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Case Number: T 1846/10 - 3.3.04

Application Number: 05023400.4

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Title of invention:
Lawsonia intracellularis cultivation, anti-Lawsonia intracellularis vaccines and diagnostic agents

Patent Proprietor:
Boehringer Ingelheim Vetmedica, Inc.

Opponents:
Intervet International B.V.
Wyeth

Headword:
Attenuated Lawsonia/BOEHRINGER

Relevant legal provisions:
EPC Art. 83, 56

Keyword:
Main request, auxiliary requests I to V:
sufficiency of disclosure - (no)
Auxiliary request VI: requirements of the EPC fulfilled-(yes)

Decisions cited:
T 0019/90

Catchword:
see points 28 to 29

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Case Number: T 1846/10 - 3.3.04

D E C I S I O N
of Technical Board of Appeal 3.3.04
of 12 March 2015

Appellant: (Opponent 2)	Wyeth Five Giralda Farms Madison, New Jersey 07940 (US)
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Party as of right: (Opponent 1)	Intervet International B.V. P.O.Box 31 5830 AA Boxmeer (NL)
Representative:	Janssen, Paulus J. P. Intervet International B.V. Intellectual Property Department Wim de Körverstraat 35 5831 AN Boxmeer (NL)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 5 July 2010 concerning maintenance of the European Patent No. 1645567 in amended form.**

Composition of the Board:

Chairwoman	G. Alt
Members:	R. Morawetz
	K. Garnett

Summary of Facts and Submissions

I. The appeal of opponent 2 (hereinafter "appellant") lies against the interlocutory decision of the opposition division to maintain European patent EP 1645567 in amended form. The patent has the title "*Lawsonia intracellularis* cultivation, anti-*Lawsonia intracellularis* vaccines and diagnostic agents".

II. Two oppositions were filed, both under Article 100(a) EPC, on the ground of lack of inventive step, and under Article 100(b) EPC. The opposition division decided that the main request met the requirements of the EPC.

III. The following documents are referred to in this decision:

D1 McOrist S. *et al.*, Infection and Immunity (1993), vol. 61, pages 4286-4292

D48 Experiments, filed by the respondent on 30 April 2010

D50 WO 2005/011731

D55 Declaration of Dr. R. Ackenbauer, submitted by the appellant with the statement of grounds of appeal

D64 Declaration of Dr. B. Eichenmüller submitted by the respondent with its letter dated 28 March 2011

D65 Kroll J.J. et al., AJVR (2004), vol. 65(5), pages 559-565

D66 Vannucci F.A. *et al.*, Vet. Micr. (2013), vol. 162, pages 265-269

D67 Declaration of Dr. S. McOrist, submitted by the respondent with its letter of 12 February 2015

D68 Smith S.H. et al., Veterinary Research (2014), vol. 45, pages 1-10

IV. In response to the appellant's statement of grounds of appeal, the patent proprietor (hereinafter "respondent") maintained its main request and submitted auxiliary requests I to III.

Independent claims 1 and 12 of the main request read as follows:

"1. A method for producing a live vaccine against *L. intracellularis* comprising the steps:
(1) cultivating attenuated *L. intracellularis* bacteria to obtain culture cells infected with *L. intracellularis*;
(2) incubating said infected cells at an oxygen concentration of less than about 18 percent while maintaining said infected cells in suspension;
(3) harvesting the attenuated *L. intracellularis* bacteria; and
(4) admixing the attenuated *L. intrallularis* bacteria with an acceptable pharmaceutical carrier.

12. A method for producing a live vaccine against *L. intracellularis*, wherein said vaccine comprises an attenuated *L. intracellularis* strain, comprising the steps:

(1) producing an attenuated *L. intracellularis* strain comprising obtaining culture cells infected with *L. intracellularis* bacteria, incubating said infected cells at an oxygen concentration of 0 percent to about 18 percent, agitating said infected cells so as to cultivate said bacteria while maintaining said infected cells in suspension, passaging at least a portion of said cultivated bacteria, harvesting at least a portion of said cultivated bacteria, and selecting for an attenuated strain to provide an attenuated *L. intracellularis* bacteria; and

(2) admixing said attenuated *L. intracellularis* in an acceptable pharmaceutical carrier."

Auxiliary request I corresponds to the main request except for the deletion of claims 1 to 11. Claim 1 of this request thus corresponds to claim 12 of the main request. Auxiliary request II corresponds to the main request, except that the culture cells recited in claims 1 and 12 of the main request have now been specified as being McCoys cells. Auxiliary request III corresponds to auxiliary request II except for the deletion of claims 1 to 11.

V. The parties were summoned for oral proceedings and were informed about the board's preliminary view in a communication pursuant Article 15(1) RPBA. The board observed *inter alia* that it appeared questionable that the strains tested in examples 5 and 6 of the patent were indeed attenuated in the sense that they were apathogenic, immunogenic and genetically stable (see paragraph 13 of the communication).

VI. In reply to the board's communication the respondent submitted auxiliary requests IV and V. Auxiliary request IV corresponds to the main request except for the deletion of claims 12 to 19. Claim 1 of auxiliary request IV is thus identical to claim 1 of the main request (see section IV above). Auxiliary request V corresponds to auxiliary request IV with the restriction of the recited culture cells to McCoys cells.

VII. Oral proceedings before the board were held on 12 March 2015 in the absence of opponent 1, who had been duly summoned and had announced its intention not attend in its letter dated 27 February 2015. During the debate regarding sufficiency of disclosure, the respondent argued that, in view of document D48, it was reasonable to infer that the strain referred to in example 6 of the patent and deposited as ATCC 55783 had been obtained after passaging the bacterial culture 40 times. After the board had announced its conclusion on the main request and indicated that it considered that the same conclusion applied to the pending auxiliary requests the respondent did not make any further submissions on the pending auxiliary requests but submitted auxiliary request VI and amended pages of the description. Claim 1 of auxiliary request VI reads as follows:

"1. A method for producing a live vaccine against *L. intracellularis*, comprising the steps:
(1) cultivating the attenuated *L. intracellularis* bacteria deposited as ATCC 55783 to obtain culture cells infected with *L. intracellularis*;
(2) incubating said infected cells at an oxygen concentration of less than about 18 percent while maintaining said infected cells in suspension;

(3) harvesting the attenuated *L. intracellularis* bacteria; and
(4) admixing the attenuated *L. intracellularis* bacteria with an acceptable pharmaceutical carrier."

At the end of the oral proceedings the chairwoman announced the board's decision.

VIII. The appellant's arguments may be summarized as follows:

Main request

Sufficiency of disclosure: claims 1 and 12

The skilled person knew at the effective date of the patent that the provision of a live attenuated vaccine required a bacterial strain satisfying the three requirements of apathogenicity, immunogenicity and genetic stability. He also knew that it was not predictable whether passaging of a bacterium *in vitro* would result in attenuation.

The patent taught in the general part that 7 to 12 passages were enough to obtain an attenuated *L. intracellularis* strain. However, from examples 5 and 6 of the patent it could not be concluded that 7 to 12 passages were indeed enough.

Example 5 of the patent provided no evidence that any of the three requirements was fulfilled by the high passage strain tested. The vaccinated group had worse enteritis than the control group and the PCR results were not a reliable indicator of efficacy in view of the results set forth in the table on page 17. Moreover, paragraph [0042] of the patent emphasised the importance of histological assays.

From the table on pages 17 and 18 it could also be understood that hamsters did not generally suffer from enteritis as none of the hamsters in Group C showed any signs of enteritis.

Thus, example 5 provided evidence that by following the teaching of the patent no attenuated *L. intracellularis* strain was obtained, in other words, that 7 to 12 passages were not enough to obtain an attenuated *L. intracellularis* strain.

The strain tested in example 6 had been cultured for 29 weeks and was not the strain deposited at the ATCC with the accession number 55783 (the deposited strain) but ISi-1. This became clear when reading paragraph [0125] of the patent. The harvested cultures referred to in this paragraph were the ISi-1 bacteria cultured for 29 weeks. It would make no technical sense to harvest the deposited strain.

The data from example 6 did not allow any conclusions to be drawn as to the protective efficacy of the vaccine culture tested since example 6 provided no information regarding gross pathology or clinical disease following dosing with strain ISi-1. There was no demonstration of the strain being apathogenic. It was not disclosed whether the development of enteritis was prevented or not in the vaccinated pigs. The absence of a positive staining in the fluorescent antibody (FA) test was no indication that the animals did not develop the disease, as could be seen from the Table on page 17. Many animals had negative FA stains despite having enteritis.

The induced immune response was not necessarily protective as 9 out of 10 unvaccinated animals also produced IgG and there was nothing to indicate that the vaccinated animals were protected by the IgG response. The IgG response could very well be a reaction of the immune system to infection with *L. intracellularis*.

The genetic stability of the strain had not been tested and no data were provided to show that the tested strain was sufficiently attenuated to be apathogenic. Thus, example 6 provided no evidence that any of the three requirements was fulfilled by the strain tested. In other words, example 6 provided no evidence that an attenuated strain of *L. intracellularis* had been provided.

From the patent it could also not be derived that more than 7 to 12 passages should be carried out. That an attenuated *L. intracellularis* suitable for a live vaccine could reliably be obtained by passaging *L. intracellularis* in vitro for 20 to 40 times was also not derivable from the patent nor from the prior art. Hence, the patent did not show a single way of carrying out the invention. On the contrary, example 5 provided evidence that the method of the patent did not work. The burden to obtain the vaccine against *L. intracellularis* was thus on the addressee of the patent. The skilled person had to perform a research program and was faced with an undue burden.

None of the documents D65, D66 and D68 were available to the person skilled in the art at the effective date of the patent. Thus, they could not possibly have rendered it plausible to the skilled person that the process of obtaining attenuated *L. intracellularis* was a highly repeatable event happening on a consistent

basis between the 20th and 40th passage of *L. intracellularis in vitro*. Document D50 provided evidence that 80 passages of *L. intracellularis* were required to obtain the B3903 vaccine (see page 30, line 6).

Auxiliary requests I to V

The same objections applied to these requests.

Auxiliary request VI

Admissibility, clarity and sufficiency of disclosure

The request was filed late and should not be admitted in the appeal proceedings.

The bacterial strain of claim 1 was not clearly defined as the genome of a bacterial strain might change as a consequence of passaging the bacteria *in vitro*.

Claim 1 encompassed the cultivation of the deposited strain. It could not be excluded that the cultivation of the deposited strain led to further mutations as it was not known whether the deposited strain was genetically stable. The thus cultivated bacteria might no longer be suitable as a vaccine.

Inventive step

No objection was raised.

Adaptation of the description

No objections were raised against the amended pages of the description filed at approximately 19:45 h during the oral proceedings before the board.

IX. The respondent's arguments may be summarized as follows:

Main request

Sufficiency of disclosure: claims 1 and 12

In order to be suitable as a vaccine, attenuated bacteria had to be apathogenic, immunogenic, and genetically stable. Attenuation was not a routine process, since the results of passaging a bacterium were not predictable. No attenuated strain of *L. intracellularis* was available at the priority date of the patent. From the prior art it was not at all clear whether *L. intracellularis* would be suitable for attenuation and as a live attenuated vaccine.

The patent provided in examples 5 and 6 two ways of carrying out the invention. In example 5 *L. intracellularis* was passaged 40 times over 29 weeks in HEp2 cell cultures. The resulting potential vaccine strain was tested in hamsters and a clear reduction of infection was seen. The reported 50% reduction of infection based on the PCR results was a valid conclusion. In contrast, no weight should be given to the histological data because according to the author of declaration D67, Prof. McOrist, it was known that hamsters tended to spontaneously develop enteritis. Therefore, the example showed that it was plausible

that attenuated bacteria suitable for a live attenuated vaccine could be made.

Example 6 had been performed with the strain deposited at the ATCC with the accession number 55783 (the deposited strain): see paragraph [0125]. The PCR data of this example coincided with the immunological data and made a protective effect plausible. The deposited strain was the master seed for a marketed vaccine, and this strain had been apathogenic, immunogenic and genetically stable for 10 years: see declaration D64. The strain disclosed in example 6 was, therefore, avirulent, immunogenic and genetically stable.

Document D48 disclosed that the culture of *L. intracellularis* in McCoys cells had led to 100% infectivity after 7 days. Because example 6 also used McCoys cells for the culture, it was reasonable to infer that the deposited strain was passaged once a week, resulting in 40 passages in total, taking into account that the bacteria were cultured for 40 weeks.

The claimed invention could be worked over the entire claimed scope. The patent showed that *L. intracellularis* could indeed be attenuated and was suitable as a live vaccine. Once the skilled person had learned from the patent that attenuated *L. intracellularis* bacteria suitable as a live vaccine could be produced, he would have understood that attenuation was possible for virtually any given *L. intracellularis* isolate. The skilled person could reproduce the process by applying routine procedures according to the teaching of the patent. The general part of the description taught in paragraph [0043] that the bacterial culture was passaged at least 7 to 12 times and that these figures could be varied as long as

selection methods were employed. The skilled person would thus passage the bacteria *in vitro* as often as necessary and test them for attenuation in suitable animal models *in vivo* until an attenuated strain was obtained. Thus, the patent provided sufficient teaching about how to prepare attenuated *L. intracellularis* strains.

Several post-published documents (see D50, D65, D66, and D68) provided evidence that a live attenuated vaccine of *L. intracellularis* could be produced. Document D66 showed that attenuation of *L. intracellularis* during repeated cell culture was a highly repeatable event happening on a consistent basis between the 20^{th} and 40^{th} passage *in vitro*.

The burden of proof to substantiate lack of sufficiency of disclosure was on the opponent. According to decision T 19/90 an opponent has to establish serious doubts, substantiated by verifiable facts, that the invention can be carried out by the average person skilled in the art without undue experimentation or inventive skill. The appellant had not carried out any experiments to show that the claimed invention could not be carried out. No evidence for failure had been provided.

Claim 1 concerned the proliferation of an existing attenuated *L. intracellularis* strain. According to the wording of the claim, "such strain is just there from the beginning". Thus, the only question to be asked was whether the steps recited in claim 1 could be carried out by the skilled person without undue burden or inventive effort.

Auxiliary requests I to V

The same arguments as submitted for the main request applied to auxiliary requests I to III. Auxiliary requests IV and V only contained claims which concerned the proliferation of an existing attenuated *L. intracellularis* strain.

Auxiliary request VI

Admissibility, clarity and sufficiency of disclosure

Auxiliary request VI addressed the conclusion reached by the board with respect to the main request and should be admitted.

Claim 1 was limited to the use of the deposited strain. That this strain was genetically unstable had not been shown.

Claim 1 related to a method for producing a vaccine. No evidence had been provided that the method could not be carried out as claimed. Declaration D64 provided evidence that the deposited strain was suitable as a vaccine.

Inventive step

Document D1, which was the closest prior art, disclosed that *L. intracellularis* was the causative agent of enteritis in pigs. The problem to be solved by the patent was the provision of a method for producing a vaccine against *L. intracellularis*. The solution consisted in the method steps of claim 1 using a specific attenuated strain.

At the effective date of the patent it was not predictable whether *L. intracellularis* could be attenuated so as to achieve a safe and efficacious vaccine. Starting from document D1, the person skilled in the art would have had no reason to expect that a live vaccine based on an attenuated *L. intracellularis* strain could be provided.

Adaptation of the description

The necessary amendments had been carried out.

X. Opponent 1, who is a party as of right in the present appeal proceedings, did not file any arguments or requests during the appeal proceedings.

XI. The appellant requested that the decision under appeal be set aside and the patent be revoked. The respondent requested that the appeal be dismissed, alternatively that the decision under appeal be set aside and the patent be maintained on the basis of one of auxiliary requests I to III as filed with its letter dated 28 March 2011, alternatively on the basis of auxiliary requests IV or V as filed with its letter of 12 February 2015, alternatively on the basis of auxiliary request VI filed during the oral proceedings of 12 March 2015.

Reasons for the Decision

Main request

Introduction

1. The invention under consideration relates to the preparation of a live vaccine against *Lawsonia intracellularis* (*L. intracellularis)* which relies on the use of attenuated *L. intracellularis* bacteria. *L. intracellularis* is the causative agent of proliferative enteropathy in pigs, also known as porcine proliferative enteropathy (PPE). Pigs affected by PPE have characteristic lesions in the ileum and colon.

2. Attenuation is the process by which the virulence of pathogenic microorgansisms is reduced. Attenuation is achieved by classical attenuation via passage (empirical) or directed attenuation through molecular biology methods (rationally designed). Classical attenuation relies on random mutations in populations of microorgansisms and subsequent screening of the resultant organisms in animals to assess virulence.

3. To be suitable as a live vaccine strain, the attenuated bacteria must fulfil the following three criteria: (i) apathogenicity, which means that they do not cause the disease; (ii) be suitable and retain immunogenicity, which means that they induce protective immunity in the animal host, and (iii) genetic stability, which means that they do not revert to being pathogenic or conversely become too attenuated. This was undisputed among the parties.

Sufficiency of disclosure (Article 83 EPC): claims 1 and 12

4. The subject-matter of claims 1 and 12 relates to a method for producing a live vaccine against *L. intracellularis* (see section IV above for the complete wording of the claims). While claim 1 comprises the step of cultivating attenuated *L. intracellularis* bacteria to obtain culture cells infected with *L. intracellularis*, claim 12 comprises the step of producing an attenuated *L. intracellularis* strain. To carry out the methods of both claims, the person skilled in the art must thus be in a position to obtain/produce attenuated *L. intracellularis* bacteria.

5. For the requirement of sufficiency of disclosure to be fulfilled the European patent application or European patent must disclose the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art (Article 83 EPC, Article 100(b) EPC).

6. It is established jurisprudence of the Boards of Appeal that the invention must be sufficiently disclosed at the effective date of the application, based on the application as a whole, including the examples and taking into account the common general knowledge of the skilled person. At least one way of enabling the person skilled in the art to carry out the invention must be disclosed, although this will be sufficient only if it allows the invention to be performed over the whole range claimed (see Case Law of the Boards of Appeal of the EPO, 7th edition 2013, section II.C).

7. It was common ground among the parties that, prior to the effective date of the patent, no attenuated strain of *L. intracellularis* was available to the public. It

was also common ground that it is not possible to predict for a given bacterium whether or not it can be attenuated so as to achieve a safe and efficacious vaccine. Thus, although the skilled person knew before the effective date of the patent that passaging of bacteria in *in vitro* cultures may lead to attenuation of virulence, he could not be sure that he would obtain an attenuated strain suitable for a vaccine by culturing and serially passaging *L. intracellularis*.

8. The board concludes from the above that the skilled person, wanting to carry out the claimed invention, could not rely on his common general knowledge or the prior art to obtain suitable attenuated *L. intracellularis* bacteria. Under these circumstances, the patent must provide the necessary guidance for the successful implementation of the claimed invention (see point 6 above).

9. Concerning the guidance provided by the patent for obtaining attenuated strains of *L. intracellularis*, page 3, lines 3 to 4 of the description teach that:

> "(...) the bacteria are continuously cultured for at least about 6 to 8 months while being passaged at least about 7 to 12 times to produce an attenuated strain for use as a vaccine."

10. The culture conditions are explained in paragraphs [0020] through [0036] of the patent, while paragraph [0037] establishes the frequency of passaging as follows:

> "Depending upon the rate at which the culture cells become infected, passage to fresh cells generally occurs between about every 2 to about 5 weeks.

> Assuming that the culture cells become at least 70% infected within 2-3 weeks, preferably passage occurs between about every 3 to 4 weeks."

11. Finally, paragraph [0043] of the patent states that:

> "Generally, an attenuated immunogenic *L. intracellularis* strain is produced after continuous culture for between at least about 150 and 250 days, during which time the culture is passaged at least about 7 to about 12 times."

12. Pursuant to the patent, serially passaged bacteria are tested in host animals for signs of attenuation. To this end animals are orally vaccinated with the bacteria and challenged 28 days later with bacteria from a less passaged virulent culture of *L. intracellularis*. The infected animals are necropsied 21 days after challenge and the small intestines observed for gross lesions as well as microscopic lesions. PCR and fluorescent antibody (FA) assays should also be performed. Pursuant to paragraph [042]:

> "(a)bout eighty percent of the control animals will show gross or microscopic lesions and test positive for the presence of *L. intracellularis* in the mucosal cells of the intestines using either PCR or FA testing methods. Vaccinated animals will have normal mucosal surfaces as determined by histological observations and will be negative by PCR testing."

13. In summary, the general part of the description teaches that in order to obtain an attenuated *L. intracellularis* strain, the bacteria are passaged at least about 7 to about 12 times *in vitro* to produce an

attenuated strain and then tested in host animals for signs of attenuation.

14. The patent further comprises six examples, of which examples 5 and 6 are of relevance for the present issue. These two examples concern vaccine efficacy experiments carried out in hamsters and pigs, respectively.

15. In example 5, a continuous culture of *L. intracellularis* grown in HEp-2 cells for 29 weeks and passed to new HEp-2 cells every 2-3 weeks is used as the vaccine culture (see paragraph [0106]). In this context, the board notes that the assertion by the respondent that the strain was passed 40 times is contradicted by the explicit disclosure in paragraph [0106]. Indeed, the preparation of the attenuated *L. intracellularis* strain in example 5 is carried out in accordance with the instructions from the general part of the description (see points 9 to 13 above): 29 weeks correspond to 203 days, or almost 7 months, which is indeed between 6 to 8 months or between 150 and 250 days. The number of passages during this period is not explicitly indicated in example 5 but can be calculated to be 11 or 12 on the basis of the indication that the culture is passed every 2 to 3 weeks. This is within the interval of "at least about 7 to about 12 times" mentioned in paragraph [0043] of the patent (see point 11 above).

16. In example 6, ISi-1 was grown continuously in pure culture for 29 weeks in McCoys cells in spinner flasks. The number of passages is not explicitly indicated but

pursuant to paragraph [0043] the number of passages during 29 weeks or 203 days will have been around 10 (see point 11 above).

17. The board concludes from the above that the patent consistently teaches, both in the general part of the description and in the relevant examples, that in order to produce an attenuated strain for use as a vaccine, *L. intracellularis* is passaged at least about 7 to about 12 times. The question which thus arises is whether the skilled person, by following this guidance, will reliably obtain an attenuated *L. intracellularis* strain suitable for use in a live vaccine.

18. In the study reported in example 5 two groups of hamsters (A and B) were challenged with pure cultures of low passage virulent *L. intracellularis* strain N343. While group A had been vaccinated with pure cultures of a high passage strain of *L. intracellularis* prior to the challenge, the control group B had not received any vaccine culture prior to the challenge.

19. The results obtained are set forth in the table of pages 17 to 18 of the patent and summarized in paragraphs [0103] and [0104], where it is stated that:

> "PCR results indicated the presence of *L. intracellularis* in the intestinal mucosas of 100% of the Group A hamsters 21 days post-vaccination. Group B hamsters were all negative at 21 days post-vaccination. Twenty-one days post-challenge 50% of the hamsters were PCR positive in Group A 100% were positive in Group B. Histopathology of the sections indicated mild to severe lesions in 50% of animals in Group A and mild lesions in 50% Group B 21 days post-

challenge. No animals demonstrated lesions 21 days post-vaccination. Group C animals did not have lesions at 21 days post-challenge. FA and silver stains were not able to demonstrate the presence of *L. intracellularis* in any of the sections. In conclusion, a 50% reduction of infection was observed in hamsters vaccinated with a high passage strain of *L. intracellularis* as demonstrated by PCR."

20. From the data reported in the table on page 17 for the individual animals of Groups A and B it can be seen, that in both groups 5 hamsters out of 15 developed enteritis. The number of animals that developed enteritis was thus the same in the vaccinated and in the control group. Moreover, the histological lesions were more severe in the animals that had received the high passage strain (Group A). This is not the result the skilled person would expect for an effective vaccine strain.

21. In that context, the board is not persuaded by the respondent's argument that the PCR data of example 5 (see point 19 above) would indicate to the skilled person that the tested high passage strain was indeed suitable as a vaccine.

22. Paragraph [0042] of the patent sets forth the criteria for evaluation of the results achieved by the vaccines prepared according to the invention (see point 12 above). Thus, vaccinated animals should be negative by PCR. However, not all animals of Group A of example 5 did test negative by PCR. In fact, 50% of the animals were positive by PCR. The high passage strain of

example 5 therefore did not fulfil the criteria set by the patent itself.

23. Concerning the clinical observation that enteritis appeared to be more severe in the vaccinated animals, the respondent argued that hamsters tended to develop enteritis spontaneously, and that therefore the histological data in the hamster model would be disregarded by the person skilled in the art.

24. The board does not find this argument persuasive either. Not only was the respondent's argument not supported by any evidence, it was in fact contradicted by the evidence provided by the patent itself in the context of group C of example 5. Group C was given challenge strain N101494, to compare relative virulence to strain N343. None of the hamsters of group C developed any enteritis and thus also no "spontaneous enteritis": see the table bridging pages 17 and 18. Also with regard to the histological observations, the high passage strain of example 5 therefore did not fulfil the criteria set by the patent itself (see point 12 above).

25. The board is also not persuaded by the respondent's argument that the skilled person would have simply taken the indication of "at least about 7 to about 12 times" of paragraph [0043] (see point 11 above) to represent a lower limit and would have done as many passages of the *in vitro* culture as were necessary and tested them for attenuation in suitable animal models to obtain an attenuated *L. intracellularis* strain.

26. The skilled person is taught by the patent that he has to test the passaged strain, but only to confirm attenuation (see point 12 above). Bearing in mind that

the *L. intracellularis* strain suitable to carry out the invention must not only be less virulent than the corresponding wild type strain, but must also fulfill the additional two criteria of appropriate immunogenicity and genetic stability (see point 3 above), the skilled person would know that the number of passages required a delicate balance between reducing pathogenicity and maintaining immunogenicity. Relying on the guidance provided by the patent and not knowing why the strain used in example 5 did not protect the vaccinated animals he would have had no reason to assume that the number of passages had to be increased. In view of this, the person skilled in the art would not be inclined to consider the intervals of "at least about 7 to about 12 times", "6 to 8 months" or "150 to 250 days" disclosed in the description of the patent (see points 9, 10 and 11 above) as mere lower limits but would have understood these indications as concrete ranges.

27. The board concludes from the above (see points 8 to 26), that example 5 of the patent represents evidence that the skilled person, by following the guidance of the patent, would fail to obtain an attenuated strain of *L. intracellularis* suitable for the preparation of a live vaccine.

28. The respondent submitted that for an objections of lack of sufficiency of disclosure to succeed there must be serious doubts, substantiated by verifiable facts, that the invention can in fact be carried out by the average person skilled in the art without undue experimentation or inventive skills (see decision T 19/90, reasons, point 3.3). Since the burden of proof lies with the appellant (the opponent), it was necessary to provide

experimental evidence that the invention could not be carried. This had not been done.

29. In the board's judgement, verifiable facts are (i) that example 5 of the patent was carried out in line with the general teaching of the patent, (ii) that the percentage of animals which developed enteritis in example 5 was the same between the group vaccinated with the culture of a high passage strain of *L. intracellularis* and the non-vaccinated group, and (iii) that the histological lesions were worse in the vaccinated group (the evidence supporting this fact being in paragraph [0103] and the table on page 17 of the patent). Therefore, the board concludes that example 5 of the patent provides verifiable facts which raise serious doubts that the invention can in fact be carried out by the average person skilled in the art without undue experimentation or inventive skills by following the guidance provided in the patent. Under these circumstances, no additional experimental evidence from the appellant is required as it can rely on the evidence provided by the patent itself. The board concludes that the appellant has discharged its burden of proof.

30. The respondent relied on example 6 of the patent, the *L. intracellularis* strain deposited at the ATCC under accession number 55783 and post-published documents D50, D65, D66, and D68 to argue that the patent provided at least one way to perform the claimed invention and that it could be performed over the whole scope claimed.

31. As regards example 6, the parties disagreed on whether or not the example had been performed with the strain deposited at the ATCC under accession number 55783. The

board observes that pursuant to paragraph [0125]:

> "[t]he live vaccine was prepared at the NOBL Laboratories Research and Development facility and identified as experimental serial ISi-1. ISi-1 (strain N343) was isolated from a pig and grown continuously in pure culture for 29 weeks. The vaccine was grown in McCoys cells in spinner flasks at reduced oxygen until approximately 100% infection was observed. A sample of the high passage strain N343 used for ISi-1 was passed an additional 11 weeks ("N343NP40wk") and deposited under the Budapest Treaty on May 22, 1996 in the ATCC, 12301 Parklawn Drive, Rockville, Maryland U.S.A. 20852 and assigned Accession Number 55783. The cultures were harvested by centrifuging at 3000 x g for 20 minutes."

32. In the board's view this paragraph discloses that ISi-1, the vaccine tested in example 6, was grown in pure culture for 29 weeks, after which it was harvested. It also discloses that a sample of the culture was passed for an additional 11 weeks, thus 40 weeks in total, and was then deposited. It would not make any technical sense to read the paragraph to mean that the deposited strain was harvested. The board concludes that the experiment of example 6 was not performed with the deposited strain, but with ISi-1, which had been cultured for 29 weeks.

33. The respondent's argument based on the premise that example 6 was performed with the deposited strain, which had been shown (see declaration D64) to be apathogenic, immunogenic and stable, thus fails. At the effective date of the patent the skilled person would have understood that the *L. intracellularis* tested in

example 6, namely ISi-1, has been generated in line with the general teaching of the patent. The post-published evidence relating to the deposited strain thus cannot be relied on in the context of example 6.

34. Regarding the results of example 6 in relation to apathogenicity, immunogenicity and genetic stability, the board notes, first of all, that no information concerning gross pathology or clinical disease following dosing with ISi-1 is provided. Moreover, no direct comparison between the pathogenicities of N343 and its progeny ISi-1 has been carried out. The only histological data referred to are FA stains (see paragraph [0138]). From example 5 it is however apparent that a negative FA stain does not necessarily correlate with the absence of enteritis (see the Table on page 17). Thus while all 15 animals of group A and of group B of example 5 had negative FA stains, 5 animals of each group had enteritis. A negative FA stain is thus not a reliable evidence for satisfactory protection against PEE or apathogenicity.

35. The PCR data provided for example 6 cannot not be considered as a reliable indicator of apathogenicity of the vaccine either, since negative PCR results - as negative FA stains - are not necessarily correlated with the absence of enteritis. Indeed, at least one vaccinated hamster of example 5 (ID A-10 in the table on page 17) has developed enteritis despite having a negative PCR result. No data are thus provided in example 6 to indicate that ISi-1 is sufficiently attenuated to be apathogenic. Therefore, the requirement of apathogenicity was not demonstrated.

36. With regard to the IgG response tested in example 6, the board notes that no conclusion as regards

protection can be drawn from the disclosure that 8 out of 10 vaccinated pigs showed serum IgG production, since 9 out of 10 of the control animals also showed a serum IgG response. The observed IgG response might very well be the reaction of the immune system to infection with *L. intracellularis* without being necessarily protective. Moreover, no evidence of prevention or reduction of gross pathology or clinical disease is provided. No data are thus provided in example 6 to conclude that ISi-1 induces a protective immune response.

37. The board concludes from the above that from the results provided in example 6 it can neither be concluded that the high passage strain ISi-1 is apathogenic nor that it induces protective immunity. And as the genetic stability of the strain was not tested, no conclusion about that can be drawn either.

38. Hence, the board considers that example 6 cannot be relied upon to argue that the patent teaches at least one way to obtain an attenuated *L. intracellularis* strain suitable as a live vaccine.

39. As regards the respondent's reliance on the deposited strain as evidence that the guidance provided by the patent was sufficient to carry out the invention, the board notes the following. The board accepts the respondent's argument that the deposited strain was likely to have been passed 40 times. Document D48 discloses that the culture of *L. intracellularis* in McCoys cells leads to 100% infectivity after 7 days. Because example 6 also uses McCoys cells for the culture of *L. intracellularis*, and cells are passed once 100% infectivity is reached, it is safe to assume

that the deposited bacteria were passed once a week, which results in 40 passages in 40 weeks.

40. However, the board observes that the patent itself does not allow such a conclusion to be drawn (see points 9 to 13 and 31 to 32 above), and document D48 was not available to the public at the effective date of the patent. Thus, assuming that the deposited strain mentioned in paragraph [0125] of the patent was indeed passed 40 times, this means that it was not prepared according to the teaching of the patent (see point 17 above) and thus cannot be taken as evidence that the guidance provided by the patent suffices to put the claimed invention into practice over the whole scope claimed.

41. The respondent further submitted that once the skilled person had learned from the patent's teaching that *L. intracellularis* could indeed be attenuated by passaging and selection and that attenuated *L. intracellularis* was suitable as a live vaccine, this teaching could be reproduced by a skilled person by applying routine procedures according to the teaching of the patent. In this context the respondent relied on documents D50, D65, D66 and D68 as providing evidence that live attenuated vaccines of *L. intracellularis* had been produced after the effective date. It was also submitted that according to document D66 attenuation of *L. intracellularis* during repeated cell culture passage was a highly repeatable event happening on a consistent basis between the 20^{th} and 40^{th} passage of *L. intracellularis in vitro*.

42. The board has already concluded above that the patent teaches consistently that at least about 7 to about 12 passages should be carried out to obtain an attenuated

L. intracellularis strain (see point 17). Thus, it does not help the respondent's case that, after the effective date of the patent, attenuated *L. intracellularis* strains were obtained by carrying out many more passages of the *L. intracellularis* bacteria *in vitro.* Even if the deposited strain had been passaged 40 times - that information is not derivable from the patent (see point 40 above). That attenuation is a repeatable event that occurs between the 20^{th} and 40^{th} passage of *L. intracellularis in vitro* is not disclosed in the patent either. Finally, the board considers that document D50 provides evidence that certain *L. intracellularis* strains required up to 80 passages to become attenuated (see page 30, line 6). Thus, it appears in any case questionable that obtaining attenuated *L. intracellularis* is a highly repeatable event happening on a consistent basis between the 20^{th} and 40^{th} passage of the bacteria *in vitro*.

43. In conclusion, the board, having regard to the facts and arguments presented to it, concludes that no evidence has been provided that by following the teaching of the contested patent the person skilled in the art would succeed in producing an attenuated *L. intracellularis* strain. On the contrary, example 5 constitutes evidence that he would fail and thus raises serious doubts that the invention can in fact be carried out by the average person skilled in the art without undue experimentation or inventive skills.

44. After realising that the instructions concerning passaging the *L. intracellularis* bacteria in the patent were insufficient to reliably obtain an attenuated *L. intracellularis* strain suitable as a live vaccine, the skilled person would recognize the need to perform

further research. In the board's judgement, this additional research involves an undue burden in view of the technical context of the present case, which is characterized by the unpredictability of attenuation of *L. intracellularis* (see point 7 above) and the requirement of performing *in vivo* tests in suitable host animals (see point 12 above).

45. The respondent's final argument that claim 1 concerned the proliferation of an existing attenuated *L. intracellularis* strain and that according to the wording of the claim, "such strain is just there from the beginning", cannot succeed either. The first step of the method of claim 1 requires that attenuated *L. intracellularis* bacteria be cultivated. It is undisputed that no attenuated *L. intracellularis* bacteria were available to the public at the effective date of the patent (see point 7 above). It has also been established above (see points 8 to 43) that the guidance provided by the patent does not allow the skilled person to obtain an attenuated *L. intracellularis* strain without undue burden or inventive step. And in the board's view, an attenuated *L. intracellularis* strain certainly does not become available to the public by the mere exercise of drafting a claim which requires that attenuated *L. intracellularis* bacteria be cultivated. The steps recited in claim 1 can thus not be carried out by the skilled person without undue burden or inventive effort.

46. The board concludes from the above that the patent does not disclose the claimed invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art. The main request fails to meet the requirements of Article 83 EPC.

Auxiliary requests I to V

Sufficiency of disclosure (Article 83 EPC)

47. In the board's judgement the above considerations under Article 83 EPC as regards claims 1 and 12 of the main request (see points 8 to 46) apply, *mutatis mutandis*, to claim 1 of Auxiliary requests I to V.

Auxiliary request VI

Admissibility, Article 84 and Article 83 EPC

48. Auxiliary request VI was filed during the oral proceedings before the board, after the board had announced its conclusion as regards sufficiency of disclosure of the main request and auxiliary requests I to V.

49. Claim 1 of auxiliary request VI is based on claim 1 of auxiliary request IV and relates to a method for producing a live vaccine against *L. intracellularis* which relies on the *L. intracellularis* strain deposited under ATCC 55783 as the starting material (see section VII above for the complete wording of the claim). The restriction of the attenuated *L. intracellularis* bacteria to the deposited strain represented an amendment to the respondent's case. Pursuant to Article 13 RPBA any amendment to a party's case after it has filed its grounds of appeal or reply may be

admitted and considered at the board's discretion. The discretion is to be exercised in view of *inter alia* the complexity of the new subject-matter submitted, the current state of the proceedings and the need for procedural economy.

50. The appellant requested that the request be not admitted into the appeal proceedings. It was submitted that auxiliary request VI was filed at a late stage, that claim 1 had an unclear scope and that its subject-matter was insufficiently disclosed. Both these objections were based on the submission that the deposited strain might be genetically unstable. The appellant also stated that otherwise it had no objections against this request.

51. The board observes that lateness by itself is no reason not to admit the request into the appeal proceedings. The restriction of the attenuated *L. intracellularis* bacteria to the deposited strain which is indicated in the patent (see paragraph [0012]) to be the preferred attenuated *L. intracellularis* strain also cannot be considered surprising in the board's judgement. There is also no evidence on file that the *L. intracellularis* strain deposited as ATCC 55783 is genetically unstable. Therefore, the board was not persuaded by the appellant's objections under Articles 83 and 84 EPC.

52. The board considered that, although late filed, the amendment was not particularly complex and could reasonably be dealt with without adjourning the oral proceedings. In the exercise of its discretion under Article 13 RPBA the board thus decided to admit the auxiliary request VI in the appeal proceedings.

Articles 76(1), 123(2) and (3), and 54 EPC

53. The appellant had no objections under Articles 76(1), 123(2), 123(3) or 54 EPC and the board is satisfied that the requirements of these Articles are met by the subject-matter of auxiliary request VI.

Inventive step (Article 56 EPC)

54. Document D1 represents the closest prior art with respect to the invention. This document identifies *L. intracellularis* as the pathogen responsible for proliferative enteropathy in pigs and discloses its cultivation *in vitro*.

55. Starting from document D1, the problem to be solved by the patent can be formulated as the provision of a method for producing a vaccine against *L. intracellularis, i.e.* against porcine proliferative enteropathy.

56. The proposed solution consists in a method which rests on the cultivation of the *L. intracellularis* strain with the deposit number ATCC 55783. Pursuant to the patent, the deposited strain is the preferred attenuated strain N3434NP40wk (see paragraph [0012]). This strain is used as the master seed of a commercial vaccine and has been apathogenic, immunogenic and genetically stable for 10 years: see declaration D64.

57. Faced with the above problem, the person skilled in the art might have expected that repeated passaging of *L. intracellularis* was likely to reduce its virulence. However, as explained in point 7 above, it was not possible to predict what the result of passaging an *L. intracellularis* isolate might be. In fact, the

skilled person knew that passaging of a bacterium does not necessarily, and certainly does not always, lead to an attenuated bacterium suitable as a live vaccine, i.e. being not only apathogenic, but also immunogenic and genetically stable.

58. In view of this, the person skilled in the art starting from document D1 would have had to do further research with no particular reason to expect success or guarantee that an attenuated *L. intracellularis* strain suitable for the production of a vaccine could indeed be produced. Under these circumstances, the finding of a specific attenuated *L. intracellularis* strain which is indeed suitable as a live vaccine, is to be considered surprising.

59. For these reasons the board concludes that the subject-matter of claim 1 involves an inventive step. The same applies to independent claim 11 of auxiliary request VI, which shares with claim 1 the inventive technical feature of the attenuated *L. intracellularis* strain with the deposit number ATCC 55783. Depended claims 2 to 10 are inventive by the same token. The requirements of Article 56 EPC are fulfilled.

Adaptation of the description

60. At the oral proceedings, the respondent filed amended pages 2 to 21 of the description to bring it into line with auxiliary request VI. No objections were raised by the appellant and the board is also satisfied that the amended description meets the requirements of the EPC.

61. With its letter dated 20 March 2015 the respondent filed a typed version of amended pages 2 to 6 of the description. This version has not been checked by the

board as it was submitted after the board took its decision, which is based on the amended pages of the description submitted during the oral proceedings.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the opposition division with the order to grant a patent on the following basis:

(1) Claims 1 to 11 according to auxiliary request VI filed during the oral proceedings on 12 March 2015;

(2) The amended description pages numbered 2 to 21 as filed at approximately 19:45 h during the oral proceedings of 12 March 2015.

The Registrar: The Chairwoman:

P. Cremona G. Alt

Decision electronically authenticated