pigs or the immunological relatedness of the various serotypes based on these two virulence factors.

14. Document (4) is in the Board's opinion not conclusive as far as the number, the nature and the distribution of the molecules responsible for these two activities among the various serotypes are concerned.

For instance Table 8 indicates that some serotypes have both activities. If these activities are assumed to be carried on the same protein in these serotypes, then this protein must be different for each serotype, since the hemolysin/cytotoxin ratio varies from one serotype to the other. In other words, this protein must be serotype dependent. Another explanation could be that hemolysin and cytotoxin are carried on two different molecules. This second explanation is corroborated by Table 2, which describes the kinetics of production of hemolytic and cytotoxic activity in culture supernatant and shows that the activities of hemolysin and cytotoxin do not evolve in a parallel manner as would be expected for two activities carried on the same protein. If these hemolysin and cytotoxin activities are carried on different molecules, then the serotype-dependent difference in the hemolysin/cytotoxin ratio

can be explained either by a serotype-dependent difference in the concentration of these molecules or a serotype-dependent difference in the nature of these molecules. In the former case, the same hemolysin and the same cytotoxin are present in each serotype exhibiting both activities, but their respective concentrations vary from serotype to serotype, whereas in the latter case each serotype has its own particular hemoylsin and cytotoxin.

Therefore, the information contained in document (4) does not allow the skilled person to conclude that these two activities, when simultaneously present in a given serotype, are not carried on two different molecules, which is acknowledged by the authors of document (4) on page 1057: "*...Until the hemolytic or neutrophil-toxic substance has been purified, it cannot be determined whether one molecule is responsible for both activities and whether different strains produce different toxins...*".

Document (17) is also of no help for the skilled person, since it does not demonstrate that both activities are carried on a single protein, but only states that both activities are isolated in ion exchange chromatography as a single peak. However, in particular in the absence of information concerning the chromatographic resin, the column size, the elution buffer and/or the elution rate, ie parameters known to exert an influence on the separation power, it cannot be excluded that two different proteins, each carrying one of the two activities, are eluted as a single peak.

The skilled person, summarizing the reliable information which can be retrieved from the combined

and to some extent confusing teaching of documents (3) and (4), would only know that two kinds of proteinaceous virulence factors are secreted in the culture medium of *H. pleuropneumoniae*, ie the hemolysin and the cytotoxin, but would have no information on the number, the nature and the distribution among the various serotypes of the proteins responsible for these virulence factors. The skilled person would also be unable to retrieve from documents (3) and (4) any information concerning the immunological relatedness of the serotypes in view of these virulence factors.

15. In view of this inconclusive prior art, two "routes" would have been possible for the skilled person: the "biochemical" route or the "immunological" route.

16. The "biochemical" route consists in giving an answer to the question concerning the number, the nature and the distribution of the proteins responsible for the hemolytic and cytotoxic activities among the various serotypes. It implies the purification of each of these molecules from each serotype and their precise structural characterization, possibly down to the level of the determination of their amino acid sequence in order to reach a satisfying level of certainty when assessing identity, similarity or difference between the serotypes. This route, although it may probably be carried out using well-known routine techniques, would nevertheless be cumbersome, time-consuming, expensive and at variance with industrial/commercial considerations.

17. Furthermore, the skilled person interested in the provision of a vaccine is, in the Board's view, not a biochemist, but an immunologist or a team, the leader

of which is an immunologist. The reason therefor lies in the fact that the provision of a vaccine does not demand the knowledge of the precise nature of the proteins responsible for the hemolytic and cytotoxic activities, but puts the accent on the knowledge of the immunological reactivity of the various serotypes and their possible relatedness in view of these virulence factors.

18. Therefore, the Board is of the opinion that the skilled person would have favoured the "immunological" route, ie the determination of a possible cross-neutralisation between the various serotypes in order to obtain a reduced number of ingredients necessary for the preparation of the vaccine. This route could be reduced to practice without any undue effort: the skilled person, knowing from documents (3) and/or (4) the excellular character of hemolysin and cytotoxin, would only need to produce antisera against the culture supernatants of each of the 12 different serotypes and study their neutralizing effect on the hemolytic and/or cytotoxic activities contained in the supernatant of the culture of the 12 serotypes.

In the worst (but theoretical) case, there would be no cross-neutralization and the vaccine composition should contain the proteinaceous material of the supernatant of each of the 12 serotypes. This may result in viscosity or solubility problems and would place the hemolysin and cytotoxin, as far as the practical suitability for the provision of a vaccine composition is concerned, on the same level as LPS, OMPs, capsules and whole-cell bacterins (cf supra, points 5-10). Nevertheless, hemolysin and cytotoxin would, in this case, still be more advantageous than LPS, capsules,

OMPs and whole-cell bacterins for the preparation of a vaccine composition because of their extracellular character (cf supra, point 11).

19. However, the skilled person would have been confident of finding at least a certain amount of cross-reaction among the serotypes, because, although the bacterial classification is not only based on genetic considerations, it nevertheless reflects genetic relatedness among the strains of a serotype, among serotypes and/or even within a species. This is confirmed by document (13) (page 56) and document (14).

20. Therefore, in the Board's opinion, the "immunological" route, as opposed to the "biochemical" route, gives the answer required for the provision of a vaccine composition in a simple, fast and obvious manner and is nothing other than the straightforward and natural development of the prior art.

21. The "immunological" route is the way which has been followed in the patent in suit and has led to the vaccine described in the claims of the patent in suit. The claims of the patent in suit, which thus represent nothing else than the normal development of the prior art, lack inventive step and do not meet the requirements of Article 56 EPC.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The patent is revoked.

The Registrar: The Chairwoman:

P. Cremona U. Kinkeldey