EXHIBIT 1

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

INTERVET INC., d/b/a MERCK ANIMAL HEALTH and THE ARIZONA BOARD OF REGENTS, on behalf of The University of Arizona,	)	
Plaintiffs/Counterclaim Defendants,	)	C.A. No. 11-595-LPS
v.	)	**JURY TRIAL DEMANDED**
BOEHRINGER INGELHEIM VETMEDICA, INC.,	)	**PUBLIC VERSION**
Defendant/Counterclaim Plaintiff.	)	

BOEHRINGER INGELHEIM VETMEDICA, INC.'S FIRST AMENDED ANSWER AND COUNTERCLAIMS TO INTERVET'S COMPLAINT FOR PATENT INFRINGEMENT

Boehringer Ingelheim Vetmedica, Inc. ("Vetmedica") hereby answers the Complaint for Patent Infringement filed by Intervet Inc., d/b/a Merck Animal Health and the Arizona Board of Regents, on behalf of the University of Arizona (collectively "Intervet"). Vetmedica denies the allegations and characterizations in the Complaint unless expressly admitted herein in the following paragraphs.

THE PARTIES

1. Vetmedica lacks sufficient information to admit or deny the allegations of paragraph 1 and therefore denies them.

2. Vetmedica lacks sufficient information to admit or deny the allegations of paragraph 2 and therefore denies them.

3. Vetmedica admits that it is a corporation organized and existing under the laws of Delaware, having a principal place of business in St. Joseph, Missouri. Vetmedica further admits that it manufactures, markets, and sell products, and conducts business in the United

States. Vetmedica further admits that it conducts business and markets and sells products in Delaware. Vetmedica denies the remaining allegations in paragraph 3.

NATURE OF THE ACTION

4. Vetmedica admits that Intervet purports to bring an action for patent infringement of United States Patent No. 5,610,059 ("the '059 Patent" or "the Patent-in-Suit") arising under the patent laws of the United States, Title 35 of the United States Code. Vetmedica denies that Intervet has stated any claim upon which relief can be granted, and denies the remaining allegations in paragraph 4.

JURISDICTION AND VENUE

5. Vetmedica admits that subject matter jurisdiction in this Court is proper pursuant to 28 U.S.C. §§ 1331 and 1338(a). Vetmedica denies the remaining allegations set forth in paragraph 5.

6. Vetmedica admits that it is incorporated in this District and conducts business in this District. Vetmedica further admits that for purposes of this action it is subject to personal jurisdiction in this District. Vetmedica lacks sufficient information to admit or deny the remaining allegations of paragraph 6 and therefore denies them.

7. Admitted.

THE PATENT

8. Vetmedica admits that the '059 Patent on its face states that it issued on March 11, 1997, names Lynn A. Joens and Robert D. Glock as inventors, and is entitled "Etiological Agent for Porcine Enteritis." Vetmedica further admits that a copy of the '059 Patent is attached to the Complaint as Exhibit A. Vetmedica lacks sufficient information to admit or deny the remaining allegations of paragraph 8 and therefore denies them.

9. Vetmedica lacks sufficient information to admit or deny the allegations of paragraph 9 and therefore denies them.

CLAIM FOR RELIEF

INFRINGEMENT OF THE '059 PATENT

10. This paragraph contains no factual allegations. Vetmedica incorporates by reference its answers to paragraphs 1 through 9 of the Complaint.

11. Denied.

12. Denied.

13. Denied.

14. Denied.

ANSWER TO PLAINTIFF'S PRAYER FOR RELIEF

Vetmedica denies that Intervet is entitled to any relief whatsoever in this action, either as requested in Intervet's Complaint or otherwise. Vetmedica further denies each and every allegation of Intervet's Complaint as set forth herein or to which Vetmedica has not specifically responded.

VETMEDICA'S DEFENSES

Vetmedica alleges and asserts the following defenses in response to the allegations in the Complaint, undertaking the burden of proof only as to those defenses deemed affirmative defenses by law. Vetmedica reserves the right to amend its Answer, including its defenses, based upon subsequent information.

FIRST DEFENSE

(Failure to State a Claim)

1. The Complaint fails to state a claim upon which relief may be granted.

SECOND DEFENSE

(Noninfringement of United States Patent No. 5,610,059)

2. Vetmedica has not infringed, contributed to the infringement of, or induced others to infringe any valid and enforceable claim of the '059 Patent, either literally or under the doctrine of equivalents.

THIRD DEFENSE

(No willful infringement of United States Patent No. 5,610,059)

3. Vetmedica has not willfully infringed any valid or enforceable claim of the '059 Patent and Intervet is not entitled to any enhanced damages.

FOURTH DEFENSE

(Invalidity of United States Patent No. 5,610,059)

4. The claims of the '059 Patent are invalid for failure to meet one or more of the conditions for patentability specified in Title 35 of the United States Code, including Sections 101, 102, 103, and 112.

FIFTH DEFENSE

(Prosecution History Estoppel)

5. Intervet's claims are barred in whole or in part by the doctrine of prosecution history estoppel.

SIXTH DEFENSE

(Statutory limitations on Damages and Costs)

6. Intervet's claims for damages and costs, if any, are statutorily limited by the provisions of 35 U.S.C. §§ 286, 287, and/or 288.

SEVENTH DEFENSE

(Laches and Other Equitable Defenses)

7. On information and belief, the relief sought by Intervet is barred, in whole or in part, under the doctrines of laches, waiver, equitable estoppel, and/or unclean hands for, among other reasons, unreasonable delay in filing suit against Vetmedica.

EIGHTH DEFENSE

(No Irreparable Harm)

8. Intervet is not entitled to injunctive relief against Vetmedica because any alleged injury to Intervet as a result of Vetmedica's alleged activities is not immediate or irreparable, and Intervet has an adequate remedy at law.

NINTH AFFIRMATIVE DEFENSE

(Unenforceability Due to Inequitable Conduct)

9. The application resulting in the '059 Patent was filed on November 9, 1992. The '059 Patent issued on March 11, 1997.

10. Nicholas Seay of the law firm Quarles & Brady LLP prosecuted the application that resulted in the '059 patent.

11. Lynn Joens and Robert Glock are identified as the inventors on the face of the '059 Patent.

12. The University of Arizona purports to be the assignee of the '059 Patent.

13. On information and belief, Lynn Joens was employed by the University of Arizona while the application resulting in the '059 patent was being prosecuted.

14. In connection with the application resulting in the '059 Patent, Lynn Joens signed a declaration in which he acknowledged his duty to disclose to the Patent Office information known to him to be material to patentability in accordance with 37 C.F.R. § 1.56.

15. In connection with the application resulting in the '059 Patent, Robert Glock signed a declaration in which he acknowledged his duty to disclose to the Patent Office information known to him to be material to patentability in accordance with 37 C.F.R. § 1.56.

16. Nicholas Seay understood his duty to disclose to the Patent Office information known to him to be material to patentability in accordance with 37 C.F.R. § 1.56.

17. Nicholas Seay informed Lynn Joens and Robert Glock of their duty of disclosure.

18. Attached as Exhibit A hereto is a true and correct copy of an article published in September 1991, Stills, H.F., Isolation of an Intracellular Bacterium from Hamsters (Mesocricetus auratus) with Proliferative Ileitis and Reproduction of the Disease with a Pure Culture, Infection and Immunity, Vol. 59, No. 9, p. 3227-3236 (1991) (hereinafter referred to as "Stills 1991").

19. Stills 1991 is prior art to the '059 patent under 35 U.S.C. § 102(b).

20. Stills 1991 discloses a process for isolating an intracellular bacterium that causes proliferative ileitis and a process for culturing that bacterium.

21. [redacted]

22. [redacted]

23. Attached hereto as Exhibit B is a true and correct copy of L.A. Joens, et. al, *Induction of Gross and Microscopic Lesions of Porcine Proliferative Enteritis By Lawsonia Intracellaris*, AJVR, Vol. 58, No. 10, October 1997 (hereinafter referred to as "Joens 1997").

24. Joens 1997 is an article co-authored by Lynn Joens, Robert Glock, and Stuart Nibbelink.

25. Joens 1997 states, *inter alia*, that:

> Stills isolated a bacterium from the enterocytes of hamsters with proliferative enteritis and propagated the organism in a continuous epithelial cell line. The organism was described as a curved rod, with a trilaminar membrane, having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long. Stills induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular agent. A bacterium having the same measurements has also been cultured in a rat enterocyte cell line under an atmosphere of O2:CO2:N2 (8:8:84%).[14] The bacterium was isolated from intestinal homogenates of a pig with proliferative ileitis, using methods similar to that of Stills [1991]. * * * Numerous research workers have attempted to isolate the PPE organism from the intestinal contents of pigs. Stills' work with hamsters with ileitis provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora. These methods were adapted for isolation of a bacterial agent from necrotic intestinal lesions typical of proliferative ileitis in the pigs of this study. The original isolation and growth of the organism was accomplished, using an atmosphere of 02:C02:H2:N2 (6:7:7:80%), as originally described by Stills [1991].

26. Joens 1997 was received for publication on or around September 3, 1996.

27. On information and belief, Joens 1997 was submitted for publication while the application resulting in the '059 patent was being prosecuted.

28. On information and belief, during prosecution of the application resulting in the '059 patent, the named authors of Joens 1997, including Lynn Joens and Robert Glock, were aware of the following and at least one draft or version of Joens 1997 disclosing the following:

(a) Stills 1991;

(b) that prior to November 9, 1991, Stills had isolated a bacterium from the intestinal cells of hamsters with proliferative enteritis, propagated the organism in a Henle 407 cell line, and induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular bacterium;

(c) that the organism isolated by Stills was a curved rod having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long;

(d) that the organism isolated by Stills was intracellular;

(e) that Stills 1991 and Stills' work with hamsters with ileitis prior to November 9, 1991 provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora; and

(f) that Lynn Joens and/or Robert Glock used the methods described in Stills 1991 to purportedly isolate *Lawsonia intracellularis*.

29. Lynn Joens did not disclose Stills 1991 to the PTO in connection with the application that resulted in the '059 patent.

30. Lynn Joens did not disclose to the PTO in connection with the application that resulted in the '059 patent Joens 1997, any draft or version of Joens 1997, or any of the following information:

(a) that prior to November 9, 1991, Stills had isolated a bacterium from the intestinal cells of hamsters with proliferative enteritis, propagated the organism in a Henle 407 cell line, and induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular bacterium;

(b) that the organism isolated by Stills was a curved rod having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long;

(c) that the organism isolated by Stills was intracellular;

(d) that Stills 1991 and Stills' work with hamsters with ileitis prior to November 9, 1991 provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora; and

(e) that Lynn Joens and/or Robert Glock used the methods described in Stills 1991 to purportedly isolate *Lawsonia intracellularis*.

31. Robert Glock did not disclose Stills 1991 to the PTO in connection with the application that resulted in the '059 patent.

32. Robert Glock did not disclose to the PTO in connection with the application that resulted in the '059 patent Joens 1997, any draft or version of Joens 1997, or any of the following information:

(a) that prior to November 9, 1991, Stills had isolated a bacterium from the intestinal cells of hamsters with proliferative enteritis, propagated the organism in a Henle 407 cell line, and induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular bacterium;

(b) that the organism isolated by Stills was a curved rod having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long;

(c) that the organism isolated by Stills was intracellular;

(d) that Stills 1991 and Stills' work with hamsters with ileitis prior to November 9, 1991 provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora; and

(e) that Lynn Joens and/or Robert Glock used the methods described in Stills 1991 to purportedly isolate *Lawsonia intracellularis*.

33. The University of Arizona did not disclose Stills 1991 to the PTO in connection with the application that resulted in the '059 patent.

34. The University of Arizona did not disclose to the PTO in connection with the application that resulted in the '059 patent Joens 1997, any draft or version of Joens 1997, or any of the following information:

(a) that prior to November 9, 1991, Stills had isolated a bacterium from the intestinal cells of hamsters with proliferative enteritis, propagated the organism in a Henle 407 cell line, and induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular bacterium;

(b) that the organism isolated by Stills was a curved rod having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long;

(c) that the organism isolated by Stills was intracellular;

(d) that Stills 1991 and Stills' work with hamsters with ileitis prior to November 9, 1991 provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora; and

(e) that Lynn Joens and/or Robert Glock used the methods described in Stills 1991 to purportedly isolate *Lawsonia intracellularis*.

35. Nicholas Seay did not disclose Stills 1991 to the PTO in connection with the application that resulted in the '059 patent.

36. Nicholas Seay did not disclose to the PTO in connection with the application that resulted in the '059 patent Joens 1997, any draft or version of Joens 1997, or any of the following information:

(a) that prior to November 9, 1991, Stills had isolated a bacterium from the

intestinal cells of hamsters with proliferative enteritis, propagated the organism in a Henle 407 cell line, and induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular bacterium;

(b) that the organism isolated by Stills was a curved rod having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long;

(c) that the organism isolated by Stills was intracellular;

(d) that Stills 1991 and Stills' work with hamsters with ileitis prior to November 9, 1991 provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora; and

(e) that Lynn Joens and/or Robert Glock used the methods described in Stills 1991 to purportedly isolate *Lawsonia intracellularis*.

37. On information and belief, Lynn Joens, in breach of the duty of candor and good faith required by 37 C.F.R. § 1.56 and with specific intent to deceive the Examiner, failed to disclose during prosecution of the '059 Patent Stills 1991, [redacted], Joens 1997, any draft or version of Joens 1997, and any of the following information:

(a) that prior to November 9, 1991, Stills had isolated a bacterium from the intestinal cells of hamsters with proliferative enteritis, propagated the organism in a Henle 407 cell line, and induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular bacterium;

(b) that the organism isolated by Stills was a curved rod having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long;

(c) that the organism isolated by Stills was intracellular;

(d) that Stills 1991 and Stills' work with hamsters with ileitis prior to November 9, 1991 provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora; and

(e) that Lynn Joens and/or Robert Glock used the methods described in Stills 1991 to purportedly isolate *Lawsonia intracellularis*.

38. On information and belief, Robert Glock, in breach of the duty of candor and good faith required by 37 C.F.R. § 1.56 and with specific intent to deceive the Examiner, failed to disclose during prosecution of the '059 Patent Stills 1991, Joens 1997, any draft or version of Joens 1997, and any of the following information:

(a) that prior to November 9, 1991, Stills had isolated a bacterium from the intestinal cells of hamsters with proliferative enteritis, propagated the organism in a Henle 407 cell line, and induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular bacterium;

(b) that the organism isolated by Stills was a curved rod having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long;

(c) that the organism isolated by Stills was intracellular;

(d) that Stills 1991 and Stills' work with hamsters with ileitis prior to November 9, 1991 provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora; and

(e) that Lynn Joens and/or Robert Glock used the methods described in Stills 1991 to purportedly isolate *Lawsonia intracellularis*.

39. On information and belief, the University of Arizona, in breach of the duty of candor and good faith required by 37 C.F.R. § 1.56 and with specific intent to deceive the

Examiner, failed to disclose during prosecution of the '059 patent Stills 1991, [redacted], Joens 1997, any draft or version of Joens 1997, and any of the following information:

(a) that prior to November 9, 1991, Stills had isolated a bacterium from the intestinal cells of hamsters with proliferative enteritis, propagated the organism in a Henle 407 cell line, and induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular bacterium;

(b) that the organism isolated by Stills was a curved rod having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long;

(c) that the organism isolated by Stills was intracellular;

(d) that Stills 1991 and Stills' work with hamsters with ileitis prior to November 9, 1991 provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora; and

(e) that Lynn Joens and/or Robert Glock used the methods described in Stills 1991 to purportedly isolate *Lawsonia intracellularis*.

40. Based on Lynn Joens's, Robert Glock's, and the University of Arizona's failures to disclose Stills 1991, [redacted], Joens 1997, any draft or version of Joens 1997, and any of the following information:

(a) that prior to November 9, 1991, Stills had isolated a bacterium from the intestinal cells of hamsters with proliferative enteritis, propagated the organism in a Henle 407 cell line, and induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular bacterium;

(b) that the organism isolated by Stills was a curved rod having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long;

(c) that the organism isolated by Stills was intracellular;

(d) that Stills 1991 and Stills' work with hamsters with ileitis prior to November 9, 1991 provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora; and

(e) that Lynn Joens and/or Robert Glock used the methods described in Stills 1991 to purportedly isolate *Lawsonia intracellularis*,

the '059 Patent is unenforceable due to inequitable conduct.

41. Specifically, under the pleading requirements set forth by the United States Court of Appeals for the Federal Circuit in *Exergen*, 575 F.3d 1312 regarding Stills 1991:

(a) "[W]ho both knew of the material information and deliberately withheld or misrepresented it" (*Exergen*, 575 F.3d at 1329)

42. Lynn Joens and Robert Glock knew of, and on information and belief, deliberately withheld from the PTO Stills 1991.

43. Lynn Joens knew of, and on information and belief, deliberately withheld from the PTO the fact that he used Stills 1991 to obtain the invention claimed in the '059 patent.

(b) What "claims, and which limitations in those claims, the withheld references [or misrepresentation] are relevant to" (*id.*)

44. Stills 1991 anticipates under 35 U.S.C. § 102(b) and/or renders obvious under 35 U.S.C. § 103, and is therefore relevant to, all limitations of all claims (1-4) of the '059 patent.

45. Claim 1 of the '059 patent requires: "A biologically pure culture of a mammalian host cell infected by an obligate intracellular bacterium." Stills 1991 discloses a biologically

pure culture of a mammalian host cell infected by an obligate intracellular bacterium. *See, e.g.*, Stills 1991 at Title, Abstract.

46. Claim 1 of the '059 patent requires that the bacterium "causes porcine proliferative enteritis after inoculation into pigs." Stills 1991 discloses that its bacterium causes proliferative enteritis after inoculation in hamsters, *e.g.*, Stills 1991 at *Abstract*, 3234, and that "porcine proliferative enteropathy" and hamster proliferative enteritis possibly had the "same etiology," *id.* at 3227.

47. Claim 1 of the '059 patent requires that the culture have "all of the identifying characteristics of ATCC Accession No. 55370." Plaintiffs do not treat this as a separate limitation on their claims. Regardless, Stills 1991 discloses characteristics of the culture that the applicants allegedly deposited with the ATCC as Accession No. 55370 that are identified in the '059 patent specification and by the applicants in the file history. *Compare, e.g.* Stills 1991 at *Abstract*, 3234, *with* '059 Patent at 1:6–9, 7:38–8:4, '059 File History, 12-19-1994 Amend., at 4–5, *and* '059 File History, 05-31-1994 Amend., at 5, 7.

48. Claim 2 of the '059 patent requires that "the mammalian host cell is the human intestinal cell line Henle 407." Stills 1991 discloses that its mammalian host cell line was a Henle 407 cell line. E.g., Stills 1991 at *Abstract*, 3228.

49. Claim 3 of the '059 patent requires that "the obligate intracellular bacterium that causes porcine proliferative enteritis is a bacterium having dimensions in the range of 0.24-0.27 micron x 1.4-2.4 micron." Stills 1991 discloses that its bacterium had dimensions in the range of "0.26 to 0.34 [micron] in width" and "1.4 to 1.7 [micron] in length." *E.g.*, Stills 1991 at *Abstract*, 3231.

50. Claim 4 of the '059 patent requires that "the obligate intracellular bacterium that causes porcine proliferative enteritis is isolated from enterocytes of a pig having porcine proliferative enteritis." Stills 1991 discloses that its bacterium causes proliferative enteritis after inoculation in hamsters, *e.g.*, Stills 1991 at *Abstract*, 3234, and that "porcine proliferative enteropathy" and hamster proliferative enteritis possibly had the "same etiology," *id.* at 3227.

(c) "**Where** in th[e] references the material information is found" (*id.*)

51. Material information is found throughout Stills 1991, including, for example, in Stills 1991 at *Title, Abstract*, 3227-28, and 3233-34.

52. Among other things, Stills 1991 discloses a method for isolating an intracellular bacterium from hamsters and pigs with proliferative enteritis and reproducing proliferative enteritis with a pure culture of that bacterium. Stills 1991 also discloses a pure culture of the isolated intracellular bacterium hosted in Henle 407 cells.

53. Paragraphs 44-51 above are hereby incorporated by reference as if fully restated herein.

(d) "'**How**' an examiner would have used [the] information in assessing the patentability of the claims (*Exergen*, 575 F.3d at 1329-1330).

54. Had the Examiner known about Stills 1991, the Examiner would have used such information to find the claims of the '059 Patent anticipated and/or rendered obvious under 35 U.S.C. §§ 102 and/or 103. For example, the Examiner examining the application resulting in the '059 patent stated that she had allowed the '059 patent because the "closest prior art does not teach or suggest the isolation of a microorganism in a mammalian host cell line wherein the microorganism causes porcine proliferative enteritis (PPE). The claimed PPE-causing agent is

an obligate, intracellular microorganism. The microorganism taught by the closest prior art causes only symptoms of PPE and is not an intracellular organism." '059 patent file history, 08/28/1996 Notice of Allowance, at 2-3. Stills 1991 teaches and suggests what the Examiner explicitly believed was missing from the prior art that was in front of her and justified allowing the '059 patent over that art. Stills 1991 also would have contradicted arguments made by the applicants to obtain the '059 patent, including but not limited to their arguments that "it is clear that even for a period of at least nine years after the publication of Lomax, the art was unable to culture any organism necessary and sufficient to cause PPE", there was no "suggestion to be gleaned from the art to isolate an unknown species of bacterium," and that one of "the strongest arguments in support of the newly isolated nature of the [claimed] strain [was] that the prior art does not describe an obligate intracellular organism that can alone induce PPE." *See*, '059 file history, 6/6/1994 Resp. to Office Action, at 4-12.

55. Stills 1991 teaches and suggests the isolation of a microorganism (a bacterium) in a mammalian host cell line, including a Henle 407 cell line. *See, e.g.*, Stills 1991, at Title, Abstract, 3227-38.

56. Stills 1991 teaches and suggests that its bacterium causes proliferative enteritis after inoculation in hamsters, *e.g.*, Stills 1991 at *Abstract*, 3234, and that "porcine proliferative enteropathy" and hamster proliferative enteritis possibly had the "same etiology," *id.* at 3227.

57. Stills teaches and suggests that the proliferative enteritis-causing bacterium was an obligate, intracellular microorganism. *See e.g.*, Stills 1991 at *Title, Abstract*, 3227-28, 3234 (describing bacterium's intracellular nature and discussing inability to propagate bacterium on artificial media).

58. Paragraphs 44-51 above are hereby incorporated by reference as if fully restated herein.

(e) The "**when**... of the material misrepresentation or omission committed before the PTO" (*id.* at 1327)

59. The omissions of Stills 1991 committed before the PTO by Lynn Joens and Robert Glock occurred during the entirety of the period beginning on the date the application resulting in the '059 patent was filed and continuing until the '059 Patent issued: November 9, 1992 through March 11, 1997.

(f) **Knowledge** "of the withheld material information or of the falsity of the material misrepresentation" (*id.* at 1328)

60. On information and belief, Lynn Joens knew of and understood the materiality of Stills 1991 [redacted] and his co-authoring of Joens 1997 (received for publication in 1996) discussing and citing Stills 1991. Robert Glock also knew of and understood the materiality of Stills 1991. Robert Glock [redacted], and co-authored Joens 1997 discussing and citing Stills 1991. In Joens 1997, Lynn Joens and Robert Glock stated, *inter alia*, that "Stills' work with hamsters with ileitis provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora. These methods were adapted for isolation of a bacterial agent from necrotic intestinal lesions typical of proliferative ileitis in the pigs of [their] study."

(g) "Specific **intent** to deceive the PTO" (*id.* at 1327).

61. On information and belief, Lynn Joens and Robert Glock acted with specific intent to deceive the PTO. Lynn Joens's and Robert Glock's specific intent to deceive the PTO when failing to disclose material information during prosecution of the '059 Patent is supported by, among other things, the facts that 1) Lynn Joens and Robert Glock knew about Stills 1991 and its materiality; [redacted] 3) Lynn Joens and Robert Glock stated and understood that "Stills' work with hamsters with ileitis provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora. These methods were adapted for isolation of a bacterial agent from necrotic intestinal lesions typical of proliferative ileitis in the pigs of this study. The original isolation and growth of the organism was accomplished, using an atmosphere of 02:C02:H2:N2 (6:7:7:80%), as originally described by Stills [1991]."; 4) Lynn Joens and Robert Glock were informed of and acknowledged their duty of disclosure to the PTO; 5) Stills 1991 anticipates and/or renders obvious all of the '059 patent claims; 6) that, on information and belief, the inventors wanted the application for the '059 Patent to issue as a patent in order to, *inter alia*, be compensated and get credit therefor; and 7) no other credible explanation exists for the inventors' failure to disclose Stills 1991 even though they based their work on it, used its methods, and acknowledge that they did no more than adapt the proven Stills 1991 method.

62. Specifically, under the pleading requirements set forth by the United States Court of Appeals for the Federal Circuit in *Exergen*, 575 F.3d 1312 regarding Joens 1997:

(a) "**[W]ho** both knew of the material information and deliberately withheld or misrepresented it" (*Exergen*, 575 F.3d at 1329)

63. Lynn Joens and Robert Glock knew of, and on information and belief, deliberately withheld from the PTO Joens 1997 (received for publication in 1996) and the following information contained within Joens 1997:

(a) that prior to November 9, 1991, Stills had isolated a bacterium from the intestinal cells of hamsters with proliferative enteritis, propagated the organism in a Henle 407 cell line, and induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular bacterium;

(b) that the organism isolated by Stills was a curved rod having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long;

(c) that the organism isolated by Stills was intracellular;

(d) that Stills 1991 and Stills' work with hamsters with ileitis prior to November 9, 1991 provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora; and

(e) that Lynn Joens and/or Robert Glock used the methods described in Stills 1991 to purportedly isolate *Lawsonia intracellularis*.

(b) **What** "claims, and which limitations in those claims, the withheld references [or misrepresentation] are relevant to" (*id.*)

64. Joens 1997 (received for publication in 1996) and the following information contained within Joens 1997 is relevant to all claims of the '059 patent (claims 1-4):

(a) that prior to November 9, 1991, Stills had isolated a bacterium from the intestinal cells of hamsters with proliferative enteritis, propagated the organism in a Henle 407 cell line, and induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular bacterium;

(b) that the organism isolated by Stills was a curved rod having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long;

(c) that the organism isolated by Stills was intracellular;

(d) that Stills 1991 and Stills' work with hamsters with ileitis prior to November 9, 1991 provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora; and

(e) that Lynn Joens and/or Robert Glock used the methods described in Stills 1991 to purportedly isolate *Lawsonia intracellularis*.

(c) "**Where** in th[e] references the material information is found" (*id.*)

65. Material information is found throughout Joens 1997 (received for publication in 1996), including, for example, the information found on pages 1125 and 1130 of Joens 1997. Joens 1997 discloses:

(a) Stills 1991;

(b) that prior to November 9, 1991, Stills had isolated a bacterium from the intestinal cells of hamsters with proliferative enteritis, propagated the organism in a Henle 407 cell line, and induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular bacterium;

(c) that the organism isolated by Stills was a curved rod having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long;

(d) that the organism isolated by Stills was intracellular;

(e) that Stills 1991 and Stills' work with hamsters with ileitis prior to November 9, 1991 provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora; and

(f) that Lynn Joens and/or Robert Glock used the methods described in Stills 1991 to purportedly isolate *Lawsonia intracellularis*.

(d)"'**How'** an examiner would have used [the] information in assessing the patentability of the claims (*Exergen*, 575 F.3d at 1329-1330).

66. Had the Examiner known about Joens 1997 (received for publication in 1996), or any of the following information contained within Joens 1997:

(a) that prior to November 9, 1991, Stills had isolated a bacterium from the intestinal cells of hamsters with proliferative enteritis, propagated the organism in a Henle 407 cell line, and induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular bacterium;

(b) that the organism isolated by Stills was a curved rod having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long;

(c) that the organism isolated by Stills was intracellular;

(d) that Stills 1991 and Stills' work with hamsters with ileitis prior to November 9, 1991 provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora; and

(e) that Lynn Joens and/or Robert Glock used the methods described in Stills 1991 to purportedly isolate *Lawsonia intracellularis*,

the Examiner would have used such information to find the claims of the '059 Patent anticipated and/or rendered obvious under 35 U.S.C. §§ 102 and/or 103. For example, the PTO Examiner stated that she had allowed the '059 patent because the "closest prior art does not teach or suggest the isolation of a microorganism in a mammalian host cell line wherein the microorganism causes porcine proliferative enteritis (PPE). The claimed PPE-causing agent is

an obligate, intracellular microorganism. The microorganism taught by the closest prior art causes only symptoms of PPE and is not an intracellular organism." '059 patent file history, 08/28/1996 Notice of Allowance, at 2-3. Joens 1997 (received for publication in 1996) and the foregoing information contained within Joens 1997 would have demonstrated to the Examiner that Stills 1991 teaches and suggests what the Examiner explicitly believed was missing from the prior art that was in front of her and justified allowing the '059 patent over that art. It also would have contradicted arguments made by the applicants to obtain the '059 patent, including but not limited to their arguments that "it is clear that even for a period of at least nine years after the publication of Lomax, the art was unable to culture any organism necessary and sufficient to cause PPE", there was no "suggestion to be gleaned from the art to isolate an unknown species of bacterium," and that one of "the strongest arguments in support of the newly isolated nature of the [claimed] strain [was] that the prior art does not describe an obligate intracellular organism that can alone induce PPE." *See*, '059 file history, 6/6/1994 Resp. to Office Action, at 4-12.

(e) The "**when**... of the material misrepresentation or omission committed before the PTO" (*id.* at 1327)

67. The omissions of Joens 1997 (received for publication in 1996) and the information contained within Joens 1997 committed before the PTO by Lynn Joens and Robert Glock occurred during the entirety of the period beginning on the date Joens 1997 was drafted and continuing until the '059 Patent issued: at least as early as September 3, 1996 through March 11, 1997.

(f) **Knowledge** "of the withheld material information or of the falsity of the material misrepresentation" (*id.* at 1328)

68. On information and belief, Lynn Joens and Robert Glock knew of and understood the materiality of Joens 1997 (received for publication in 1996) and the information contained within Joens 1997 at least through and a result of their co-authorship of Joens 1997 and their use of the Stills 1991 method in connection with their own work.

(g) "Specific **intent** to deceive the PTO" (*id.* at 1327).

69. On information and belief, Lynn Joens and Robert Glock acted with specific intent to deceive the PTO. Lynn Joens's and Robert Glock's specific intent to deceive the PTO when failing to disclose material information during prosecution of the '059 Patent is supported by, among other things, the facts that 1) Lynn Joens and Robert Glock knew about Joens 1997 (received for publication in 1996) and the information contained within Joens 1997 and their materiality; 2) Lynn Joens and Robert Glock were informed of and acknowledged their duty of disclosure to the PTO; 3) Stills 1991 anticipates and/or renders obvious all of the '059 patent claims and Joens 1997 and the information contained within Joens 1997 would have demonstrated to the Examiner that this was so; 4) Lynn Joens and Robert Glock stated in Joens 1997 that "Stills' work with hamsters with ileitis provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora. These methods were adapted for isolation of a bacterial agent from necrotic intestinal lesions typical of proliferative ileitis in the pigs of this study. The original isolation and growth of the organism was accomplished, using an atmosphere of 02:C02:H2:N2 (6:7:7:80%), as originally described by Stills [1991]."; 5) that, on information and belief, the inventors wanted the application for the '059 Patent to issue as a patent in order to, *inter alia*, be compensated and get credit therefor; and 6) no other credible explanation exists for the inventors' failure to disclose Joens 1997.

VETMEDICA'S COUNTERCLAIMS

Vetmedica for its Counterclaims against Intervet hereby demands a jury trial and alleges as follows:

PARTIES

1. Vetmedica is a corporation organized and existing under the laws of the State of Delaware, having a place of business in St. Joseph, Missouri.

2. Intervet alleges in its Complaint that the Arizona Board of Regents, on behalf of The University of Arizona ("the University") is a constitutionally-created corporate body, an instrumentality of the state of Arizona, having a principal place of business at 1401 East University Blvd, Administration Bldg – Room 103, Tucson, Arizona 85721-0066.

3. Intervet alleges in its Complaint that Intervet Inc., d/b/a Merck Animal Health is a corporation organized and existing under the laws of Delaware with its principal place of business at 556 Morris Avenue, Summit, New Jersey.

NATURE OF COUNTERCLAIMS

4. Vetmedica brings an action for a declaration of patent noninfringement, invalidity, and unenforceability arising under the Declaratory Judgment Act, 28 U.S.C. § 2201 *et. seq.* and the patent laws of the United States, 35 U.S.C. § 1 *et. seq.*

JURISDICTION AND VENUE

5. This Court has subject matter jurisdiction over these Counterclaims pursuant to the Declaratory Judgment Act, 28 U.S.C. §§ 2201-02, and under the Patent Laws of the United States, 28 U.S.C. §§ 1331 and 1338(a).

6. Intervet has consented to personal jurisdiction and venue in this judicial district with respect to Vetmedica's Counterclaims by voluntarily appearing before this Court and filing its Complaint against Vetmedica here.

7. Intervet alleges that the University is the assignee and sole owner of the '059 patent, that Intervet Inc., d/b/a Merck Animal Health is the exclusive licensee under the '059 patent, and that Plaintiffs have the right to sue to enforce the '059 patent.

8. Intervet alleges that Vetmedica infringes the '059 patent. Vetmedica denies that it infringes any claim of the '059 patent and that the claims of the '059 patent are invalid.

9. There is an actual justiciable controversy between Vetmedica and Intervet concerning the noninfringement, invalidity, and unenforceability of the '059 Patent.

FIRST COUNTERCLAIM

(Declaratory Judgment of Noninfringement of United States Patent No. 5,610,059)

10. Vetmedica incorporates its responses to Intervet's Complaint, its Defenses, and Paragraphs 1 through 9 of these Counterclaims as if fully set forth herein.

11. An actual and justiciable controversy exists between Vetmedica and Intervet concerning the non-infringement of the '059 Patent.

12. Vetmedica has not infringed, contributed to the infringement of, or induced others to infringe any valid and enforceable claim of the '059 Patent, either literally or under the doctrine of equivalents, willfully or otherwise.

13. Vetmedica is entitled to a judgment from this Court that Vetmedica has not infringed, contributed to the infringement of, or induced others to infringe any valid and enforceable claim of the '059 Patent, either literally or under the doctrine of equivalents.

SECOND COUNTERCLAIM

(Declaratory Judgment of Invalidity of United States Patent No. 5,610,059)

14. Vetmedica incorporates its responses to Intervet's Complaint, its Defenses, and Paragraphs 1 through 13 of these Counterclaims as if fully set forth herein.

15. An actual and justiciable controversy exists between Vetmedica and Intervet concerning the invalidity of the '059 Patent.

16. The claims of the '059 Patent are invalid for failure to meet the requirements of the patent laws of the United States, including, but not limited to, 35 U.S.C. §§ 101, 102, 103, and 112.

17. Vetmedica is entitled to a judgment from this Court that each asserted claim of the '059 Patent is invalid for failure to meet the requirements of the patent laws of the United States, including, but not limited to, 35 U.S.C. §§ 101, 102, 103, and 112.

THIRD COUNTERCLAIM

(Unenforceability Due to Inequitable Conduct)

18. Vetmedica incorporates its responses to Intervet's Complaint, its Defenses, and Paragraphs 1 through 17 of these Counterclaims as if fully set forth herein.

19. An actual and justiciable controversy exists between Vetmedica and Intervet concerning the unenforceability of the '059 Patent.

20. The application resulting in the '059 Patent was filed on November 9, 1992. The '059 Patent issued on March 11, 1997.

21. Nicholas Seay of the law firm Quarles & Brady LLP prosecuted the application that resulted in the '059 patent.

22. Lynn Joens and Robert Glock are identified as the inventors on the face of the '059 Patent.

23. The University of Arizona purports to be the assignee of the '059 Patent.

24. On information and belief, Lynn Joens was employed by the University of Arizona while the application resulting in the '059 patent was being prosecuted.

25. In connection with the application resulting in the '059 Patent, Lynn Joens signed a declaration in which he acknowledged his duty to disclose to the Patent Office information known to him to be material to patentability in accordance with 37 C.F.R. § 1.56.

26. In connection with the application resulting in the '059 Patent, Robert Glock signed a declaration in which he acknowledged his duty to disclose to the Patent Office information known to him to be material to patentability in accordance with 37 C.F.R. § 1.56.

27. Nicholas Seay understood his duty to disclose to the Patent Office information known to him to be material to patentability in accordance with 37 C.F.R. § 1.56.

28. Nicholas Seay informed Lynn Joens and Robert Glock of their duty of disclosure.

29. Attached as Exhibit A hereto is a true and correct copy of an article published in September 1991, Stills, H.F., Isolation of an Intracellular Bacterium from Hamsters (Mesocricetus auratus) with Proliferative Ileitis and Reproduction of the Disease with a Pure Culture, Infection and Immunity, Vol. 59, No. 9, p. 3227-3236 (1991) (hereinafter referred to as "Stills 1991").

30. Stills 1991 is prior art to the '059 patent under 35 U.S.C. § 102(b).

31. Stills 1991 discloses a process for isolating an intracellular bacterium that causes proliferative ileitis and a process for culturing that bacterium.

[redacted]

34. Attached hereto as Exhibit B is a true and correct copy of L.A. Joens, et. al, *Induction of Gross and Microscopic Lesions of Porcine Proliferative Enteritis By Lawsonia Intracellaris*, AJVR, Vol. 58, No. 10, October 1997 (hereinafter referred to as "Joens 1997").

35. Joens 1997 is an article co-authored by Lynn Joens, Robert Glock, and Stuart Nibbelink.

36. Joens 1997 states, *inter alia*, that:

> Stills isolated a bacterium from the enterocytes of hamsters with proliferative enteritis and propagated the organism in a continuous epithelial cell line. The organism was described as a curved rod, with a trilaminar membrane, having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long. Stills induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular agent. A bacterium having the same measurements has also been cultured in a rat enterocyte cell line under an atmosphere of O2:CO2:N2 (8:8:84%).[14] The bacterium was isolated from intestinal homogenates of a pig with proliferative ileitis, using methods similar to that of Stills [1991]. * * * Numerous research workers have attempted to isolate the PPE organism from the intestinal contents of pigs. Stills' work with hamsters with ileitis provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora. These methods were adapted for isolation of a bacterial agent from necrotic intestinal lesions typical of proliferative ileitis in the pigs of this study. The original isolation and growth of the organism was accomplished, using an atmosphere of 02:C02:H2:N2 (6:7:7:80%), as originally described by Stills [1991].

37. Joens 1997 was received for publication on or around September 3, 1996.

38. On information and belief, Joens 1997 was submitted for publication while the application resulting in the '059 patent was being prosecuted.

39. On information and belief, during prosecution of the application resulting in the '059 patent, the named authors of Joens 1997, including Lynn Joens and Robert Glock, were aware of the following and at least one draft or version of Joens 1997 disclosing the following:

(a) Stills 1991;

(b) that prior to November 9, 1991, Stills had isolated a bacterium from the intestinal cells of hamsters with proliferative enteritis, propagated the organism in a Henle 407 cell line, and induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular bacterium;

(c) that the organism isolated by Stills was a curved rod having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long;

(d) that the organism isolated by Stills was intracellular;

(e) that Stills 1991 and Stills' work with hamsters with ileitis prior to November 9, 1991 provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora; and

(f) that Lynn Joens and/or Robert Glock used the methods described in Stills 1991 to purportedly isolate *Lawsonia intracellularis*.

40. Lynn Joens did not disclose Stills 1991 to the PTO in connection with the application that resulted in the '059 patent.

41. Lynn Joens did not disclose to the PTO in connection with the application that resulted in the '059 patent Joens 1997, any draft or version of Joens 1997, or any of the following information:

(a) that prior to November 9, 1991, Stills had isolated a bacterium from the intestinal cells of hamsters with proliferative enteritis, propagated the organism in a Henle 407 cell line, and induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular bacterium;

(b) that the organism isolated by Stills was a curved rod having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long;

(c) that the organism isolated by Stills was intracellular;

(d) that Stills 1991 and Stills' work with hamsters with ileitis prior to November 9, 1991 provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora; and

(e) that Lynn Joens and/or Robert Glock used the methods described in Stills 1991 to purportedly isolate *Lawsonia intracellularis*.

42. Robert Glock did not disclose Stills 1991 to the PTO in connection with the application that resulted in the '059 patent.

43. Robert Glock did not disclose to the PTO in connection with the application that resulted in the '059 patent Joens 1997, any draft or version of Joens 1997, or any of the following information:

(a) that prior to November 9, 1991, Stills had isolated a bacterium from the intestinal cells of hamsters with proliferative enteritis, propagated the organism in a Henle 407 cell line, and induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular bacterium;

(b) that the organism isolated by Stills was a curved rod having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long;

(c) that the organism isolated by Stills was intracellular;

(d) that Stills 1991 and Stills' work with hamsters with ileitis prior to November 9, 1991 provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora; and

(e) that Lynn Joens and/or Robert Glock used the methods described in Stills 1991 to purportedly isolate *Lawsonia intracellularis*.

44. The University of Arizona did not disclose Stills 1991 to the PTO in connection with the application that resulted in the '059 patent.

45. The University of Arizona did not disclose to the PTO in connection with the application that resulted in the '059 patent Joens 1997, any draft or version of Joens 1997, or any of the following information:

(a) that prior to November 9, 1991, Stills had isolated a bacterium from the intestinal cells of hamsters with proliferative enteritis, propagated the organism in a Henle 407 cell line, and induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular bacterium;

(b) that the organism isolated by Stills was a curved rod having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long;

(c) that the organism isolated by Stills was intracellular;

(d) that Stills 1991 and Stills' work with hamsters with ileitis prior to November 9, 1991 provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora; and

(e) that Lynn Joens and/or Robert Glock used the methods described in Stills 1991 to purportedly isolate *Lawsonia intracellularis*.

46. Nicholas Seay did not disclose Stills 1991 to the PTO in connection with the application that resulted in the '059 patent.

47. Nicholas Seay did not disclose to the PTO in connection with the application that resulted in the '059 patent Joens 1997, any draft or version of Joens 1997, or any of the following information:

(a) that prior to November 9, 1991, Stills had isolated a bacterium from the intestinal cells of hamsters with proliferative enteritis, propagated the organism in a Henle 407 cell line, and induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular bacterium;

(b) that the organism isolated by Stills was a curved rod having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long;

(c) that the organism isolated by Stills was intracellular;

(d) that Stills 1991 and Stills' work with hamsters with ileitis prior to November 9, 1991 provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora; and

(e) that Lynn Joens and/or Robert Glock used the methods described in Stills 1991 to purportedly isolate *Lawsonia intracellularis*.

48. On information and belief, Lynn Joens, in breach of the duty of candor and good faith required by 37 C.F.R. § 1.56 and with specific intent to deceive the Examiner, failed to disclose during prosecution of the '059 Patent Stills 1991, [redacted], Joens 1997, any draft or version of Joens 1997, and any of the following information:

(a) that prior to November 9, 1991, Stills had isolated a bacterium from the intestinal cells of hamsters with proliferative enteritis, propagated the organism in a Henle 407 cell line, and induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular bacterium;

(b) that the organism isolated by Stills was a curved rod having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long;

(c) that the organism isolated by Stills was intracellular;

(d) that Stills 1991 and Stills' work with hamsters with ileitis prior to November 9, 1991 provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora; and

(e) that Lynn Joens and/or Robert Glock used the methods described in Stills 1991 to purportedly isolate *Lawsonia intracellularis*.

49. On information and belief, Robert Glock, in breach of the duty of candor and good faith required by 37 C.F.R. § 1.56 and with specific intent to deceive the Examiner, failed to disclose during prosecution of the '059 Patent Stills 1991, Joens 1997, any draft or version of Joens 1997, and any of the following information:

(a) that prior to November 9, 1991, Stills had isolated a bacterium from the intestinal cells of hamsters with proliferative enteritis, propagated the organism in a Henle 407 cell line, and induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular bacterium;

(b) that the organism isolated by Stills was a curved rod having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long;

(c) that the organism isolated by Stills was intracellular;

(d) that Stills 1991 and Stills' work with hamsters with ileitis prior to November 9, 1991 provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora; and

(e) that Lynn Joens and/or Robert Glock used the methods described in Stills 1991 to purportedly isolate *Lawsonia intracellularis*.

50. On information and belief, the University of Arizona, in breach of the duty of candor and good faith required by 37 C.F.R. § 1.56 and with specific intent to deceive the Examiner, failed to disclose during prosecution of the '059 patent Stills 1991, [redacted], Joens 1997, any draft or version of Joens 1997, and any of the following information:

(a) that prior to November 9, 1991, Stills had isolated a bacterium from the intestinal cells of hamsters with proliferative enteritis, propagated the organism in a Henle 407 cell line, and induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular bacterium;

(b) that the organism isolated by Stills was a curved rod having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long;

(c) that the organism isolated by Stills was intracellular;

(d) that Stills 1991 and Stills' work with hamsters with ileitis prior to November 9, 1991 provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora; and

(e) that Lynn Joens and/or Robert Glock used the methods described in Stills 1991 to purportedly isolate *Lawsonia intracellularis*.

51. Based on Lynn Joens's, Robert Glock's, and the University of Arizona's failures to disclose Stills 1991, [redacted], Joens 1997, any draft or version of Joens 1997, and any of the following information:

(a) that prior to November 9, 1991, Stills had isolated a bacterium from the intestinal cells of hamsters with proliferative enteritis, propagated the organism in a

Henle 407 cell line, and induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular bacterium;

(b) that the organism isolated by Stills was a curved rod having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long;

(c) that the organism isolated by Stills was intracellular;

(d) that Stills 1991 and Stills' work with hamsters with ileitis prior to November 9, 1991 provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora; and

(e) that Lynn Joens and/or Robert Glock used the methods described in Stills 1991 to purportedly isolate *Lawsonia intracellularis*,

the '059 Patent is unenforceable due to inequitable conduct.

52. Specifically, under the pleading requirements set forth by the United States Court of Appeals for the Federal Circuit in *Exergen*, 575 F.3d 1312 regarding Stills 1991:

(a) "[W]ho both knew of the material information and deliberately withheld or misrepresented it" (*Exergen*, 575 F.3d at 1329)

53. Lynn Joens and Robert Glock knew of, and on information and belief, deliberately withheld from the PTO Stills 1991.

54. [redacted]

(b) What "claims, and which limitations in those claims, the withheld references [or misrepresentation] are relevant to" (*id.*)

55. Stills 1991 anticipates under 35 U.S.C. § 102(b) and/or renders obvious under 35 U.S.C. § 103, and is therefore relevant to, all limitations of all claims (1-4) of the '059 patent.

56. Claim 1 of the '059 patent requires: "A biologically pure culture of a mammalian host cell infected by an obligate intracellular bacterium." Stills 1991 discloses a biologically pure culture of a mammalian host cell infected by an obligate intracellular bacterium. *See, e.g.,* Stills 1991 at Title, Abstract.

57. Claim 1 of the '059 patent requires that the bacterium "causes porcine proliferative enteritis after inoculation into pigs." Stills 1991 discloses that its bacterium causes proliferative enteritis after inoculation in hamsters, *e.g.*, Stills 1991 at *Abstract*, 3234, and that "porcine proliferative enteropathy" and hamster proliferative enteritis possibly had the "same etiology," *id.* at 3227.

58. Claim 1 of the '059 patent requires that the culture have "all of the identifying characteristics of ATCC Accession No. 55370." Plaintiffs do not treat this as a separate limitation on their claims. Regardless, Stills 1991 discloses characteristics of the culture that the applicants allegedly deposited with the ATCC as Accession No. 55370 that are identified in the '059 patent specification and by the applicants in the file history. *Compare, e.g.* Stills 1991 at *Abstract*, 3234, *with* '059 Patent at 1:6–9, 7:38–8:4, '059 File History, 12-19-1994 Amend., at 4–5, *and* '059 File History, 05-31-1994 Amend., at 5, 7.

59. Claim 2 of the '059 patent requires that "the mammalian host cell is the human intestinal cell line Henle 407." Stills 1991 discloses that its mammalian host cell line was a Henle 407 cell line. E.g., Stills 1991 at *Abstract*, 3228.

60. Claim 3 of the '059 patent requires that "the obligate intracellular bacterium that causes porcine proliferative enteritis is a bacterium having dimensions in the range of 0.24-0.27 micron x 1.4-2.4 micron." Stills 1991 discloses that its bacterium had dimensions in the range of

"0.26 to 0.34 [micron] in width" and "1.4 to 1.7 [micron] in length." *E.g.*, Stills 1991 at *Abstract*, 3231.

61. Claim 4 of the '059 patent requires that "the obligate intracellular bacterium that causes porcine proliferative enteritis is isolated from enterocytes of a pig having porcine proliferative enteritis." Stills 1991 discloses that its bacterium causes proliferative enteritis after inoculation in hamsters, *e.g.*, Stills 1991 at *Abstract*, 3234, and that "porcine proliferative enteropathy" and hamster proliferative enteritis possibly had the "same etiology," *id.* at 3227.

(c) "**Where** in th[e] references the material information is found" (*id.*)

62. Material information is found throughout Stills 1991, including, for example, in Stills 1991 at *Title, Abstract*, 3227-28, and 3233-34.

63. Among other things, Stills 1991 discloses a method for isolating an intracellular bacterium from hamsters and pigs with proliferative enteritis and reproducing proliferative enteritis with a pure culture of that bacterium. Stills 1991 also discloses a pure culture of the isolated intracellular bacterium hosted in Henle 407 cells.

64. Paragraphs 56-61 above are hereby incorporated by reference as if fully restated herein.

(d) "'**How**' an examiner would have used [the] information in assessing the patentability of the claims (*Exergen*, 575 F.3d at 1329-1330).

65. Had the Examiner known about Stills 1991, the Examiner would have used such information to find the claims of the '059 Patent anticipated and/or rendered obvious under 35 U.S.C. §§ 102 and/or 103. For example, the Examiner examining the application resulting in the '059 patent stated that she had allowed the '059 patent because the "closest prior art does not teach or suggest the isolation of a microorganism in a mammalian host cell line wherein the

microorganism causes porcine proliferative enteritis (PPE). The claimed PPE-causing agent is an obligate, intracellular microorganism. The microorganism taught by the closest prior art causes only symptoms of PPE and is not an intracellular organism." '059 patent file history, 08/28/1996 Notice of Allowance, at 2-3. Stills 1991 teaches and suggests what the Examiner explicitly believed was missing from the prior art that was in front of her and justified allowing the '059 patent over that art. Stills 1991 also would have contradicted arguments made by the applicants to obtain the '059 patent, including but not limited to their arguments that "it is clear that even for a period of at least nine years after the publication of Lomax, the art was unable to culture any organism necessary and sufficient to cause PPE", there was no "suggestion to be gleaned from the art to isolate an unknown species of bacterium," and that one of "the strongest arguments in support of the newly isolated nature of the [claimed] strain [was] that the prior art does not describe an obligate intracellular organism that can alone induce PPE." *See*, '059 file history, 6/6/1994 Resp. to Office Action, at 4-12.

66. Stills 1991 teaches and suggests the isolation of a microorganism (a bacterium) in a mammalian host cell line, including a Henle 407 cell line. *See, e.g.*, Stills 1991, at Title, Abstract, 3227-38.

67. Stills 1991 teaches and suggests that its bacterium causes proliferative enteritis after inoculation in hamsters, *e.g.*, Stills 1991 at *Abstract*, 3234, and that "porcine proliferative enteropathy" and hamster proliferative enteritis possibly had the "same etiology," *id.* at 3227.

68. Stills teaches and suggests that the proliferative enteritis-causing bacterium was an obligate, intracellular microorganism. *See e.g.*, Stills 1991 at *Title, Abstract*, 3227-28, 3234 (describing bacterium's intracellular nature and discussing inability to propagate bacterium on artificial media).

69. Paragraphs 56-61 above are hereby incorporated by reference as if fully restated herein.

(e) The "**when**... of the material misrepresentation or omission committed before the PTO" (*id.* at 1327)

70. The omissions of Stills 1991 committed before the PTO by Lynn Joens and Robert Glock occurred during the entirety of the period beginning on the date the application resulting in the '059 patent was filed and continuing until the '059 Patent issued: November 9, 1992 through March 11, 1997.

(f) **Knowledge** "of the withheld material information or of the falsity of the material misrepresentation" (*id.* at 1328)

71. On information and belief, Lynn Joens knew of and understood the materiality of Stills 1991 at least through [redacted] and his co-authoring of Joens 1997 (received for publication in 1996) discussing and citing Stills 1991. Robert Glock also knew of and understood the materiality of Stills 1991. Robert Glock [redacted] and co-authored Joens 1997 discussing and citing Stills 1991. In Joens 1997, Lynn Joens and Robert Glock stated, *inter alia*, that "Stills' work with hamsters with ileitis provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora. These methods were adapted for isolation of a bacterial agent from necrotic intestinal lesions typical of proliferative ileitis in the pigs of [their] study."

(g) "Specific **intent** to deceive the PTO" (*id.* at 1327).

72. On information and belief, Lynn Joens and Robert Glock acted with specific intent to deceive the PTO. Lynn Joens's and Robert Glock's specific intent to deceive the PTO when failing to disclose material information during prosecution of the '059 Patent is supported by, among other things, the facts that 1) Lynn Joens and Robert Glock knew about Stills 1991 and its materiality; 2) [redacted] 3) Lynn Joens and Robert Glock stated and understood that "Stills' work with hamsters with ileitis provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora. These methods were adapted for isolation of a bacterial agent from necrotic intestinal lesions typical of proliferative ileitis in the pigs of this study. The original isolation and growth of the organism was accomplished, using an atmosphere of 02:C02:H2:N2 (6:7:7:80%), as originally described by Stills [1991]."; 4) Lynn Joens and Robert Glock were informed of and acknowledged their duty of disclosure to the PTO; 5) Stills 1991 anticipates and/or renders obvious all of the '059 patent claims; 6) that, on information and belief, the inventors wanted the application for the '059 Patent to issue as a patent in order to, *inter alia*, be compensated and get credit therefor; and 7) no other credible explanation exists for the inventors' failure to disclose Stills 1991 even though they based their work on it, used its methods, and acknowledge that they did no more than adapt the proven Stills 1991 method.

73. Specifically, under the pleading requirements set forth by the United States Court of Appeals for the Federal Circuit in *Exergen*, 575 F.3d 1312 regarding Joens 1997:

> (a) "**[W]ho** both knew of the material information and deliberately withheld or misrepresented it" (*Exergen*, 575 F.3d at 1329)

74. Lynn Joens and Robert Glock knew of, and on information and belief, deliberately withheld from the PTO Joens 1997 (received for publication in 1996) and the following information contained within Joens 1997:

(a) that prior to November 9, 1991, Stills had isolated a bacterium from the intestinal cells of hamsters with proliferative enteritis, propagated the organism in a Henle 407 cell line, and induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular bacterium;

(b) that the organism isolated by Stills was a curved rod having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long;

(c) that the organism isolated by Stills was intracellular;

(d) that Stills 1991 and Stills' work with hamsters with ileitis prior to November 9, 1991 provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora; and

(e) that Lynn Joens and/or Robert Glock used the methods described in Stills 1991 to purportedly isolate *Lawsonia intracellularis*.

(b) **What** "claims, and which limitations in those claims, the withheld references [or misrepresentation] are relevant to" (*id.*)

75. Joens 1997 (received for publication in 1996) and the following information contained within Joens 1997 is relevant to all claims of the '059 patent (claims 1-4):

(a) that prior to November 9, 1991, Stills had isolated a bacterium from the intestinal cells of hamsters with proliferative enteritis, propagated the organism in a Henle 407 cell line, and induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular bacterium;

(b) that the organism isolated by Stills was a curved rod having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long;

(c) that the organism isolated by Stills was intracellular;

(d) that Stills 1991 and Stills' work with hamsters with ileitis prior to November 9, 1991 provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora; and

(e) that Lynn Joens and/or Robert Glock used the methods described in Stills 1991 to purportedly isolate *Lawsonia intracellularis*.

(c) "**Where** in th[e] references the material information is found" (*id.*)

76. Material information is found throughout Joens 1997 (received for publication in 1996), including, for example, the information found on pages 1125 and 1130 of Joens 1997. Joens 1997 discloses:

(a) Stills 1991;

(b) that prior to November 9, 1991, Stills had isolated a bacterium from the intestinal cells of hamsters with proliferative enteritis, propagated the organism in a Henle 407 cell line, and induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular bacterium;

(c) that the organism isolated by Stills was a curved rod having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long;

(d) that the organism isolated by Stills was intracellular;

(e) that Stills 1991 and Stills' work with hamsters with ileitis prior to November 9, 1991 provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora; and

(f) that Lynn Joens and/or Robert Glock used the methods described in Stills 1991 to purportedly isolate *Lawsonia intracellularis*.

(d)"'**How**' an examiner would have used [the] information in assessing the patentability of the claims (*Exergen*, 575 F.3d at 1329-1330).

77. Had the Examiner known about Joens 1997 (received for publication in 1996), or any of the following information contained within Joens 1997:

(a) that prior to November 9, 1991, Stills had isolated a bacterium from the intestinal cells of hamsters with proliferative enteritis, propagated the organism in a Henle 407 cell line, and induced lesions in the small intestine of hamsters after oral inoculation with Henle 407 cells containing the intracellular bacterium;

(b) that the organism isolated by Stills was a curved rod having dimensions 0.26 to 0.34μm wide by 1.4 to 1.7 μm long;

(c) that the organism isolated by Stills was intracellular;

(d) that Stills 1991 and Stills' work with hamsters with ileitis prior to November 9, 1991 provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora; and

(e) that Lynn Joens and/or Robert Glock used the methods described in Stills 1991 to purportedly isolate *Lawsonia intracellularis*,

the Examiner would have used such information to find the claims of the '059 Patent anticipated and/or rendered obvious under 35 U.S.C. §§ 102 and/or 103. For example, the PTO Examiner stated that she had allowed the '059 patent because the "closest prior art does not teach or suggest the isolation of a microorganism in a mammalian host cell line wherein the microorganism causes porcine proliferative enteritis (PPE). The claimed PPE-causing agent is an obligate, intracellular microorganism. The microorganism taught by the closest prior art causes only symptoms of PPE and is not an intracellular organism." '059 patent file history,

08/28/1996 Notice of Allowance, at 2-3. Joens 1997 (received for publication in 1996) and the foregoing information contained within Joens 1997 would have demonstrated to the Examiner that Stills 1991 teaches and suggests what the Examiner explicitly believed was missing from the prior art that was in front of her and justified allowing the '059 patent over that art. It also would have contradicted arguments made by the applicants to obtain the '059 patent, including but not limited to their arguments that "it is clear that even for a period of at least nine years after the publication of Lomax, the art was unable to culture any organism necessary and sufficient to cause PPE", there was no "suggestion to be gleaned from the art to isolate an unknown species of bacterium," and that one of "the strongest arguments in support of the newly isolated nature of the [claimed] strain [was] that the prior art does not describe an obligate intracellular organism that can alone induce PPE." *See*, '059 file history, 6/6/1994 Resp. to Office Action, at 4-12.

(e) The "**when**... of the material misrepresentation or omission committed before the PTO" (*id.* at 1327)

78. The omissions of Joens 1997 (received for publication in 1996) and the information contained within Joens 1997 committed before the PTO by Lynn Joens and Robert Glock occurred during the entirety of the period beginning on the date Joens 1997 was drafted and continuing until the '059 Patent issued: at least as early as September 3, 1996 through March 11, 1997.

(f) **Knowledge** "of the withheld material information or of the falsity of the material misrepresentation" (*id.* at 1328)

79. On information and belief, Lynn Joens and Robert Glock knew of and understood the materiality of Joens 1997 (received for publication in 1996) and the information contained within Joens 1997 at least through and a result of their co-authorship of Joens 1997 and their use of the Stills 1991 method in connection with their own work.

(g) "Specific **intent** to deceive the PTO" (*id.* at 1327).

80. On information and belief, Lynn Joens and Robert Glock acted with specific intent to deceive the PTO. Lynn Joens's and Robert Glock's specific intent to deceive the PTO when failing to disclose material information during prosecution of the '059 Patent is supported by, among other things, the facts that 1) Lynn Joens and Robert Glock knew about Joens 1997 (received for publication in 1996) and the information contained within Joens 1997 and their materiality; 2) Lynn Joens and Robert Glock were informed of and acknowledged their duty of disclosure to the PTO; 3) Stills 1991 anticipates and/or renders obvious all of the '059 patent claims and Joens 1997 and the information contained within Joens 1997 would have demonstrated to the Examiner that this was so; 4) Lynn Joens and Robert Glock stated in Joens 1997 that "Stills' work with hamsters with ileitis provided researchers with a proven method for isolation of intracellular agents without risk of contamination by normal intestinal flora. These methods were adapted for isolation of a bacterial agent from necrotic intestinal lesions typical of proliferative ileitis in the pigs of this study. The original isolation and growth of the organism was accomplished, using an atmosphere of 02:C02:H2:N2 (6:7:7:80%), as originally described by Stills [1991]."; 5) that, on information and belief, the inventors wanted the application for the '059 Patent to issue as a patent in order to, *inter alia,* be compensated and get credit therefor; and 6) no other credible explanation exists for the inventors' failure to disclose Joens 1997.

PRAYER FOR RELIEF

WHEREFORE, Vetmedica prays for the following judgment and relief:

A. An judgment denying and dismissing all of Intervet's claims against Vetmedica with prejudice and denying Intervet's request for damages and injunctive relief;

B. A declaration that Vetmedica has not infringed and does not infringe, any valid or enforceable claim of the '059 Patent;

C. A declaration that the '059 Patent is invalid;

D. A declaration that the '059 Patent is unenforceable;

E. A declaration that this case is exceptional within the meaning of 35 U.S.C. § 285 and an award to Vetmedica of its reasonable attorneys' fees, expenses, and costs incurred in connection with this action; and

F. Any other equitable and legal relief that this Court deems just and proper.

JURY DEMANDED

Pursuant to Federal Rule of Civil Procedure 38, Vetmedica hereby respectfully requests a jury trial on all issues and claims so triable.

Respectfully submitted,

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EXHIBIT A

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Isolation of an Intracellular Bacterium from Hamsters (*Mesocricetus auratus*) with Proliferative Ileitis and Reproduction of the Disease with a Pure Culture

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An intracellular bacterium was isolated from hamsters (*Mesocricetus auratus*) with proliferative ileitis. The organism was isolated in Intestine 407 and GPC-16 cell cultures (incubated in a microaerophilic atmosphere) from isolated and lysed epithelial cells from hamsters with proliferative ileitis. The bacterium measured 1.4 to 1.7 μm in length by 0.26 to 0.34 μm in width, was slightly curved, and had an irregular trilaminar cell wall. Inoculation of hamsters with a cell culture lysate containing the organism or a 0.65-μm-pore-size filtrate of an infected-cell lysate resulted in the typical lesions of proliferative ileitis in approximately 50% of the animals in 28 days. Hamsters inoculated with uninfected cells or a 0.2-μm-pore-size filtrate of an infected-cell lysate remained uninfected. Attempts to propagate the organism on cell-free media have been unsuccessful.

Proliferative ileitis (transmissible ileal hyperplasia, wet tail) is a serious infectious disease of hamsters characterized by hyperplasia of the ileal epithelium and pyogranulomatous inflammation. Light microscopic studies have revealed a gram-negative, slightly curved bacillus within the hyperplastic ileal epithelial cells (6–8, 19). Ultrastructurally, the intracellular bacterium resembles members of the genus *Campylobacter* (2, 9, 20).

Campylobacter jejuni has been repeatedly associated with proliferative ileitis by microbiologic studies (10, 11). Experimental production of proliferative ileitis by inoculation with *C. jejuni* orally (1, 10, 11) or by surgically inoculating the ileum (6) has been uniformly unsuccessful. Proliferative ileitis has also been experimentally produced by using an infected homogenate free of *C. jejuni* (17). *Campylobacter hyointestinalis* (3, 5), *Campylobacter cinaedi* (4), and several additional *Campylobacter*-like organisms (18) have also been isolated from hamsters, but attempts to reproduce the disease with these organisms, as well as with *Campylobacter mucosalis* and *Campylobacter coli*, have also been unsuccessful (15). Porcine proliferative enteropathy is a disease of pigs with similar lesions and possibly the same etiology as hamster proliferative ileitis (15). Antigenic (14) and DNA (16) analysis of a preparation of intracellular organisms recovered from pigs with porcine proliferative enteropathy has suggested that the intracellular organism is unrelated to the presently described *Campylobacter* spp. and may not be a member of the genus *Campylobacter*.

In this paper we report the isolation of an organism in cell culture from hamsters with proliferative ileitis, reproduction of proliferative ileitis in hamsters following oral inoculation with the organism, and reisolation of the organism. Serological evidence of infection and immunohistochemical stains supporting the role of this organism in the pathogenesis of proliferative ileitis of hamsters are also provided.

MATERIALS AND METHODS

Isolation procedures. The aseptically removed ilea of three hamsters with experimentally induced proliferative ileitis (21 days postinoculation of infected ileal homogenate) provided the source material. The ilea were cut into 2.5-cm sections, opened longitudinally, and repeatedly washed with Hank's balanced salt solution (HBSS) without Ca^{2+} or Mg^{2+} with 1 mM EDTA (HBSS-EDTA) to remove the intestinal contents. The mucus was removed by incubating the ileal sections in 20% (wt/vol) *N*-acetyl-cysteine (Sigma Chemical Co., St. Louis, Mo.) for 45 min at 37°C with agitation. The supernatant was decanted, and the sections were washed twice in HBSS-EDTA and then three times in HBSS. The sections were then transferred to 20 ml of hyaluronidase (1 mg/ml) in HBSS with 5% (wt/vol) bovine serum albumin (BSA) and incubated at 37°C for 2 h with agitation to free the epithelial cells. The resulting solutions were successively filtered through a sterilized glass bead-filled syringe, a 105-μm-pore-size polypropylene mesh, and a 52-μm-pore-size nylon mesh (Spectra/Mesh; Spectrum Medical Industries, Inc., Los Angeles, Calif.) to remove the larger tissue fragments. The crude cell suspensions were centrifuged (400 × *g* for 10 min) and resuspended in HBSS twice. Following a final centrifugation, the cell pellets were resuspended in 50 ml of Leibovitz L-15 media (Sigma) with 10% fetal calf serum, 100 μg of gentamicin sulfate (Sigma) per ml, and 2.5 μg of amphotericin B (Sigma) per ml and incubated overnight at 4°C with agitation.

The cells were separated by centrifugation (400 × *g* for 10 min) and washed three times in HBSS-EDTA with 5% BSA and resuspended in a minimal volume of HBSS-EDTA with 5% BSA. The cell suspensions were then layered onto preformed (15 min at 15,000 × *g* and at 4°C) 30% Percoll (Pharmacia LKB Biotechnology, Inc., Piscataway, N.J.) gradients in HBSS-EDTA with 5% BSA and centrifuged at 400 × *g* for 15 min. The epithelial cell bands (density, 1.035 g/ml) were suspended in HBSS with 5% BSA and washed four times in HBSS with 5% BSA followed by centrifugation (400 × *g*, 10 min). The cell pellets were resuspended in HBSS with 5% BSA and 0.2% Triton X-100 and incubated at 37°C for 30 min with agitation to effect cell lysis. The suspensions were then centrifuged (400 × *g*, 10 min) to remove cell fragments, and the supernatants were filtered through stacked 20-μm-pore-size nylon (Micron Separations, Inc., Westboro, Mass.) and 0.65-μm-pore-size nitro-

cellulose filters (Whatman Co., Clifton, N.J.). The filtrates were centrifuged at 10,000 × *g* for 15 min at 4°C, and the resulting bacterial pellets were washed once in HBSS, recentrifuged, and resuspended in HBSS.

Microbiologic procedures. Three Trypticase soy agar–5% sheep blood plates were inoculated with 250 μl of each bacterial suspension. All plates were incubated at 37°C. One plate was incubated at ambient atmosphere, one was incubated in an atmosphere consisting of approximately 6% O_2, 7% CO_2, 7% H_2, and 80% N_2 obtained as described previously (5), and the remaining plate was incubated anaerobically. The aerobic and microaerobic plates were examined daily for growth, while the anaerobic plate was examined every 3 days for growth. All plates were held for a minimum of 12 days prior to being discarded.

Cell culture isolation procedures. All cell lines were obtained from the American Type Culture Collection (Rockville, Md.). Culture media, heat-inactivated fetal calf serum, media supplements, and additives were obtained from Sigma.

Intestine 407 human embryonic intestinal cells (ATCC CCL 6) and GPC-16 guinea pig colonic adenocarcinoma cells (ATCC CCL 242) were grown in Dulbecco's modified Eagle's medium with 4,500 mg of glucose per liter, 110 mg of sodium pyruvate per liter, 584 mg of L-glutamine per liter, and 10% fetal calf serum (DME). HaK Syrian hamster kidney cells were grown in Eagle's minimum essential media with Hank's salts and 10% fetal calf serum. Vero African green monkey kidney cells were grown in medium 199 containing Hank's salts and 10% fetal calf serum. All cell lines were plated onto 75-cm^2 culture flasks (Corning Glass Works, Corning, N.Y.) in the respective media and incubated at 37°C in a 5% CO_2 atmosphere until approximately 50% confluent. Each cell line was then inoculated with 500 μl of each isolated bacterial suspension and incubated at 37°C in a 6% CO_2, 6% O_2, 88% N_2 atmosphere. Cell cultures were split 2:1 by scraping on days 4 and 9 of incubation and examined daily by phase microscopy (Olympus INT; Olympus Corp., Lake Success, N.Y.) for evidence of bacterial growth. All cell cultures were maintained for a minimum of 14 days before negatives were discarded.

Cell cultures with intracellular bacterial growth were continued in cell culture by scraping and splitting through four doublings. The cell culture supernatant was used to infect additional flasks of the same cell line to expand the culture. Infected cells were frozen at −80°C in the respective media with 10% dimethyl sulfoxide for reference and later use.

Inocula preparation. Four inocula were prepared for the infection studies. The inoculum for the control group (group A) was prepared from two 75-cm^2 flasks of confluent uninfected Intestine 407 cells. The cells were mechanically separated from the flask by scraping, concussion, and vortexing. Triton X-100 was then added to the cell media suspension to a concentration of 0.2% (vol/vol), and the suspension was incubated at 37°C with agitation for 20 min. The suspension was then centrifuged (400 × *g*, 20 min), and the supernatant was filtered through a 20-μm-pore-size nylon filter (Micron Separations, Inc.). The resulting filtrate was centrifuged at 11,000 × *g* for 20 min at 4°C, and the resulting pellet was resuspended in 10 ml of DME.

The infected inocula were prepared in a similar manner except that a total of six 75-cm^2 flasks were processed. Following the filtration through a 20-μm-pore-size nylon filter, the infected inocula were divided into three portions. The group B inoculum was processed identically to the group A control inoculum. The group C inoculum was filtered through a 0.65-μm-pore-size cellulose filter (Whatman) prior to final centrifugation, and the group D inoculum was similarly filtered through a 0.2-μm-pore-size cellulose filter (Whatman) prior to final centrifugation. All inocula were held at 4°C after preparation and were utilized within 3 h of preparation.

Animals. Four adult Syrian hamsters (Hsd:SYR) with 17-day-old litters were purchased from a commercial supplier whose colony had no previous history of proliferative ileitis. The weanling hamsters were separated from their dams, sexed, weighed, and ear tagged (National Band and Tag Co., Newport, Ky.) upon arrival at our facility and placed into group cages by gender. Postinoculation, hamsters were housed in conventional polycarbonate rodent cages and provided a commercial rodent diet (RMH 3000; Agway, Inc., Syracuse, N.Y.) and water ad libitum. Each hamster was weighed at 3- to 5-day intervals by placing the animal in a clean pretared container.

Animal inoculation. The weanling hamsters were divided into four groups with a conscious attempt to equalize the sex and weight distribution among the groups. Each hamster was given 7.5 mg of cimetidine (Tagamet; SK&F Lab Co., Cidra, Puerto Rico) intraperitoneally 30 min prior to inoculation to induce temporary achlorhydria. Following oral inoculation by gavage, each hamster was given 0.2 mg of dexamethasone (Azium; Schering Corp., Kenilworth, N.J.) intramuscularly.

Control group A hamsters (five male and three female) were inoculated with 1 ml of a cell-free filtrate from uninfected Intestine 407 cells. Group B hamsters (five male and three female) were inoculated with 1 ml of a cell-free filtrate from infected Intestine 407 cells. Groups C and D hamsters (four male and four female [each]) were inoculated with 0.65-μm- and 0.2-μm-pore-size filtrates from infected Intestine 407 cells.

Necropsy and tissue processing. Hamsters were killed by an intraperitoneal injection of 25 mg of sodium pentobarbital at day 28 postinoculation. A terminal blood sample of 2 ml was drawn from the right ventricle for serum titer determination by enzyme-linked immunosorbent assay (ELISA), and the ileum was aseptically removed. A small section of the ileum was fixed in McDowell-Trump fixative (13) for light and electron microscopy. The remainder of the ileum was either frozen (−80°C) for later use or further processed for organism reisolation. Sections for light microscopy were routinely processed, embedded in paraffin, cut at 4 μm, and stained with hematoxylin and eosin and with Warthin-Starry silver stains (12) for microscopic evaluation. Light microscopic sections were coded and evaluated without knowledge of experimental group assignment.

Reisolation procedures. Reisolation procedures were identical to those utilized in the initial isolation with the exception that only Intestine 407 cells were inoculated for organism reisolation. The ilea of three hamsters from group C (0.65-μm-pore-size filtrate inoculated) and two hamsters from group A (control cell inoculated) were independently processed for reisolation of the organism.

Transmission electron microscopy. Hamster ileal sections fixed in McDowell-Trump fixative were washed twice in 0.122 M Millonig's buffer, immersed in 1.33% osmium tetroxide in 0.122 M Millonig's buffer, dehydrated in graded ethanol solutions, cleared in propylene oxide, and embedded in Epon 812. Alkaline toluidine blue-stained thick sections were examined for selection of thin sections. Thin sections were mounted on 300-mesh copper grids, stained with lead

citrate-uranyl acetate, and examined with a Philips 300 electron microscope.

Infected Intestine 407 cells (passage 13) for electron microscopy were removed from the culture flask by scraping, pelleted by centrifugation (400 × *g*, 10 min), and fixed in 2.5% glutaraldehyde in Millonig's buffer. The cells were then processed as described above except for the necessity of pelleting the cells by centrifugation between steps.

ELISA technique. The terminal serum samples, day 28 postinfection, were evaluated for serum titer to the organism by ELISA. A crude whole-cell preparation was used as the antigen in the ELISA procedures. Supernatant from infected Intestine 407 cell cultures (passages 25 to 32) was centrifuged at 400 × *g* for 10 min to remove large particulates and cells. The supernatant was then centrifuged at 12,000 × *g* for 15 min at 4°C to pellet the bacteria. The pellet was washed once with phosphate-buffered saline (PBS; 0.01 M., pH 7.4, with 0.02% NaN_3), recentrifuged, resuspended in PBS to a density corresponding to a McFarland standard of 2, and refrigerated until used. *C. jejuni* (ATCC 29428), isolated from brucella broth, was similarly processed as a control antigen.

Ninety-six-well assay plates (E.I.A. Microtiter Plates; Flow Laboratories, McLean, Va.) were coated with a 1:64 dilution of both the isolated bacterial antigen and *C. jejuni* at 100 μl per well and dried overnight at 37°C. Wells were incubated with 100 μl of absolute methanol containing 0.3% H_2O_2 for 30 min at 37°C to inactivate endogenous peroxidase activity. The plates were then washed twice in ELISA wash buffer (0.15 M NaCl, 0.1 M Tris-HCl, 0.02% Tween 20 [pH 7.4]) and incubated for 1 h at room temperature with 200 μl of PBS containing 0.20% (vol/vol) Tween 20 and 2.5% (wt/vol) nonfat powdered milk per well. Plates were washed twice with ELISA wash, and 100 μl of test sera diluted from 1:100 to 1:12,800 in PBS was applied for 1 h at room temperature. After being washed three times, plates were incubated for 1 h at room temperature with 100 μl of peroxidase-labeled goat anti-hamster immunoglobulin G (IgG) diluted 1:2,500 in ELISA wash per well. Following an additional three washes, bound antibody was detected by the addition of 100 μl of 2,2'-azinobis(3-ethylbenzthiazoline sulfonic acid) substrate (Sigma) for 15 min, and the A_{405} was determined with an automated ELISA reader (Titertek Multiscan; Flow Laboratories). All assays were performed in duplicate and were repeated if values differed by greater than 10%. Absorbance values greater than 0.2 absorbance units above that of the control (*C. jejuni*) were considered positive. Pooled sera from adult female hamsters from a closed colony without a history of proliferative ileitis were included as a negative control with all tests.

Polyclonal antibody production. The bacterial isolate was purified from Intestine 407 cell culture (passage 15 and 16) for antibody production. Culture supernatant was centrifuged at 400 × *g* for 10 min to remove cell debris, and the resulting supernatant was filtered through a 0.65-μm-pore-size cellulose acetate filter (Whatman). The filtrate was then centrifuged (12,000 × *g* for 15 min at 4°C), and the pelleted organisms were washed once in PBS, recentrifuged, and resuspended in 10 ml of PBS containing 4% formalin. Following overnight incubation at 4°C, the organisms were again isolated by centrifugation, washed once in Dulbecco's PBS, recentrifuged, and resuspended in Dulbecco's PBS to a concentration approximating a McFarland standard of 0.5. The organism suspension was then emulsified with an equal volume of Freund's complete adjuvant (Sigma Chemical Co.).

Two male rabbits were utilized for antibody production. Each rabbit was injected with a total of 0.5 ml of the adjuvant-organism mixture divided into 10 sites intradermally on the back. A booster injection of 1.0 ml of the organism in Dulbecco's PBS was given subcutaneously at 40 days postinjection, and sera were collected 7 days later.

The IgG fraction of the rabbit antisera was purified by precipitation with ammonium sulfate (33% final concentration) followed by ion-exchange chromatography on DEAE cellulose (Whatman DE-52) equilibrated and eluted with 0.0175 M phosphate buffer, pH 6.3. The resulting IgG fractions were then reprecipitated with ammonium sulfate, isolated by centrifugation, and desalted on a Sephadex G25 column (Pharmacia LKB Biotechnology, Inc.) equilibrated and eluted with PBS.

Immunohistochemistry. Fixed ileal sections embedded in paraffin and cut to 4 μm were utilized for immunohistochemistry. Sections were routinely deparaffinized in xylene and rehydrated through decreasingly concentrated ethanol solutions. The sections were then incubated for 1 h in 0.03% H_2O_2 in absolute methanol to remove endogenous peroxidase activity. Following washing in PBS, the sections were incubated for 1 h in a blocking solution of 0.2% (vol/vol) Tween 20 and 2.5% (wt/vol) nonfat dry milk in PBS. Following an additional wash, the rabbit anti-organism antibody was applied at a dilution of 1:100 in PBS, and the slides were incubated overnight at 4°C in a humidified chamber. The slides were then washed four times in ELISA wash and incubated for 3 h with affinity-purified peroxidase-labeled goat anti-rabbit IgG (Jackson Immunoresearch) diluted 1:500 in ELISA wash. The sections were then washed four times in ELISA wash, and bound antibody was detected with a commercial peroxidase detection system for immunohistochemistry (HistoMark Black; Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.) by following the manufacturer's directions. Controls to ensure specificity included both infected and uninfected Intestine 407 cells, preimmunization rabbit sera, and organism-adsorbed immune rabbit antibody.

RESULTS

Isolation and propagation of the organism. Inoculation of cell cultures with two of the bacterial suspensions resulted in heavy bacterial contamination and discarding of the cell cultures after 18 h of incubation. Cell cultures inoculated with the remaining bacterial suspension appeared to be free of bacterial contamination, incubation was continued, and the flasks were split (2:1) on day 4. Intracellular organisms were first observed in both the Intestine 407 and GPC-16 cell lines by phase microscopy on day 8 following inoculation. Clusters of minute organisms within the cells were readily identified by their rapid motility. The clusters of organisms were extremely variable in size, from barely discernible within the cell cytoplasm at a ×400 magnification to large clusters completely filling the distended cells (Fig. 1). Occasional cells with small clusters of organisms were observed in mitosis with the cell cluster apparently transferring to one of the daughter cells. No evidence of intracellular bacteria was observed in either the HaK or Vero cell lines.

The organism continued to multiply in the original cell cultures (with additional 2:1 splittings on days 14, 18, and 20) through day 19 postinoculation (Intestine 407) and day 21 postinoculation (GPC-16), when the cultures were contaminated. The organism was readily and continually (more than 40 passages) propagated by infecting confluent Intestine 407 cell cultures with the supernatant from infected cultures (1



FIG. 1. Intestine 407 cells 48 h after infection with the intracellular organism. Approximately 90% of the cells are infected, and the monolayer has begun to degenerate. Numerous motile organisms are present within vacuoles in the cells (arrows). Phase-contrast microscopy. Bar = 10 μm.

ml of infected supernatant per 25-cm^2 surface area). During the first five passages in cell culture, intracellular organisms could be detected by phase microscopy within 72 h of infection, with the interval between infection and microscopic detection decreasing in later passages. Cell culture monolayer viability decreased from 10 days (passage 1) to 6 days by passage 5. Following the initial five passages in cell culture, organisms were detected by phase microscopy at 24 h post-cell culture infection, and monolayer destruction was evident in 4 to 6 days following infection. No changes in bacterial morphology were evident following up to 40 passages in cell culture. Growth of the organism appeared to be substantially greater on the Intestine 407 cell line, and passage 12 of this line was utilized for inoculum preparation.

Necropsy results. The gastrointestinal tract of all control-inoculated (group A) and 0.2-μm-pore-size filtrate-inoculated (group D) hamsters appeared grossly normal at necropsy (Fig. 2). No evidence of epithelial cell proliferation or intracellular bacteria was present on light microscopic examination of hematoxylin- and eosin-stained or Warthin-Starry-stained ileal sections from hamsters in either of these groups.

In four (two male and two female) of the eight group B hamsters (cell-free infected inoculate) and four (three male and one female) of the eight group C hamsters (0.65-μm-pore-size filtrate), the ileum was grossly thickened (Fig. 3). Ileal wall thicknesses varied from 2 to 5 mm in these cases. Ileal wall necrosis and abscessation with secondary fibrinopurulent peritonitis was present in three of these hamsters (one from group B and two from group C). Light microscopic examination of ileal sections from hamsters with grossly evident ileal thickening revealed immature and disorganized villus epithelial cells with mitotic figures extending from the crypt region up the entire length of the villus (Fig. 4). Numerous small curved bacteria were visible in the apical cytoplasm of the epithelial cells in silver-stained ileal sections of all hamsters with grossly evident ileal thickening (Fig. 5). A mixed inflammatory cell infiltrate of neutrophils and macrophages was present in the lamina propria of all hamsters with gross lesions. Mild focal epithelial cell proliferation and intracellular bacteria were present in an addi-



FIG. 2. Gastrointestinal tract from hamster inoculated with the 0.2-μm-pore-size filtrate (group D). No thickening is present. A 1-cm scale is shown.

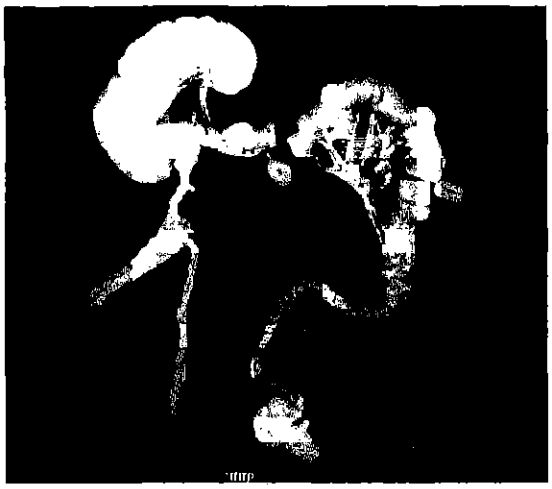


FIG. 3. Gastrointestinal tract from group C hamster inoculated with a 0.65-μm-pore-size filtrate. Ileum and terminal jejunum are severely thickened with lumenal constriction. A 1-cm scale is shown.

tional two (one from group B and one from group C) of the eight hamsters that appeared normal at gross necropsy.

Reisolation results. Three of the five bacterial suspensions inoculated into Intestine 407 cell cultures resulted in heavy bacterial contamination at 18 h of incubation, and these cultures were discarded. The remaining two cell cultures (one group A and one group C) remained free of bacterial contamination through day 7 of incubation when intracellular organisms were detected by phase microscopy in the group C (0.65-μm-pore-size filtrate) inoculated cell culture. Intracellular bacteria were not detected in the group A (control) inoculated cell culture through 14 days of incubation.

Microbiologic results. Of the eight bacterial suspensions, three from the original isolation and five from reisolation, only those resulting in contamination of the cell cultures produced any growth on Trypticase soy agar–5% sheep blood plates. *Pseudomonas aeruginosa*, one to eight colonies per 250 μl, was isolated from all five contaminated suspensions. No other isolates were detected by any of the incubation conditions.

Transmission electron microscopy results. Numerous intracellular bacteria were observed within the cytoplasm of ileal epithelial cells from hamsters inoculated with the 0.65-μm-pore-size filtrate (group C). The bacteria were free within the epithelial cell cytoplasm (Fig. 6). Macrophages with intracellular bacteria both free in the cytoplasm and in phagosomes were present adjacent to epithelial cells. The bacteria were typically slightly curved on longitudinal sections and measured 1.4 to 1.6 μm in length by 0.25 to 0.38 μm in width. The bacterial cell wall appeared trilaminar and irregular. No flagella were observed.

Examination of infected Intestine 407 cells revealed the presence of bacteria morphologically identical to those seen in the ileal cells of infected hamsters (Fig. 7). The bacteria in the Intestine 407 cells measured 1.4 to 1.7 μm in length by 0.26 to 0.34 μm with a similar trilaminar irregular cell wall. A majority of the bacteria in the Intestine 407 cells were free within the cell cytoplasm, although some were membrane bound within vacuoles.

ELISA results. Serum titers of hamsters from group A (control inoculated) ranged from <1:100 to 1:1,600, with a median titer of 1:400. The serum titers from the group D (0.2-

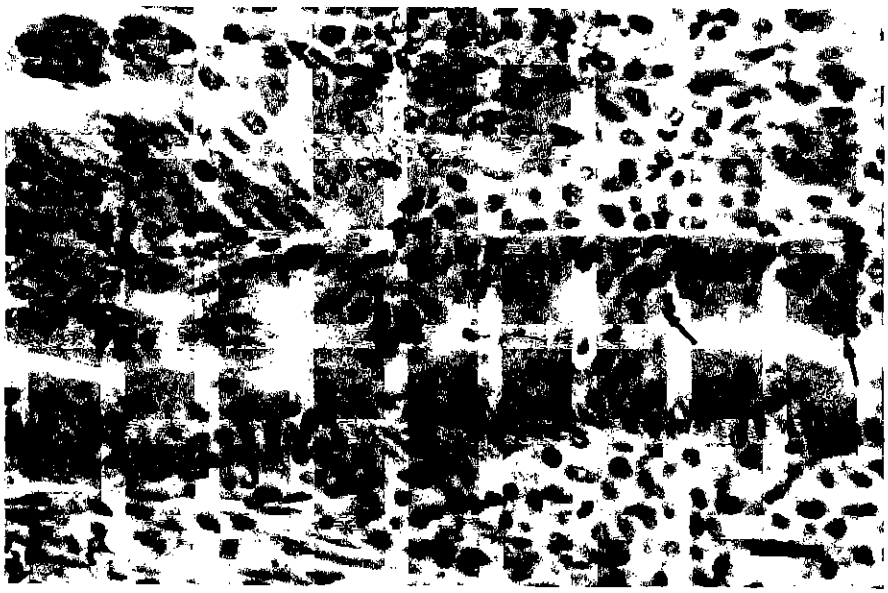


FIG. 4. Ileal section from hamster infected with a 0.65-μm-pore-size filtered inoculate. Immature epithelial cells with numerous mitotic figures (arrows) are visible extending the length of the villus. Hematoxylin and eosin stain. Bar = 20 μm.



FIG. 5. Ileal section from hamster infected with a 0.65-μm-pore-size filtered inoculate. Numerous slightly curved bacteria are present in the apical cytoplasm of the epithelial cells and extend into the lumen (arrows). Warthin-Starry silver stain. Bar = 10 μm.

μm-pore-size filtrate inoculated) hamsters ranged from <1:100 to 1:800, with a median titer of 1:400. Serum titers of hamsters from group C (0.65-μm-pore-size filtrate inoculated) ranged from 1:1,600 to >1:12,800, with a median titer of 1:6,400, while the titers of the group B hamsters ranged from 1:1,600 to >1:12,800, with a median of 1:3,200. The serum titers of the group B and group C hamsters were significantly ($P < 0.01$, Mann-Whitney test) higher than the

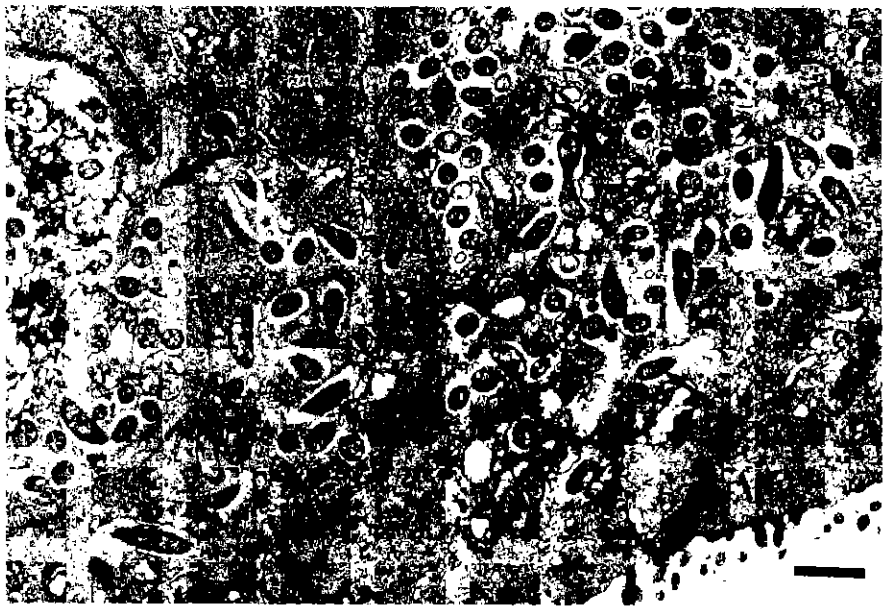


FIG. 6. Transmission electron micrograph of the ileal epithelium of a hamster inoculated with a 0.65-μm-pore-size filtrate (group C). Numerous slightly curved bacteria are present within the cytoplasm of the epithelial cells (small arrows). A macrophage with both free intracytoplasmic bacteria and bacteria within a phagosome is also present (large arrow). Bar = 1 μm.



FIG. 7. An electron micrograph of infected Intestine 407 cells. Numerous slightly curved bacteria are free within the cell cytoplasm (small arrows). A membrane-bound vacuole filled with bacteria is also present (large arrow). Bar = 1 μm.

titers of either group A or group D hamsters (Fig. 8). The highest titers, ranging from 1:6,400 to >1:12,800, were recorded from hamsters with grossly evident ileal thickening at necropsy. The pooled negative control sera consistently titered at <1:100.

Immunohistochemistry results. Organisms labeled with the polyclonal rabbit anti-organism antibody were present in the ileal sections from all 10 hamsters with histologic evidence of proliferative ileitis. Organisms were primarily confined to the apical portions of ileal epithelial cells, with few organisms being present in the lamina propria or within the lumen (Fig. 9). The distribution of organisms corresponded to the distribution of silver-stained organisms seen on the Warthin-Starry-stained sections from the same hamster. No stained organisms were present within the ileal epithelial cells from any of the eight group A or eight group D hamsters (Fig. 10).

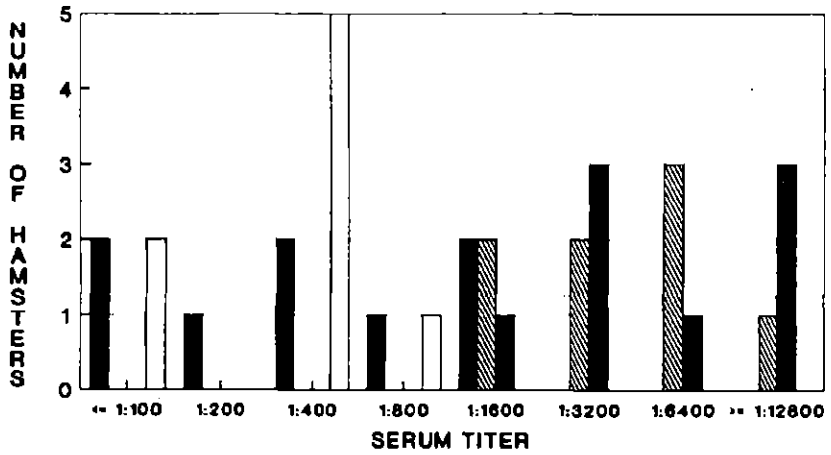


FIG. 8. The distribution of serum titers to the intracellular organism for the four experimental groups is shown. Serum titers for group A (■; control filtrate) and group D (⊔; 0.2-μm-pore-size filtrate) range from ≤1:100 to 1:1,600, while titers from group B (▨; infected filtrate) and group C (▩; 0.65-μm-pore-size filtrate) range from 1:1,600 to ≥1:12,800.



FIG. 9. An immunoperoxidase-stained ileal section from a group C (0.65-μm-pore-size filtrate inoculated) hamster. Intracellular bacteria specifically labeled with the rabbit anti-organism antibody are primarily confined to the apical portions of the epithelial cells (arrows), corresponding to the location of silver-stained bacteria on Warthin-Starry-stained sections (from the hamster in Fig. 5). Bar = 10 μm.

Organisms specifically labeled with the polyclonal rabbit anti-organism antibody were also present in the infected Intestine 407 cells (Fig. 11). No reactivity was present with uninfected Intestine 407 cells, and reactivity was completely eliminated by adsorption of the rabbit antisera with formalin-fixed organisms.

DISCUSSION

The organism isolated in this study appears to fulfill the criteria to be considered the etiologic agent of hamster proliferative ileitis. The organism was isolated from hamsters with proliferative ileitis and was propagated by utilizing cell culture methodology. The typical lesions of proliferative ileitis were successfully reproduced in hamsters inoculated with the infected cell-free filtrate and the 0.65-μm-pore-size filtrate, while littermates given the uninfected control cell-free filtrate and 0.2-μm-pore-size filtrate remained free of disease. The organism was then reisolated from one of the 0.65-μm-pore-size filtrate-inoculated hamsters which developed proliferative ileitis. The organism was not recovered from hamsters inoculated with uninfected control cell filtrate or the 0.2-μm-pore-size filtrate.

The possible involvement of a virus or other organism coisolated with the bacteria as the etiologic agent is unlikely. The 0.2-μm-pore-size filtrate did not reproduce the disease, suggesting that the causative organism is larger than 0.2 μm and is probably not a virus. Extensive transmission electron microscopic examinations have also failed to detect any viral particles or other bacteria within the infected Intestine 407 cells. The nearly identical ultrastructural morphology of the bacterium within the infected Intestine 407 cells and infected hamster ileal epithelium supports the role of the bacterium in disease pathogenesis.

The significantly elevated serum titers in hamsters infected with the organism, compared with those of hamsters given the uninfected cell-free filtrate or the 0.2-μm-pore-size filtrate and the association of the highest titers with gross lesions of proliferative ileitis support the involvement of this organism in disease pathogenesis. The immunohistochemistry studies using a specific polyclonal antibody confirm that the curved organisms seen in the epithelial cells of the hamsters with experimentally induced proliferative ileitis are the same that were present in the cell culture, further supporting the role of this organism in the disease pathogenesis. The lack of cross-reactivity (data not shown) of the rabbit antibody with members of the genus *Campylobacter* (*C. jejuni*, *C. hyointestinalis*, and *C. coli*) suggests that this organism is antigenically distinct and probably not a member of the genus *Campylobacter*.

As reported by other investigators (9, 14, 16, 20), flagella were not observed in any of the light or electron microscopic preparations. The organism exhibited a dramatic motility within the cytoplasm of infected Intestine 407 cells examined by phase microscopy, indicating the presence of some mechanism for motility. Further studies are necessary to more fully define the overall bacterial morphology and to elucidate the mechanism of motility.

The inability, to date, to propagate this organism on artificial media presents difficulties. The isolation procedures utilized in recovering this organism are lengthy and labor intensive and result in low levels of success because of contamination with other bacterial organisms present in the intestinal lumen. Improved isolation and propagation proce-

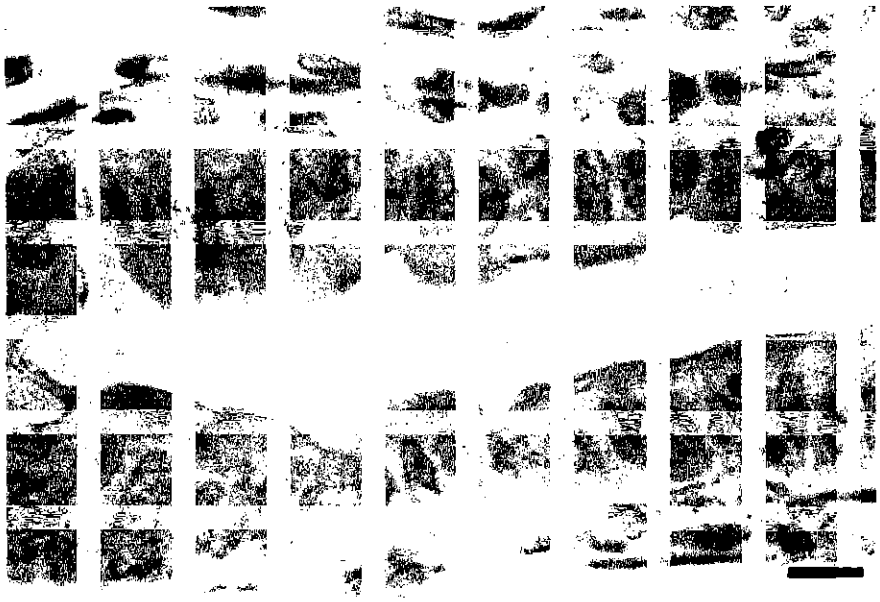


FIG. 10. An immunoperoxidase-stained ileal section from a group D (0.2-μm-pore-size filtrate inoculated) hamster. Section was stained identically to section shown in Fig. 9. No staining of intracellular bacteria is present. Bar = 10 μm.

dures are necessary to fully evaluate the role of this organism in the pathogenesis of proliferative ileitis.

Standard biochemical tests for characterization and identification are presently unfeasible, necessitating the use of other methods for classification. Genomic DNA homology and 16S rRNA analyses are needed for further classification of this organism. Information gained from these studies may also lead to successful artificial medium isolation strategies

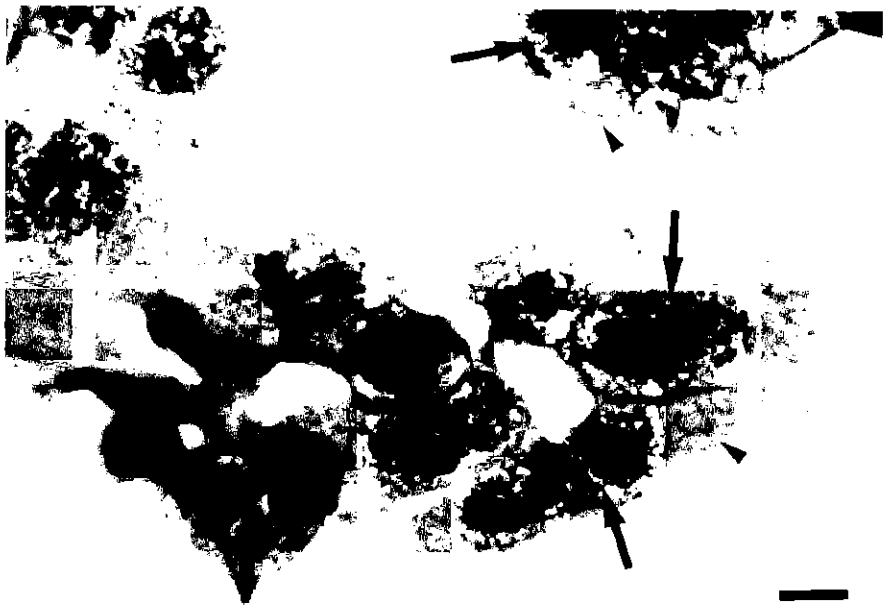


FIG. 11. Infected Intestine 407 cells stained with the rabbit anti-organism antibody and the immunoperoxidase technique. Clusters of stained intracellular bacteria are present within the cell cytoplasm (arrows). The cell nuclei (arrowheads) are displaced to the outer margin of the cells. Bar = 10 μm.

which would dramatically reduce the difficulty in studying the role of this organism in the proliferative intestinal diseases of many species.

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EXHIBIT B

THIS EXHIBIT HAS BEEN
REDACTED IN ITS ENTIRETY