bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Is Vaccination a Viable Method to Control Johne's Disease Caused by *Mycobacterium avium* subsp. *paratuberculosis*? Data from 12 Million Ovine Vaccinations and 7.6 Million Carcass Examinations in New South Wales, Australia from 1999-2009

Ian J. Links[1*¶], Laurence J. Denholm[2¶], Marilyn Evers[3¶], Lloyd J. Kingham[4], Robert J. Greenstein[5]

[1]Graham Centre for Agricultural Innovation (An alliance of Charles Sturt University and NSW Department of Primary Industries), Wagga Wagga, New South Wales, Australia

[2]NSW Department of Industry, Skills and Regional Development, Orange New South Wales, Australia

[3]Formerly NSW Department of Primary Industries, Orange, New South Wales, Australia

[4]NSW Department of Primary Industries, Orange, New South Wales, Australia

[5]James J. Peters VAMC, Bronx, New York, United States of America

*Corresponding Author

Email: ijlinks48@gmail.com (IJL)

¶ These authors contributed equally to this work.

Keywords: *

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Mycobacterium avium subsp. *paratuberculosis*; Vaccination; Emerging pathogens; Johne's disease; Ovine Johne's disease; OJD; Paratuberculosis; Sheep diseases; Australia; Abattoir surveillance; Granulomatous enteritis; Potential Zoonosis; Crohn's disease; Herd immunity; Epidemic disease control; Pandemic disease control.

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Abstract

Background

Mycobacterium avium subsp. *paratuberculosis* (MAP) causes Johne's disease (or paratuberculosis), a chronic wasting disease of ruminants and other animals resulting from granulomatous enteritis. There are increasing concerns that MAP is zoonotic. The prevalence of Johne's disease is increasing worldwide. In an attempt to control an epidemic of ovine Johne's disease (OJD) in New South Wales (NSW), a government/industry sponsored voluntary vaccination/on-farm management program commenced in 2000. We report herein an observational study of changes in disease prevalence as vaccination progressed, based on abattoir surveillance data for OJD from 1999 to 2009. We also discuss the epidemiological, policy, regulatory, research, economic and sociological elements that contributed to the development of a mature control program, whose aim was to halt the epidemic spread of OJD in a naïve sheep population.

Methods

NSW was divided into areas of "High" (HPA), "Medium" (MPA) and "Low" (LPA) OJD prevalence. A killed whole cell vaccine (Gudair®) was administered to sheep from 2000 to 2009. Trained examiners evaluated the viscera of adult sheep carcasses at slaughter for gross evidence of OJD. MAP infection was confirmed by histopathology.

Principal findings

From 2000-2009, 12 million vaccine doses were administered in NSW (91%; 10.9 million in the HPA). Many of the vaccinated flocks were suffering > 5% annual mortality in adult sheep, with some individual flocks with 10-15% losses attributable to OJD. A total of 7.6 million carcasses were examined (38%; 2.9 million from the HPA). Overall, 16% of slaughter consignments (sheep consigned to the abattoir from a single vendor) were positive for OJD, of which 94% were from the HPA. In the HPA,

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

the percentage of animals with lesions attributable to OJD at slaughter fell progressively from 2.4% (10,406/432,860) at commencement of vaccination in 2000 to 0.8% (1,573/189,564) by 2009. Herd immunity from vaccination in the HPA was estimated at 70% by 2009. This coincided with a progressive decrease in reports of clinical disease and mortalities in vaccinated flocks.

Significance

We show a decrease in the prevalence of lesions attributable to OJD in NSW concomitant with initiation of voluntary vaccination, on-farm management plans, abattoir monitoring and feedback of animal prevalence data to sheep producers. We conclude that a target of ≤ 1% regional prevalence of OJD affected sheep at slaughter is achievable using these interventions.

Introduction (See also S1 Text)

Johne's disease [1] is a chronic, incurable, fatal wasting disease of animals, particularly ruminant animals, caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Granulomatous enteritis, usually characterized by the presence of acid-fast staining MAP, is a consistent feature of the disease. Johne's disease occurs on all continents and infects domesticated [2-4] and wild animals [3-7]. Sheep with OJD in Australia are infected by the S or "sheep" strain of MAP [8] which is slow growing and fastidious in culture requirements on artificial media compared with the "cattle" or C strain isolated from bovine Johne's disease (BJD) [9]. While the C strain was cultured in Britain as early as 1912 [10], in contrast, reliable isolation of the S strain was not reported until the late-1990's, with cultures requiring incubation for up to 16 weeks [11]. Bovine Johne's disease is characterized by intractable diarrhea, leading to wasting and death. It is a major concern for the dairy industry worldwide, with disease control programs in many countries. In the USA for example, in 1997 when dairy herd MAP infection herd-level prevalence was 22% [12], the estimated cost of BJD was in excess of US$200 million /yr. By 2007 the USA herd-level prevalence had increased to 68% of dairy herds (95% of herds with >500 cattle) [2], with a probable commensurate

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

increase in cost. Additionally, there is increasing concern among some scientists and medical practitioners that MAP may be zoonotic [4, 13, 14] and a potential factor in the pathogenesis of Crohn's disease in man [3, 4, 14-23].

While diarrhea is not a feature of OJD, the slowly progressive granulomatous enteritis leads to malabsorption of nutrients and chronic weight loss. before the animal succumbs 2-6 months later. In some flocks, where lambs are exposed to heavy contamination, deaths may be evident as early as 2 years of age. More often sheep succumb at 3-5 years of age [24]. Spread of infection is by the feco-oral route, with late-stage animals excreting as many as 10^8 organisms/gm of feces [25].

Control of Johne's disease in infected herds or flocks is difficult [26], although culling of heavily infected animals or physical separation of animals with less severe infection may be partially effective [27]. In addition, vaccination against MAP may ameliorate disease [28-35] and be cost effective in combating Johne's disease [36, 37]. By delaying the onset and reducing the level of fecal shedding of MAP by infected sheep [32], vaccination may reduce the spread of infection within and between flocks [38].

The region of greatest concern for OJD at the initiation of the study in 1999 was South Eastern Australia (see map, Fig 1), where OJD detections had been increasing exponentially in the state of NSW in the two decades since first detected in 1980 [39]. In 1998, for example, 204 flocks were diagnosed with OJD, when the cumulative total of infected flocks was 441 [40-42].

Fig 1. Map of Australia showing regions of maximal OJD concern – Prevalence Areas implemented 31 March 2008. (Reprinted under a CC BY license, with permission from NSW DPI, original copyright 2008)

In the other Australian states, disease had not been detected in Victoria until 1989 in a single ewe introduced from Tasmania and was not confirmed in home-bred sheep until 1995 [43]. Infection was confirmed on mainland South Australia in 1997 while the first detection in Western Australia occurred in 2000 [41, 44].

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

A regulatory control program, based on restricting the movement of infected sheep, had commenced in NSW in 1996 [45] and this program was later expanded to a national program in 1998 in response to concern about disease spread to other states [42].

Detection initially relied on costly on-farm sampling, using serology with confirmatory histopathology on reactors or clinically affected animals. Serology was complemented with, then progressively superseded by, the more sensitive and cost effective Pooled Fecal Culture (PFC) in NSW from November 1999 [46]. The introduction of the PFC also overcame the potential liability of vaccination interfering with serological testing.

Beginning with the National Control and Evaluation Program in 1998 [47, 48], there has been a concerted attempt to evaluate the cost of OJD in Australia [49]. The sheep industry is very important to Australia, contributing as much as AU$4.3 billion in terms of direct Gross Value of Production. In 2008-09, the total number of sheep in Australia was estimated at 72 million, the gross value of sheep and lamb slaughtered was AU$2.5 billion and the gross value of wool was AU$1.8 billion. The most important states for sheep and wool are NSW, Victoria and Western Australia with NSW contributing approximately 21% of the gross national value of sheep meat and 34% of the value of wool [49].

An understanding of the complex factors in disease spread evolved over time. Straying sheep, use of common facilities including roads or yards, or local contamination of water run-off frequently led to progressive, but usually slow, spread to neighboring flocks within sub-catchments [50, 51]. In contrast, spread to new regions was due to the unwitting sale or movement of infected sheep, usually sub-clinically affected [40, 50]. There was no evidence of significant spread along major rivers ([40], by native macropods (kangaroos and wallabies) [6, 52] or rabbits [52].

Abbott & Whittington (2003) discuss in detail factors contributing to the spread of OJD and the differing environmental factors that influence potential for spread over an extended timeframe (30 years) in the HPA, MPA and LPA [53].

Vaccination was first suggested as a likely cost effective strategy for controlling OJD

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

in NSW in 1999 [54]. Subsequently, a killed whole-cell vaccine for OJD ("Gudair" ® **- CZ Veterinaria, Porriño, Pontevedra, Spain**) was approved for use under a special government permit but restricted to heavily infected flocks in NSW. Vaccination by sheep owners commenced on a voluntary basis in January 2000, in conjunction with on-farm Property Disease Management Plans (PDMPs) [48, 55]. The vaccine was subsequently registered for use in all states in April 2002. This followed release of preliminary results in controlled trials confirming the efficacy of vaccination in three heavily infected merino flocks in NSW [32]. Vaccination reduced both mortalities and the number of animals shedding MAP in their feces by 90%, as well as reducing the overall level of bacterial excretion by more than 90%.

Wide-scale monitoring for OJD of adult sheep consigned to NSW abattoirs commenced in late 1999 (this study, [51, 55-58]). The introduction of abattoir monitoring complemented existing on-farm testing. It helped determine the distribution and prevalence of this rapidly spreading disease and enabled more precise setting of prevalence area boundaries. Initially, only confirmed positive consignments were reported to the individual sheep producer [55]. However, the progressive introduction of the National Livestock Identification System (NLIS) [51], with markedly improved traceability to property-of-origin, enabled the results for both OJD positive and negative consignments to be reported to producers from January 2003.

This observational report documents the progressive changes in OJD prevalence following the sale of 12,021,963 vaccine doses and 7,635,513 carcass examinations in NSW, Australia from 1999 to 2009. During this period the sheep population of NSW decreased by 50% from approximately 35.4 million in 2000 to 17.7 million in 2009 [59-61].

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Methods

All activities and investigations were conducted in accordance with the NSW Agriculture OJD Policy Manuals issued in August 1999 and November 2001 [62, 63] and the National Johne's Disease Standard Definitions and Rules for Sheep [64-67]. Where appropriate we have defined the study parameters in accordance with established consensus-based reporting standards for diagnostic test accuracy studies for paratuberculosis in ruminants [68].

Animal ethics approval

Animal ethics approval was not required as this case study did not involve any intervention with live animals. The research procedures described in the paper did not commence until after the animals had been killed in the normal course of the meat processing and livestock slaughter system, which is subject to separate national and NSW statutory animal welfare standards.

Prevalence areas

OJD Prevalence Areas or Zones across Australia were initially established on 1st July 1999 using the results of on-farm surveillance, following tracing of sheep movements and investigation of clinical disease [55]. With the introduction of abattoir surveillance in November 1999, monitoring became more comprehensive with the boundaries of the prevalence areas progressively adjusted as OJD continued to spread and prevalence changed over time [51, 56]. For the purposes of standardization, we use the "High" (HPA), "Medium" (MPA) and "Low" (LPA) prevalence areas as implemented nationally on March 31, 2008 (Fig 1) [69]. Hence, irrespective of the date of monitoring or vaccination, the analysis of individual property data was based on the prevalence area status in March 2008.

Property identification and tracing

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

The NLIS for sheep is based on a unique Property Identification Code (PIC) that identifies the state, local government region (RLPB – Rural Lands Protection Board) and property of origin of sheep consigned for slaughter. The NLIS was progressively implemented from 2002 to 2009. By July 2007, all sheep were required to be permanently ear-tagged with the PIC identifier for the property of birth. Sheep sent for slaughter were required to be accompanied by a National Vendor Declaration which recorded details of the owner and the property from which they were consigned (including the PIC from January 2006) [56]. Introduction of the NLIS greatly facilitated tracing, recording of vaccine sales, identification of consignments at slaughter, allocation to a prevalence area and reporting of abattoir monitoring results to producers. When the PIC was unknown, monitored consignments were allocated to a prevalence area based on Locality of Origin reported by the abattoir.

Vaccine

The Spanish whole-cell oil adjuvant vaccine for OJD ("Gudair" ®), containing 2.5mg/ml of killed bovine MAP strain 316F as the sole active ingredient, is the only OJD vaccine approved for use in Australia. A single dose is considered to provide protection for the life of the animal (normally 5-6 years). Data on the number of doses of Gudair® vaccine used were obtained from two complementary sources: records of the number of vaccine doses used on each sheep property maintained for NSW Department of Primary Industries (DPI) by individual RLPBs, and vaccine sales data provided by the vaccine distributor (initially - CSL Limited, Parkville, Melbourne, subsequently Pfizer Animal Health, Sydney, Australia, now Zoetis Australia).

Vaccine sales data were consolidated into a Microsoft Access database for analysis.

Vaccine regulation (See also S2 Text)

Vaccine was initially approved in January 2000 for use on up to 50 individual properties in the NSW HPA with confirmed unacceptable losses attributable to OJD, i.e. ≥5% annual mortality. This was extended in July 2001 to 150 properties. It was approved for

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

wider use nationally in April 2002, although still subject to restrictions. Unrestricted access to vaccine for any flock in NSW was approved in July 2003 [32, 51, 55, 63, 70].

Vaccination procedures (See also S3 Text)

Vaccine was sold in 100ml or 250ml packs with each sheep receiving a 1ml dose subcutaneously in the upper neck behind the ear using an automatic syringe.

Standard Operating Procedures were developed to minimize the risk of operator self-inoculation and a national register of accidental self-inoculation was maintained by Pfizer Animal Health. To optimize the advantage of vaccination before significant exposure to MAP, it was recommended as a single dose in lambs from 4-16 weeks of age in NSW. Vaccination of older sheep was initially only permitted with approval of NSW DPI. Some producers vaccinated older lambs, hoggets (12-24 months-old) and/or 2- year-old ewes, while whole of flock vaccination was undertaken in a number of infected flocks.

Property disease management plans (PDMPs), financial assistance and producer workshops (See also S4 Text)

The concept of formal approved PDMPs was promoted through a series of workshops, commencing in February 2002, with vaccination as a key control measure complemented by a range of other management strategies. A further series of workshops was held within the HPA and MPA between July and August 2003 [66, 71].

In order to implement PDMPs, financial assistance was made available to those producers whose flocks were confirmed infected with OJD prior to November 2002. Most of this funding was utilized for purchase of vaccine in 2002 and 2003.

Abattoir monitoring

Monitoring was conducted from November 1999 to December 2009 in 18 abattoirs in

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

NSW (11), Victoria (4) and South Australia (3) which slaughtered sheep from NSW. Results were analyzed based on the calendar year of monitoring. To ensure comprehensive regional coverage in areas with differing seasonal sheep turnoff patterns, monitoring in the four major export abattoirs in NSW was maintained where possible throughout the year. These abattoirs process approximately 70% of all adult sheep slaughtered in NSW annually, with up to 10 sheep slaughtered per minute. Seasonal closure of abattoirs or other constraints meant that abattoirs were not able to be monitored continuously during the study period. The remaining 14 domestic and interstate abattoirs were monitored on a rotational basis depending on throughput of eligible age sheep.

***Direct* Consignments** of sheep ≥ 2-years-old, which were sent for slaughter from flocks in NSW as part of routine animal production turn-off, were screened for the presence of OJD using methods previously reported in detail [72-74]. Inspectors aimed to monitor 90% of sheep in all eligible direct consignments on all slaughter shifts monitored. Definition: *Direct Consignment* - derived from a single vendor/PIC/property either consigned direct to the abattoir or through a saleyard without mixing with other consignments.

Inspection, sampling & data collection (See also S5 Text)

In brief, all inspections were undertaken by qualified meat inspectors approved to monitor for OJD after completing a national training workshop. These were conducted in an abattoir that slaughtered consignments from heavily infected properties. The monitoring procedures were detailed in a comprehensive training manual [67, 75-77] . Inspectors examined visually, and by palpation, the terminal ileum and adjacent lymph nodes for changes, including thickening of the intestinal wall, enlarged lymph nodes, lymphatic cording or mesenteric edema (Figs 2-4). The inspectors performed this task at a rate of up to 10 animals/minute. The animal-level sensitivity and specificity were estimated at 70% and 97% respectively following a research trial involving 5 consignments (1131 sheep) from known high prevalence infected properties [74].

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Fig 2. Ileo-cecal area of an OJD affected sheep showing histopathology sampling sites. A: ileo-cecal valve, B: thickened terminal ileum and C: enlarged ileo-cecal lymph nodes.

Fig 3. Intestinal tract of an OJD affected sheep. Note the thickened ileum, enlarged lymph nodes, prominent lymphatic vessels and edema of the mesentery, and the raised ridges (rugae) on the mucosal surface of the opened terminal ileum.

Fig 4. Intestinal mucosa of an OJD affected sheep. Note the prominent mucosal rugae in the severely affected section of the terminal ileum (left), the segmental nature of the ileal lesion (center) and the relatively normal mucosal lining of the jejunum (right).

Animals with suspect lesions were sampled (up to a maximum of three animals from each consignment – see below "Histopathology"). Where more than three suspect sheep per consignment were observed, inspectors could accumulate specimens and submit the most severely affected viscera after the whole line had been inspected. Sampling of sheep was therefore non-random and was biased towards those with the most obvious gross pathology. the animal-level and flock-level specificity of histopathology were both assumed to be 100% [53].

Data collected by the inspectors for each consignment included: Abattoir; Date killed; Consignment reference (abattoir Lot number); Number of animals killed and inspected; Owner of the sheep; Area/Locality of origin; NLIS PIC (where available); Age of the animals (minimum 2 years or older) and the identity of the Inspector (NSW abattoirs only).

The number and percentage of individual sheep with gross lesions resembling OJD ("total lesions uncorrected") and the number of sheep sampled for histopathology were recorded for each consignment. The percentage of gross lesions was calculated from the number of sheep carcasses inspected, not the number killed. The percentage of animals with "lesions attributable to OJD" was determined pro-rata based on the results of histopathology on the animals sampled (see below "Correction of Data for Lesions Attributable to OJD").

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Data were recorded in a standardized format on inspection sheets and transmitted daily by facsimile to a central facility at Orange (1999-2003), then Wagga Wagga NSW (2003-2009). Inspection details and laboratory results were entered into a Microsoft Access database maintained by NSW DPI.

Histopathology

Histopathology is considered the "Gold Standard" diagnostic test for OJD. Sensitivity, however, was not assumed to be 100% because of the possibility that sites selected for histological examination were not representative of the sites observed with gross lesions [53]. Initially, samples from the terminal ileum, ileo-cecal valve and adjacent mesenteric lymph nodes were submitted for confirmatory histopathology (Fig 2). From 2002, however, only the terminal ileum was examined after a formal research trial demonstrated a minimal loss of sensitivity with this modification to the procedure (unpublished report to Sub-Committee on Animal Health Laboratory Standards - Sept. 2002). This change to methodology permitted the terminal ileum from three carcasses to be examined on a single microscope slide, thereby reducing laboratory costs by up to two thirds.

The suspect tissues were processed using standard procedures and the sections were stained by H&E (Hematoxylin and Eosin) and Ziehl-Neelsen for histopathogical examination and determining the presence of acid-fast mycobacteria [78, 79].

Case Definition

Throughout the term of the project the following case definition was employed:

"An OJD positive animal was defined as one with gross pathology at slaughter, confirmed by histopathology as showing granulomatous inflammation of the terminal ileum typical of OJD and presence of acid-fast organisms (AFO's) in the specific animal or another animal in the same consignment."

Classification of samples and consignments

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Samples were initially classified on the basis of histopathology (Fig 5 A-F) as:

- "Positive (Multibacillary) Histopathology" (POS) – presence of granulomatous changes typical of OJD with acid-fast organisms (AFOs) detected (Fig 5A-C), or

– "Inconclusive (Paucibacillary) Histopathology" (INC) - presence of granulomatous changes resembling OJD but no AFOs detected [80] (Fig 5D-F) or

- "Negative Histopathology" (NEG) – absence of granulomatous changes resembling OJD and no AFOs detected.

Fig 5 A-F. Histopathological changes due to OJD in the lamina propria of the terminal ileum. A) Multibacillary – masses of red staining Acid Fast Organisms (AFOs) typical of MAP bacilli (ZN X40); B) Multibacillary – aggregations of AFOs in the cytoplasm of epithelioid cells (ZN X400); C) Multibacillary – large numbers of acid fast bacilli (MAP) in the cytoplasm of epithelioid cells and interstitial connective tissue (ZN X1000); D) Paucibacillary - showing granulomatous enteritis (H&E X100); E) & F) Paucibacillary – granulomatous enteritis but no AFOs evident (ZN X400 & X1000).

Samples identified initially as "INC" were reclassified as "POS" if other samples from the same consignment were "POS". For the purpose of retrospective data analysis, remaining "INC" animals were reclassified as "POS" if OJD had been confirmed in the flock prior to monitoring or within the subsequent two years [80].

Consignments with one or more "POS" samples were classified as OJD "Positive". Consignments which remained "Inconclusive" after retrospective review of flock history were removed from the dataset. The remaining consignments with no lesions or "NEG" histopathology were classified as OJD "Negative".

Correction of data for lesions attributable to OJD (See also S5 Text)

A range of conditions, such as coccidiosis, parasitism, salmonellosis, and yersiniosis, can cause lesions which grossly resemble OJD but yield OJD "NEG" histopathology.

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Hence, the number of sheep with lesions truly attributable to OJD in "Positive" consignments was required to be estimated. This was achieved by pro-rata correction (based on the results of histopathology) of the number of animals with lesions grossly resembling OJD as originally reported by the inspector (see S5 text for examples).

The percentage of OJD-positive sheep in a consignment or prevalence area was estimated from the **"corrected"** number of positive lesions compared with the total number of sheep inspected for that consignment or period.

"Corrected" data were utilized for all analyses.

Specificity of visual inspection

The specificity of visual inspection for OJD was determined by reviewing the histopathological findings on lesions submitted to the laboratory. The relative frequency of multibacillary and paucibacillary was also examined.

Feedback to producers (See also S6 Text)

Results for the initial 4-four months were withheld pending a review of the efficacy of abattoir monitoring. Following endorsement by the NSW sheep industry consultative committee, all OJD positive results from November 1999 onwards were reported to the relevant RLPB District Veterinarian (DV) [55]. The DV in turn personally notified the owners of the sheep. Where the producer identified by tracing expressed doubt regarding the identity of their positive consignment, a risk assessment, and on-farm testing if required, was conducted to confirm or allay suspicion. Producers were notified directly by NSW DPI of all "Negative" consignments monitored after January 2003.

Reports to producers and DVs utilized a mail-merge procedure in Microsoft Word linked to the NSW PIC register in the Microsoft Access Abattoir Monitoring database.

Flock and sheep numbers

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Annual adult sheep and flock numbers (containing ≥50 adult sheep) in NSW (2000-2009) were obtained from the RLPB (later Livestock Health and Pest Authority) Annual Land & Stock Returns [59-61]. Flocks were allocated to prevalence areas based either on RLPB or by property locality in the case of RLPBs which were split by prevalence area boundaries.

Age at vaccination and cumulative (3 year) percentage of adult sheep vaccinated

The age categories vaccinated and number of doses were recorded for 2000-2002 and 2007-2009. For the purposes of analysis, data for 2003-2006 were assumed to be similar to 2007-2009. The vaccine doses sold annually as a percentage of adult sheep provide a raw estimate of annual vaccination usage. However, the vaccine is "one dose for life" and sheep may be vaccinated as lambs (<12-months-old, but recommended at <4-months-old), hoggets (12-24-months-old) or adults (≥ 2-years-old). Hence, the percentage of adult sheep currently vaccinated was determined by the cumulative number of adults remaining in the flock that were vaccinated over the preceding years. For the purposes of estimating the "cumulative" (rolling 3 year) percentage of vaccinated adult sheep (corrected for age at vaccination), it was assumed that: a) all vaccinated sheep were culled when they were nearing 5 years-old (representing 3 years of productive adult life); b) those vaccinated as lambs and hoggets contributed to the vaccinated adult numbers when 2 years-old, and c) all sheep vaccinated as adults were vaccinated when they were 2 years-old [81]. Vaccine orders per flock per year were determined from 2000-2008 (RLPB records) and 2007-2012 (Pfizer Database).

Sheep Breeds Affected

On-Farm Investigations 1982-2006

The sheep breed affected was determined for 779 NSW DPI laboratory submissions confirmed positive for OJD following on-farm sampling from 1982 to 2006.

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Abattoir Monitoring NVDs 2003-2007

In the present study, a subset of 2,249 National Vendor Declarations from 2003-2007 were identified where the sheep breed was recorded as Merino or Crossbred.

Generation of graphs

All graphs were generated from data in Excel spreadsheets (Microsoft) or Prism (GraphPad Software, Inc.).

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Results

Prevalence areas

There was a progressive increase in the detection of OJD infected flocks in NSW starting in 1980, with an exponential increase in detection over the next two decades [40, 42]. Fig 1 shows that the highest prevalence of OJD at 31 March 2008 within Australia was in NSW, Victoria, Flinders Island (Tasmania) and Kangaroo Island (South Australia). Fig 6 shows the location within NSW of the areas designated as being of High, Medium and Low prevalence for OJD. The NSW prevalence area boundaries were based on the regional prevalence and location of individual infected flocks determined by abattoir monitoring from 1999-2007. The results of abattoir monitoring from 1999-2009 (32,032 consignments) are represented in Fig 6.

Fig 6. Map of NSW showing number of abattoir consignments monitored by locality and OJD prevalence area. Shown are the total number of consignments (green disc) and numbers of "Positive" consignments (red disc) for each locality monitored from 1999-2009 (32,032 consignments). The size of the disc is indicative of the number of consignments monitored. Regions of OJD prevalence are denoted by color-coding. High Prevalence Area (HPA) is shown in dark green, Medium (MPA) in light cream and Low (LPA) in light green. (Reprinted under a CC BY license, with permission from NSW DPI, original copyright 2010)

Abattoir monitoring

Of the 32,210 consignments monitored from 1999 to 2009, 178 (0.6%) "Inconclusive" consignments were removed from the dataset as their OJD status was unable to be resolved – 1.2% (156/13,122) of consignments from the HPA, 0.3% (5/1560) of consignments from the MPA and 0.1% (17/17,528) of consignments from the LPA. The remaining 32,032 consignments classed as "Positive" or "Negative" for OJD were included in the analysis, of which 41% were from the HPA (12,966/32,032 - Table 1, Fig 6). Of the 7,635,513 inspected carcasses, 38% (2,932,170) were from the HPA

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

(Table 2). OJD was identified in 15.9% of **all** consignments (5,109/32,032 - Tables 1 and 3), of which 94% were from the HPA (4,823 /5,109; Table 3). Within the HPA itself, 37% (4,823/12,966 - Tables 1 and 3) of consignments were "Positive" for OJD.

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Table 1. Number of Sheep Consignments Monitored at Abattoirs in NSW from 1999- 2009 by Prevalence Area.

Year	High	Medium	Low	Total/year
1999	148	12	68	228
2000	2,113	287	2,291	4,691
2001	1,617	204	1,950	3,771
2002	1,820	162	1,735	3,717
2003	945	133	1,043	2,121
2004	1,119	100	1,263	2,482
2005	583	83	1,123	1,789
2006	1,570	126	1,988	3,684
2007	1,228	230	2,274	3,732
2008	1,005	165	1,974	3,144
2009	818	53	1,802	2,673
Total 1999-2009	12,966	1,555	17,511	32,032

The total number of sheep consignments monitored at abattoirs in NSW from 1999-2009. Stratification is by OJD prevalence area. Total number per year (right-hand column) and cumulative number per Prevalence Area from 1999-2009 (bottom row).

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Table 2. Number of Carcasses Inspected in NSW (1999-2009) by Prevalence Area

Year	High	Medium	Low	Total/Year
1999	32,734	3,224	20,163	56,121
2000	432,860	42,234	596,883	1,071,977
2001	344,097	27,990	507,196	879,283
2002	386,822	28,736	418,611	834,169
2003	185,452	24,851	215,753	426,056
2004	243,962	23,382	291,424	558,768
2005	151,604	19,892	289,907	461,403
2006	444,272	28,744	504,446	977,462
2007	282,677	56,805	570,022	909,504
2008	238,126	38,444	502,410	778,980
2009	189,564	11,256	480,970	681,790
Total 1999-2009	2,932,170	305,558	4,397,785	7,635,513

The numbers of sheep carcasses inspected from 1999-2009 stratified by NSW OJD Prevalence Area. Total number per year (right-hand column) and cumulative number per Prevalence Area from 1999-2009 (bottom row).

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Table 3. "Positive" Consignments in NSW (1999-2009) by Prevalence Area

Year	High	Medium	Low	Total/Year
1999	49	2	2	53
2000	494	9	15	518
2001	551	12	18	581
2002	764	14	24	802
2003	368	14	4	386
2004	465	19	8	492
2005	210	4	11	225
2006	735	8	18	761
2007	525	21	18	564
2008	388	21	20	429
2009	274	10	14	298
Total (+) 1999-2009	4,823	134	152	5,109

The numbers of "Positive" consignments where OJD was detected from1999-2009 Stratified by NSW Prevalence Area. Total number per year (right-hand column) and cumulative number per Prevalence Area from 1999-2009 (bottom row).

Of the 1,379,819 carcasses which were inspected in the "Positive" consignments, 94% (1,300,022) originated from the HPA (Table 4). Using our established criteria (see Case Definition), 50,719 animals had OJD of which 96% (48,514) originated from the HPA (Table 5). Of the 1,300,022 carcasses in OJD "Positive" consignments which originated from the HPA, 3.7% (48,514) had OJD lesions (Tables 4 and 5).

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Table 4. Total Number of Sheep Inspected in "Positive" Consignments (1999-2009) by Prevalence Area

Year	High	Medium	Low	Total/Year
1999	13,846	495	549	14,890
2000	123,390	1,180	5,748	130,318
2001	123,673	3,005	4,419	131,097
2002	177,013	3,218	6,571	186,802
2003	85,571	3,374	1,635	90,580
2004	117,282	4,690	2,996	124,968
2005	66,416	1,300	2,373	70,089
2006	251,534	1,728	6,235	259,497
2007	150,898	6,397	4,577	161,872
2008	109,918	4,934	7,335	122,187
2009	80,481	2,449	4,589	87,519
Total 1999-2009	1,300,022	32,770	47,027	1,379,819

The numbers of sheep inspected in "Positive" consignments where OJD was detected from 1999-2009 stratified by NSW Prevalence Area. Total number per year (right-hand column) and cumulative number per Prevalence Area from 1999-2009 (bottom row).

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Table 5. Total Number of Sheep with OJD Lesions in "Positive" Consignments by Prevalence Area

Year	High	Medium	Low	Total /year
1999	1,283	26	1	1,310
2000	10,406	169	74	10,649
2001	6,197	228	78	6,503
2002	10,221	124	31	10,376
2003	4,057	215	181	4,453
2004	4,607	289	15	4,911
2005	1,505	15	57	1,577
2006	3,823	20	55	3,898
2007	2,566	89	41	2,696
2008	2,276	168	120	2,564
2009	1,573	139	70	1,782
Total 1999-2009	48,514	1,482	723	50,719

The numbers of sheep detected with OJD lesions in "Positive" consignments from 1999-2009. Stratification is by NSW Prevalence Area. Total number per year (right-hand column) and cumulative number per Prevalence Area from 1999-2009 (bottom row).

During the course of the study, despite some variation between years, the percentage of consignments that were "Positive" in the HPA leveled off between 33% and 47% (Table 3 & Fig 7A) confirming that flock-level prevalence remained consistently high.

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Fig 7. Comparison of Percentage of Consignments or Percentage of Animals Positive for OJD. (A) Positive Consignments - the percentage of consignments that are positive remains relatively constant from 2001-2009 in the HPA. In the MPA there is an increase from 2005 to 2009. **(B) Positive Animals -** there is a progressive decrease in the numbers of positive animals as a percent of the total number of animals inspected in **all** consignments in the HPA. In contrast, there is a progressive increase in the percentage of positive animals from 2006 to 2009 in the MPA.

Overall, the percentage of animals that were positive for OJD in **all** consignments originating from the HPA fell progressively from 2.4% (10,406/432,860) of the carcasses inspected at commencement of vaccination in 2000, to 0.8% (1,573/189,564) of the carcasses inspected in 2009 (Tables 2 and 5; Figs 7B and 8A). However, during this period, the percentage of animals that were positive for OJD in **"Positive"** consignments from the HPA fell markedly from 8.5% (11,689/137,236) of the carcasses inspected in 1999-2000 to 2.0% (3,849/190,399) of the carcasses inspected in 2008-2009 (Tables 4 and 5; Fig 8B).

Fig 8. Comparison of Annual Vaccine Sales and Percentage of OJD Positive Lesions (1999-2009). Annual animal-level prevalence percentages are shown as vertical bars (scale on the left-hand vertical axis). Yearly sales of vaccine are shown by lines (scale on the right-hand vertical axis). **(A) All Consignments (32,032) - Stratified by Prevalence Area.** Note the progressive decline in animal-level prevalence in the HPA as vaccination progresses and the contrasting increase in animal-level prevalence in the MPA from 2006 to 2009. **(B) Positive Consignments (4,823) - High Prevalence Area.** Note the marked decline in % positive lesions from 8.5% in 1999-2000 to ~2% from 2006-2009 as vaccination progresses.

Vaccination

Vaccination began in NSW in late 1999, initially in a major vaccination trial in three heavily infected self-replacing merino flocks [32] (i.e. closed flocks with no introductions). From January 2000, vaccination was restricted to a limit of 50 heavily

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

infected flocks whose annual mortality attributable to OJD was greater than 5%. A total of 63,000 doses had been ordered for 51 flocks by the end of September 2000. By April 2002, when the Gudair® OJD vaccine was approved and registered for use in all states of Australia and restrictions eased considerably, there were 237 heavily infected flocks (>5% annual mortality) enrolled in NSW allocated 574,150 doses.

Subsequently, vaccine use became more widespread with decreasing regulatory controls, particularly in the HPA. By 2009, a total of 12,021,963 doses had been administered, of which 91% were administered in the HPA (Table 6). In the HPA, the maximum number of vaccinations in any one year occurred in 2003 (1.73 million). Subsequently, there was a gradual but variable decline in the number of doses administered annually in the HPA, falling to 0.95 million doses by 2009. In contrast, there was a progressive increase, albeit from a much lower base, in the number of vaccinations administered in the MPA, increasing 13-fold from 12,749 in 2001 to a maximum of 165,972 in 2008 (Table 6).

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Table 6. Number of Vaccine Doses Sold in NSW (1999-2009) by Prevalence Area

Year	High	Medium	Low	Total /year
1999[a]	-	-	-	-
2000	83,700	-	-	83,700
2001	400,852	12,749	-	413,601
2002	1,001,122	30,929	-	1,032,051
2003	1,730,590	75,745	13,416	1,819,751
2004	1,553,554	69,891	45,916	1,669,361
2005	1,545,161	65,117	50,073	1,660,351
2006	1,191,433	67,198	44,818	1,303,449
2007	1,261,933	147,155	88,262	1,497,350
2008	1,195,955	165,972	86,872	1,448,799
2009	950,450	93,300	49,800	1,093,550
Total 2000 - 2009	10,914,750	728,056	379,157	12,021,963

The numbers of vaccine doses sold in NSW stratified by OJD Prevalence Area. Total number per year (right-hand column) and Cumulative number per Prevalence Area from 1999-2009 (bottom row). [a]No vaccine doses were sold in 1999.

Given this decline in the animal-level prevalence of OJD in the HPA, the data were reviewed to determine whether there was any association between the use of vaccination and this trend in the regional animal-level prevalence of OJD. From the time that vaccination began in the HPA, there was a progressive decrease in the percentage of sheep with lesions per consignment (Fig 8A) and per "Positive"

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

consignment (Fig 8B). A more detailed analysis revealed a time-dependent positive correlation between the increasing number of vaccinated sheep (as vaccination of the HPA population proceeded) and a declining number of "Positive" consignments with a high percentage of infected animals (Figs 9A-F). This decrease was most pronounced for OJD "Positive" consignments categorized with >10% of positive animals. There were 28.5% of consignments in this category in 2000 falling to ~2.3% of consignments from 2005-2009 (Figs 9A and B). Furthermore, there was a progressive increase in consignments with a low percentage of infected animals as vaccination proceeded. Positive consignments with <1% of animals infected comprised ~34% in 2000 rising to ~50% by 2009 (See Figs 9C-F for trends).

Fig 9. Percentage of Lesions Attributed to OJD in Positive Consignments from the HPA Following Institution of Vaccination in 2000. (A-F) - For clarity, the Results from the HPA only are displayed. A comparison of the annual number of vaccine doses sold with the percentage of positive animals per positive consignment. In each graph, the total doses of vaccine sold are repetitively represented by a line (scale on the right-hand side vertical axis) while the percentage of positive consignments in each percentile category is shown by vertical bars (scale on the left-hand side vertical axis). (A) shows data where the percentage of animals positive per positive consignment exceeds 50%. This only occurs in 1999 and 2000, when vaccine sales were negligible. (B) and (C) show a progressive fall in the percentage of consignments where 5%-50% of animals were positive per positive consignment. In contrast, by (F) (≤1%) an increasing percentage of consignments have a low percentage of animals positive as vaccination progresses. We interpret these results as indicating improvement in the control of OJD by vaccination.

Specificity of visual inspection assessed by histopathology

Review of the histopathology results for 11,687 individual sheep in consignments derived from the HPA confirmed 9,570 as OJD positive (either multibacillary or paucibacillary), an individual animal specificity of 82%. In the MPA the specificity was 63% (271/428) and in the LPA, where inspectors were deliberately encouraged to

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

sample anything remotely suspicious, the specificity was 18% (87/1,028). The individual animal specificity was 76% for samples from the 32,032 consignments monitored from all prevalence areas in NSW.

Paucibacillary pathology attributable to OJD (typical histopathology but no acid-fast organisms) was detected in 17% (1,737/10,028) of the individual sheep sampled, while the remaining 83% were reported with the multibacillary form.

Flock and sheep numbers

The number of flocks and sheep in NSW declined by approximately 50% from 31,895 flocks and 35.4 million sheep in 2000 to 16,419 flocks and 17.7 million sheep in 2009. This decline was primarily associated with an extended period of statewide drought from 2002-2009 (the “Millennium” drought). The percentage of flocks and sheep in each prevalence area, however, remained relatively static over the decade (Table 7). The average flock size in NSW (2000-2009) was 1141 sheep, with an average of 282 sheep/flock in the HPA, 918 in the MPA and 1275 in the LPA.

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Table 7. Distribution of Flocks and Sheep in NSW (2000-2009) by Prevalence Area

	No. of Flocks				No. of Sheep			
Year	Total	High	Medium	Low	Total	High	Medium	Low
2000	31,895	37.1%	11.5%	51.4%	35,411,370	34.1%	8.4%	57.4%
2001	26,631	39.7%	10.9%	49.4%	35,353,577	34.0%	9.4%	56.5%
2002	25,277	40.6%	11.1%	48.3%	33,096,199	34.9%	10.1%	55.0%
2003	NA	NA	NA	NA	27,921,878	38.1%	9.6%	52.3%
2004	NA	NA	NA	NA	27,065,066	36.3%	8.9%	54.9%
2005	23,687	40.0%	10.6%	49.4%	26,698,537	36.3%	8.6%	55.1%
2006	24,111	40.3%	11.5%	48.2%	26,233,654	35.8%	9.0%	55.3%
2007	23,685	33.0%	15.1%	51.8%	23,418,375	31.6%	12.0%	56.4%
2008	20,670	38.8%	12.3%	48.9%	21,453,983	34.6%	9.8%	55.7%
2009	16,419	37.4%	11.6%	51.0%	17,671,195	31.7%	9.6%	58.7%
Mean	24,047	38.4%	11.8%	49.8%	27,432,383	34.8%	9.5%	55.7%

Distribution of flocks (≥50 adult sheep) and numbers of adult sheep by OJD prevalence area in NSW - 2000 to 2009 (Data from RLPB records). Flocks were allocated to prevalence areas based on the boundaries imposed nationally on 31 March 2008. NA-not available.

Age at vaccination and percentage of adult sheep vaccinated

From 2000-2002, when vaccine use was restricted to the HPA, the age at vaccination was categorized as : 75% lambs (<12-months-old), 17% hoggets (12-24-months-old) and 8% adults (≥2-years-old). The lambs at time of vaccination were further sub-categorized as: 41% at 1-4 months-old (approved vaccinates), 11% at 5-6 months and

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

23% at 7-12 months. In contrast, vaccine sales in the HPA from 2007-2009 were categorized as: 94% lambs (<12 months), 2% hoggets and 4% adults. Corresponding figures for sheep in the MPA (2007-2009) were 87%, 3% and 10% and e 90%, 1% and 9% for sheep in the LPA.

The vaccine doses sold annually as a percentage of the adult sheep population by prevalence area and the estimated "cumulative" (rolling 3 year) percentage of vaccinated adult sheep (corrected for age at vaccination) is shown in Table 8. This provides an estimate of the level of herd immunity by prevalence area as vaccination progressed over time.

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Table 8. Estimated % of Sheep Vaccinated in NSW (1999-2009) by Prevalence Area

	Vaccine Doses (Annual) as % of Adult Sheep				Cumulative (Rolling 3 Year) % of Adult Sheep Vaccinated			
Year	High	Medium	Low	All	High	Medium	Low	All
1999	-	-	-	-	-	-	-	-
2000	0.7%	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%
2001	3.3%	0.4%	0.0%	1.2%	0.5%	0.0%	0.0%	0.2%
2002	8.7%	0.9%	0.0%	3.1%	2.2%	0.1%	0.0%	0.8%
2003	16.3%	2.8%	0.1%	6.5%	6.7%	0.9%	0.0%	2.6%
2004	15.8%	2.9%	0.3%	6.2%	16.2%	2.5%	0.0%	6.1%
2005	15.9%	2.8%	0.3%	6.2%	32.1%	5.7%	0.2%	12.2%
2006	12.7%	2.8%	0.3%	5.0%	44.7%	7.6%	0.5%	16.9%
2007	17.1%	5.2%	0.7%	6.4%	64.5%	7.3%	0.9%	21.8%
2008	16.3%	7.7%	0.7%	6.8%	57.9%	10.1%	1.3%	21.6%
2009	17.0%	5.5%	0.5%	6.2%	70.8%	17.8%	1.8%	25.2%

The vaccine doses sold annually as % of adult sheep provides a raw estimate of regional vaccination level. However the vaccine is "one dose for life" and sheep may be vaccinated as lambs, hoggets or adults. Hence the % of adult flock currently of vaccinated status will comprise the cumulative number of adult sheep remaining in the flock that have been vaccinated over the preceding years (see Materials and Methods).

Annual vaccine sales in the HPA as a percentage of the adult sheep population rose rapidly (3.3% in 2001, 8.7% in 2002) before plateauing at ~16% from 2003-2009. In the MPA levels were ~3% from 2003-2006 before increasing to ~5-8% from 2007-

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

2009. Vaccine usage remained very low in the LPA (~0.3-0.7% from 2004-2009). For the whole of NSW, vaccine usage was ~6% from 2003-2009.

In contrast the "cumulative" vaccination status in the HPA reached an estimated 16% by 2004, ~45% by 2006 and ~70% in 2009. The MPA exceeded 5% "cumulative" vaccination status by 2005, 10% by 2008 and 17% by 2009 while the LPA reached 0.9% "cumulative" vaccination status in 2007 and 1.8% in 2009. The "cumulative" vaccination status for the total sheep population of NSW was estimated to exceed 2.5% in 2003, 6% in 2004, 20% in 2007 and 25% in 2009.

A total of 1320 individual flocks/properties were vaccinated in the HPA from 2000-2008 (detailed records maintained by RLPBs) and 2483 flocks from 2007-2012 (Pfizer database) – see Tables 9a and 9b.

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Table 9a. Number of Flocks Vaccinated in the High Prevalence Area 2000-2008 (RLPB Records)

Year	No. of Flocks	Total Doses	Max. Doses/ Flock	Avg. Doses/ Flock	Flocks >2000 Doses/yr	% Flocks >2000 Doses/yr
2000	55	83,700	7,700	1,522	14	9.9%
2001	184	385,400	19,700	2,095	53	18.2%
2002	431	519,450	13,600	1,205	78	11.9%
2003	611	472,550	8,000	773	49	5.7%
2004	622	374,600	6,400	602	30	3.6%
2005	536	337,800	8,000	630	26	3.6%
2006	524	311,350	8,400	594	27	4.0%
2007	319	179,700	4,750	563	16	4.4%
2008	46	19,100	1,500	415	0	0.0%
Total 2000-08	1,320	2,683,650	37,900 [a]	2,033	66 [b]	5.0% [b]

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Table 9b. Number of Flocks Vaccinated in the High Prevalence Area 2007-2012 (Pfizer Database)

Year	No. of Flocks	Total Doses	Max. Doses/Flock	Avg. Doses/ Flock	Flocks >2000 Doses/yr	% Flocks >2000 Doses/yr
2007	755	543,700	7,350	720	59	7.8%
2008	1,328	980,250	10,000	738	104	7.8%
2009	1,321	950,450	12,000	719	91	6.9%
2010	1,316	897,350	8,500	682	80	6.1%
2011	1,384	1,027,350	29,300	742	101	7.3%
2012	1,288	1,039,600	11,000	807	106	8.2%
Total 2007-12	2,483	5,438,700	33,000 [a]	2,190	116 [b]	4.7% [b]

Tables 9a & 9b: The number of flocks vaccinated and number of doses/flock/year in the NSW HPA from 2000-2012. The data in Table 9a were extracted from the RLPB records and the data in Table 9b were extracted from the Pfizer database.

[a] *Maximum number of doses/flock 2000-2008 (Table 9a) or 2007-2012 (Table 9b).*

[b] *Number and % of flocks where >8000 sheep were vaccinated in the period 2000-2008 (Table 9a) or 2007-2012 (Table 9b).*

The number of flocks vaccinated annually in the HPA (Table 9) increased progressively with the easing of restrictions on accessing vaccine. A total of 1320 individual flocks

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

were vaccinated at least once between 2000 and 2008 (400-600 flocks per year from 2002 onwards) and 2483 flocks were vaccinated at least once from 2007-2012 (~1300 flocks annually). The average number of sheep vaccinated per flock annually rose from 1522 in 2000 to 2095 in 2001 before declining to 1205 in 2002 and levelling off at ~600-800/year from 2003-2012. The percentage of these flocks with more than 2000 sheep vaccinated annually ranged from 10-18% from 2000 to 2002, reduced to ~4-6% of from 2003-2006 then rose to 6-8% from 2007-2012 after sales were permitted through rural merchandisers.

Sheep Breeds Affected

On-Farm Investigations 1982-2006

Analysis of 779 OJD positive laboratory submission from field investigations confirmed 91% of the 642 submissions with breed category defined were Merino flocks.

Abattoir Monitoring NVDs 2003-2007

A subset of 2,249 National Vendor Declarations associated with abattoir consignments from 2003 to 2007 were identified where the sheep breed was recorded as either Merino or Crossbred.

Merinos comprised 89.3% of consignments, 93.8% of 616,500 sheep inspected, 94.7% of 1036 positive consignments, 96.1% of 340,940 sheep in positive consignments and 97.5% of 6904 sheep with lesions attributable to OJD. Merinos also showed a higher proportion of heavily infected consignments with 14.6% (143/981) of consignments detected with ≥5% lesions, 4.5% (44/981) with ≥10% lesions and 0.2% (2/981) exceeding 30% lesions. This compared with positive consignments involving Crossbred sheep where 3.6% (2/55) were detected with ≥5% lesions while none exceeded 10% lesions.

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Discussion (See also S7 text)

Controlling Johne's disease is economically important for the animal production industries and for the veterinary community. In the event that MAP is established to be zoonotic, controlling Johne's disease will become important for society as a whole. Despite pilot studies showing Johne's disease can be controlled with herd, animal and feces management [27], the increasing prevalence of Johne's disease in cattle [2] and sheep [42] suggests these controls are currently ineffective in practice and this remains a serious cause for concern. Spread of infection within and between flocks and herds appears to continue despite voluntary control programs whose aims are to restrict exposure of susceptible animals to infected animals and feces.

There are no economically feasible or effective antibiotics, or similar therapeutic agents, currently available for treating MAP infection in sheep. Notwithstanding, vaccination is a potentially valuable adjunct to the control of OJD because fecal shedding of MAP by infected animals is reduced, thereby reducing environmental contamination and exposure of uninfected animals. This study provides evidence that vaccination can be used to control OJD over wide geographic regions.

Importance for the Merino wool and sheepmeat industry (See also S7a)

This manuscript extends prior observational reports [42, 49] on monitoring OJD in Australia. Abattoir monitoring has been demonstrably cost efficient and is particularly important for the Merino wool and sheepmeat industry in Australia [42]. In 2009, it was estimated that there were 77 million sheep in Australia with 26.1 million (34%) in NSW of which 21.9 million (84%) were merinos. There are numerous anecdotal, but few published reports, citing merinos in general, and superfine wool merinos in particular, as being more susceptible to OJD than first-cross or British-bred sheep [43, 82-84]. This hypothesis is supported by the analysis of the breed distribution from NSW DPI laboratory submissions where 91% of OJD positive on-farm investigations were Merinos [85]. Similarly, analysis of 2249 National Vendor Declarations confirmed

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

94.7% of OJD positive consignments and 97.5% of sheep with lesions attributable to OJD were of the Merino breed [86]. Merinos also showed a higher proportion of heavily infected consignments with 14.6% of positive consignments detected with ≥5% lesions,compared with 3.6% of consignments involving Crossbred sheep. However, these findings could be confounded by differences in flock sizes between breeds, regional differences in breed distribution or repeat investigation of individual flocks.

Economic and social impact of OJD (See also S7b)

The economic and social impact of OJD on the sheep industry in Australia from 1981 to 2009 was substantial [87, 88]. Prior to the availability of vaccine in 2000 there was no effective means of limiting the spread between and within flocks. Regulation (quarantine) placed a heavy burden on known or suspected infected properties. In addition, movement restrictions between prevalence areas led to severe impacts on trading opportunities for all flocks in affected regions.

At the same time, many infected flocks had a high annual mortality rate (≥5%) [55, 87-90] evidenced by the demand for vaccination between January 2000 and April 2002 prior to registration of the vaccine for widespread use [55].

Factors in disease spread (See also S7c)

Abattoir monitoring was critical in identifying sub-clinical disease in flocks where the owner had no other evidence of disease. This overcame the major impediment to halting unwitting spread of infection, both locally and to new regions, critical to an effective disease control program. The producer workshops and PDMPs contributed significantly to producer understanding of the epidemiology of OJD.

Cost of vaccination **(See also S7d)**

The cost of vaccination was also a significant imposition on producers at a time when they were receiving low prices for their sheep. Combined with the effects of drought,

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

an aging farming population, the increasing cost of farm inputs and low prices for sheep and wool, the added burden of OJD encouraged many producers to leave the sheep industry.

Abattoir monitoring – disease surveillance and vaccine driver

Abattoir surveillance is both sensitive and specific [55, 72-74, 80] and in NSW it has provided detailed and accurate disease surveillance data, critical for effective disease control over any large geographical region. At the same time, the abattoir surveillance data from this Government and industry partnership program has been crucial to this study of the effectiveness of vaccination for OJD.

Prior to commencement of abattoir monitoring in late 1999 and the initiation of vaccination in 2000, particularly in the HPA, the flock and animal-level prevalence of OJD in NSW had been increasing exponentially [40-42, 55, 91]. The first two months of abattoir monitoring (Nov.-Dec.1999) showed an initial peak in percentage of positive sheep from all consignments from the HPA (3.9%). This coincided with a period (late Spring, early Summer) of normally high annual turnoff of cull sheep. It was also a time when many flocks were suffering increasing clinical losses and were culling heavily infected mobs. Subsequently, animal-level prevalence in the HPA fluctuated from 2.4% in 2000 to 1.8% in 2001 before increasing to an annual peak of 2.6% in 2002. It then progressively declined to 0.8% by 2009 (Fig 7B).

This increase after the first two years of vaccination is not unexpected as access to vaccine was restricted to heavily infected flocks and only a portion of the population (primarily the newborn lambs) were vaccinated each year. In the meantime, OJD was at continued risk of spreading in the remaining unvaccinated population. Additionally, the long incubation period of OJD before detectible thickening of the terminal ileum (often 2 years or greater) dictates that sheep be at least 2-years-old to warrant monitoring at slaughter, ie young adult sheep not lambs. Breeding ewes or wool producing sheep (wethers) would normally be retained for 4-6 years to maximize productivity, while lambs/hoggets for meat production would normally be sold when

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

they are 12-18-months-old. Hence, it would be a minimum of two years before those vaccinated as lambs were included in the animals monitored at slaughter, with increasing numbers by 4-6 years after vaccination.

Concurrently, the impact of drought in NSW generally, and the HPA in particular, from 2002 onwards led to accelerated culling of non-breeding stock (wethers for wool production) and older age groups of ewes (frequently unvaccinated). The selective culling of more heavily OJD infected mobs showing clinical disease (wasting or mortalities) may have contributed to a surge in detection from 1999-2004, particularly consignments with 10% or more lesions attributable to OJD (Figs 9A & B). At the same time, culling high risk mobs would have reduced (at least temporarily) the average animal-level prevalence within these flocks.

The initial shortage of vaccine and restricted usage pending final registration in April 2002 [55] would also have delayed the overall impact of vaccination in the HPA. Financial stringency due to the drought, balanced by financial assistance to purchase vaccine, was also a significant factor in the uptake of vaccination. Thereafter, however, there was a progressive area-wide decrease in animal level disease prevalence in the HPA, as would be expected in an effective vaccination campaign. This was despite the flock-level prevalence continuing to remain very high, with more than 50% of HPA flocks estimated to be infected in 2008 based on abattoir monitoring (median 51.8%; 95% upper confidence limit – 95.6%) [49].

Concurrently with the marked decline in animal-level prevalence of sub-clinical disease evidenced by abattoir monitoring, RLPB District Veterinarians by 2007 were reporting that the level of clinical disease and mortality rates attributable to OJD within vaccinating infected flocks had also decreased markedly [57]. This decrease in clinical disease on-farm was consistent with the 90% reduction in mortality reported in the intensive vaccine trials [32].

Vaccinating infected sheep – concerns regarding exacerbation of disease (See also S7e)

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

The regulatory authorities had expressed concern that vaccinating infected animals would exacerbate the progression of disease. The findings in the "Whole-of-Flock" vaccination trial [92] were crucial to enable the initial recommended vaccination age for lambs in infected flocks to be safely extended from 4-12 weeks to 16 weeks to better fit in with routine lamb marking procedures. At the same time it enabled approval of policies to safely permit vaccination of older sheep in known infected flocks, including adult mobs known to be heavily infected. ***It is important to recognize how crucial this knowledge was in enabling a vaccination program unrestricted by age group or history of exposure.***

Vaccine – preventative or therapeutic? (See also S7f)

It remains unclear whether vaccination for OJD has a therapeutic effect (ie delay in progression of disease in infected animals or regression of pre-existing pathological changes), or whether the primary benefit is a high level of protection following vaccination prior to exposure or establishment of infection [93]. It is likely in heavily infected flocks that a high proportion of lambs are exposed to infection before they are vaccinated, while the results from the "Whole of Flock Vaccination Trial" [92] would support the concept of a therapeutic effect. Alonso et al (2012) [28] suggested a therapeutic effect for a heat killed "whole cell" vaccine in dairy cattle in Spain (Silirum® - Zoetis, USA; CZ Veterinaria, Porriño, Pontevedra, Spain). Silirum®, like Gudair®, contains 2.5mg/ml of bovine MAP strain 316F as the sole active ingredient. They reported a significant attenuation of pre-existing infection in cows naturally infected with paratuberculosis that were adults at the time of vaccination [28].

Age groups vaccinated

The primary message was to vaccinate as lambs (75% of vaccine usage 2000-2002 and 94% from 2007-2009) and preferably <4 months of age ("approved vaccinates" – 41% from 2000-2002). However, many producers throughout the period of this study, particularly those suffering heavy losses, were found to have vaccinated their whole flock or selected older age groups (17% hoggets and 8% adults from 2000-2002 falling

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

to 2% and 4% of vaccine sales in 2007-2009). Approval to vaccinate older sheep had been restricted judiciously prior to registration of the vaccine in April 2002 due to the limited number of vaccine doses available from the manufacturer. The decline in vaccination of older sheep is likely attributable to the progressive rise in fully vaccinated flocks, the decline in the number of heavily infected consignments/flocks identified, and the early detection of new infection through abattoir monitoring.

"Herd" immunity (See also S7g)

Vaccination of older lambs, hoggets and adult sheep undoubtedly contributed to a more rapid increase in "Flock/Herd Immunity", both within individual flocks and the general sheep population of the HPA. This outcome is evidenced by the "cumulative" vaccination status in the HPA reaching an estimated 16% by 2004, 45% by 2006 and 70% in 2009 (Table 8) [81]. This result is consistent with a previous estimate that ~70% of replacement sheep were being vaccinated annually in the HPA by 2007 [57]. Vaccination of older sheep is highly likely to have contributed to a more rapid decline in animal-level prevalence of OJD than might otherwise have been observed [93].

Vaccinating flocks in the HPA

The high initial annual vaccination rate per flock (av. 1,475 for 2000-2002) was attributable to the restriction of vaccine to heavily infected flocks. and the availability of financial assistance for eligible flocks. These factors provided increased incentive to vaccinate older age groups in addition to lambs. The persistent drought, and reduction in average flock size, may also have contributed to subsequent declining numbers vaccinated annually per flock (~600-800/year from 2003-2012, Tables 9a and 9b) .

Persistent flock infection/excretion

Persistent flock infection was confirmed in the present study where animal-level prevalence in the HPA declined from 2.4% in 2000 to <1% from 2005-2009 (Fig 7B) while the infected flock-level prevalence (based on % of consignments positive)

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

remained above 33% from 2001 to 2009 (Fig 7A).

These results are consistent with the findings of Windsor (2014) who reported that 81% (30/37) of known infected commercial flocks monitored in a research trial using PFC in NSW and Victoria continued to shed MAP. This was despite significant declines in estimated OJD prevalence following vaccination as lambs for ≥5 years [94]. They considered these flocks at risk of spreading disease or suffering recrudescence of losses if vaccination were to cease and they advised flock managers to persist with vaccination. They also reported a decline in animal-level prevalence from a pre-vaccination median of 2.7% (95% confidence interval: 1.4 - 6.9) to a post-vaccination median of 0.7% (0.4 - 1.3).

Factors contributing to decrease in regional animal-level prevalence

The decrease in OJD regional animal-level prevalence in the HPA that we observed from 2003 onwards could have resulted from causes other than vaccination. For example, NSW was subject to an unusually long and severe drought during the study period. Termed the "Millennium" drought it ran from 2002 to 2009. However, there is some evidence to refute this alternative interpretation of the data. In contrast to the fall in OJD animal-level prevalence (and static flock-level prevalence) in NSW, there was a marked and progressive increase in the flock-level prevalence of OJD in the adjacent state of Victoria during the period leading up to 2008-2009 [49]. The drought was equally severe in Victoria as in NSW during this time. Conversely, vaccine sales in Victoria were low and abattoir monitoring was conducted on a more limited basis than in NSW [49].We conclude that the decrease in OJD that we documented in NSW cannot solely be ascribed to diminished rainfall. In fact hand feeding of hay and grain from the ground due to the drought could have increased opportunities for infection by the feco-oral route. Further, the decrease in OJD in the HPA of NSW was not replicated in the MPA where vaccination was progressing more slowly.

Improved management practices aimed at OJD control could also explain, in whole or

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

in part, the observed fall in animal level prevalence in the HPA. The provision of financial assistance in 2002-03 was contingent on development of a formal PDMP tailored for each property. These were aimed at promoting those management practices associated with improved on-farm control of OJD, in addition to the widespread application of funding to the purchase of vaccine. It is likely that vaccination and improved management yielded a complementary benefit [92]. Certainly, an improved understanding of age susceptibility was incorporated into management practices to minimize exposure of highly susceptible lambs to pasture contaminated by adult sheep more likely to be excreting MAP.

It is unlikely that many producers implemented targeted on-farm management and control strategies prior to OJD being confirmed in their flock. From 2000 onwards, abattoir monitoring progressively assumed greater importance in confirming the presence of OJD in individual flocks. It complemented (then replaced) more targeted (but costly) surveillance strategies such as investigation of clinical disease and the testing of flocks identified following tracing of sheep movements or identified as neighbors of known infected flocks.

Prevalence areas – role of abattoir monitoring

During the period of this study, the areas that were declared as HPA and MPA for OJD were progressively modified [49, 51, 55, 56, 69], with areas of lower risk transitioning to higher risk following increased abattoir detection of OJD-infected consignments. Vaccination of sheep in the MPA was not permitted until 2003 and was uncommon until 2007. The animal-level prevalence of OJD was only observed to decline in areas where vaccination was common – the NSW HPA. The HPA was also the area where the feedback of abattoir OJD surveillance data to sheep producers was the most effective in demonstrating the severity of the problem of sub-clinical disease.

A primary aim of the abattoir monitoring is to advise producers in a timely fashion of the results of their monitoring. This advice is particularly important for those infected flocks where abattoir monitoring can detect infection when there is no evidence of

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

clinical disease. Feedback to producers also provides quantitative information on the level of infection in the consignment monitored. This promotes the uptake of vaccine and PDMPs in newly detected flocks, while also providing a measure of the progress in controlling sub-clinical disease in those flocks already vaccinating.

In the LPA, the primary aim of abattoir monitoring is early detection of sub-clinical infection so that the risk to other flocks can be promptly neutralized. Concurrently, notification of negative monitoring results provides confidence to individual producers that (a) their flocks are being monitored, (b) their results are negative, and (c) disease control measures based on prevalence areas and biosecurity are built on sound principles.

NLIS and traceability to property of origin

The results of this study also demonstrate many of the benefits available from the NLIS livestock tracing system now in place in Australia. In fact, the need for improved traceability required by abattoir monitoring for OJD [72] accelerated the implementation of NLIS for sheep. It also provided a comprehensive audit mechanism of PIC details recorded on National Vendor Declarations at slaughter [56]. The NLIS enables every animal slaughtered in Australia to be traced back to the consignment property, as well as all other properties on which the animal has been resident, including the property of birth. This level of information is essential when conducting tracing of long incubation diseases. Importantly, the NLIS facilitates data feedback to the owner, which alerts them to the disease status of their animals and promotes rational and logical management decisions on how best to control diseases such as OJD. In particular, NLIS enabled vaccination programs to be targeted at infected flocks and allowed OJD prevalence areas to be accurately defined. This in turn permitted risk-based trading systems to be implemented, without unnecessary restriction of animal movements [42].

Sensitivity and specificity (See also S7h)

The sensitivity and specificity of abattoir monitoring was unknown at the

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

commencement of monitoring in 1999. However it was clear within weeks that the procedure was capable of detecting sub-clinical OJD (often in a high proportion of animals) in many consignments from the HPA, while consignments from the LPA were routinely negative. A research trial was incorporated into the routine abattoir monitoring in 1999-2000, to determine the sensitivity and specificity of visual/tactile inspection of viscera for lesions suggestive of OJD, with follow-up histopathology on 3 animals [72]. This study estimated consignment sensitivity of 97%, and a practical working sensitivity of 90%, in consignments of >300 adult sheep, for any sheep population in which OJD has been recognized for many years (see S7h for details).

Nonetheless, some reports highlighted perceived limitations of abattoir monitoring. These included concerns about low sensitivity of visual inspection [53], concerns which were subsequently allayed by a second research trial conducted in April 2002 in one of the NSW abattoirs participating in the current study [73, 74]. This confirmed individual animal sensitivity of abattoir inspection compared with histopathology of 74-87% (depending on the inspector), and specificity of 97-98% in high prevalence consignments. Individual animal sensitivity was estimated at ~50% in low prevalence consignments (including infected consignments from low prevalence areas).

Reports from both trials emphasized the importance of maximizing sensitivity and specificity by using experienced qualified meat inspectors who had undergone specific training in inspection, sampling and tracing procedures for OJD in the abattoir [72-74]. All inspectors participating in the current study satisfied these requirements.

A review of histopathological findings from NSW and interstate abattoirs in the present study confirmed the high specificity of visual inspection for detection of OJD in the HPA (82%). In contrast, the specificity was 18% in the LPA where inspectors were deliberately encouraged to sample anything remotely suspicious of OJD [80].

There remains no clear scientific explanation as to why the majority of sheep sampled (83%) exhibit multibacillary pathology while others in the same consignment exhibit paucibacillary pathology. It is clear that inspectors are capable of detecting both forms

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

of pathology, with no obvious gross or microscopic differences other than the overt presence or absence of acid-fast organisms on histopathological examination [80].

Limitations

Classifying consignments with paucibacillary pathology as OJD positive where the property had a prior or subsequent (within 2 years) OJD confirmation is justified as a practical way of categorizing the data for a disease with a long incubation period (years) and where a relatively high percentage of cases are paucibacillary. Using these criteria there were only 178 (0.6%) consignments from NSW culled from the dataset due to unresolved status, and only 17 (0.1%) of these consignments derived from the LPA where a positive detection is of more critical importance.

Limiting the number of sheep to be sampled to a maximum of three per consignment was dictated by economic considerations, and was supported by the high individual animal sensitivity as evidenced in the abattoir sensitivity trials [72-74]. While increasing the sample size to 6 or 9 [53] would have increased flock-level sensitivity in low prevalence flocks and the LPA in particular, it would have markedly increased the cost. This issue was dealt with for consignments from the LPA by encouraging sampling of any viscera showing signs remotely suggestive of OJD while retaining a maximum sample size of three. Similarly, concerns that other conditions grossly resembling OJD would reduce sensitivity by displacing samples with OJD [53] was found not to be a major issue. Inspectors proved highly skilled in differentiating subtle changes due to early cases of OJD from normal intestines (and other conditions) as a result of their training and the inspection of large numbers of viscera on a daily basis. Inspectors would put up to three viscera aside for later sampling, but often replaced them with more advanced examples as inspection progressed. Most difficulty was reported with inspection of lambs and hoggets under 2 years of age, where the intestinal immunity was actively maturing (evidenced by slight thickening of the terminal ileum) in response to internal parasites and common intestinal flora. In light of the long incubation period of OJD, this issue was minimized by restricting OJD inspection to sheep ≥2 years of age to maximize sensitivity, specificity and cost effectiveness. Abbott

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

and Whittington (2003) discuss in detail factors likely to influence sensitivity and specificity[53].

Reducing the tissues processed from three initially (terminal ileum, ileo-cecal valve and adjacent mesenteric lymph nodes) to a single tissue per animal (terminal ileum) in 2002 enabled samples from up to 3 animals to be processed per microscope slide. This reduced the laboratory costs by up to two-thirds with minimal loss of sensitivity (unpublished report to Animal Health Committee Sub-Committee on Animal Health Laboratory Standards - Sept. 2002).

The number of infected animals per consignment was estimated by pro-rata "correction" of the number of sheep detected with lesions, according to the proportion of samples positive for OJD on histopathology. This was premised on a random distribution of OJD positive animals amongst those detected with gross lesions in the consignment, and that the animals sampled were representative of the animals with lesions. This was considered the most reasonable, practical and economical approach to standardizing the data and was applied consistently throughout the study.

Support by abattoir management

The cooperation of abattoir management was crucial in facilitating the activities of the inspectors, including provision of safe sample handling facilities and supply of NLIS and PIC/Owner information. They also played a critical role in minimizing the risk, during transport and lairage, of sheep from another consignment being accidentally mixed with the consignment to be monitored. This support was critical, as there was zero tolerance for error in property and sample identification, as any mistaken identity would have wrongly penalized the producer and rapidly discredited the program.

Producer acceptance (trust) of abattoir monitoring results

Initially, abattoir monitoring was used as a screening test with follow-up on-farm confirmatory testing (serology and histopathology). However, as producer confidence

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

in the results increased and the impact of a positive diagnosis decreased (vaccination, financial assistance, improved trading opportunities), the majority of producers in the HPA (and the MPA) accepted the abattoir monitoring result as definitive, further reducing the cost of on-farm confirmatory testing. Positive abattoir monitoring results from the LPA were always followed up by on-farm risk assessment and appropriate testing to confirm or allay suspicion [95].

Saleyard consignments (See also S7i)

Concerns were raised about producers avoiding monitoring by selling through saleyards and their sheep being slaughtered in mixed consignments from multiple vendors [41, 50]. This was considered a factor in missed detection of some smaller individual infected flocks, however the impact was minimized with the cooperation of the saleyard and transport operators. The integrity of the majority of larger individual property consignments sold through saleyards was able to be carefully maintained up to the time of slaughter. This segregation enabled them to be classified as “direct” single property consignments and included in the study. This approach was particularly important for regions distant from an abattoir where it was more cost effective for producers to sell through saleyards.

Regional prevalence data and surveillance

The ability to monitor very large numbers of direct consignments from all sheep producing regions of NSW throughout the year provided comprehensive detailed regional prevalence data. Identification of the precise location of positive flocks was critical for the review and placement of prevalence area boundaries [62, 91]. The aim was to maximize control over infected flocks while minimizing the regulatory impact on trading for flocks considered unlikely to be infected.

Ongoing and effective disease surveillance is necessary to be able to determine the efficacy, or lack thereof, of control interventions such as vaccination. More importantly, ongoing and effective disease surveillance is necessary to be able to rapidly provide

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

sheep producers with evidence of recent infection in their flocks. It is likely that the lag phase in detection, due to the long incubation period of OJD, has most impact in regions where OJD is newly emerging. However, the comprehensive centralized monitoring afforded by abattoir surveillance, offers the best opportunity for early and more cost effective detection compared with more targeted, labor intensive, on-farm surveillance procedures. This evidence also provides the best motivation for a producer to initiate vaccination – well before OJD mortality rises to a level where there is high level and widespread environmental contamination on the property due to increased infection rates.

Accidental operator self-inoculation

Minimizing the risk of accidental self-inoculation with Gudair® was also a primary aim of the vaccination program. The risk was mitigated by a concerted effort by Pfizer, NSW DPI, RLPBs and the sheep industry to communicate the nature of the risk through training programs for producers and vaccine merchandisers, and through standardized vaccine handling and vaccination procedures. Development by Pfizer of the novel "Sekurus"® vaccinator syringe with a protective shroud and double locking mechanism, and increasing the awareness within the rural medical community were critical initiatives. Many producers minimized their personal risk by employing contract vaccinators. Nonetheless small numbers of producers suffered severe impact from accidental self-inoculation [96, 97] and fear of self-inoculation caused some producers to avoid vaccinating their flocks.

Vaccine abscesses (See also S7j)

The risk of vaccine abscesses [32] was also of serious concern to the sheep industry both from an animal welfare perspective and the potential for abscesses to impact on abattoir processing. Producer workshops and a standardized vaccination procedure requiring sub-cutaneous injection high on the neck (at the base of the ear) were effective in minimizing untoward outcomes. From commencement of monitoring in 1999, producers were notified directly by the abattoir if any abscesses were detected in neck

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

muscle at slaughter.

Potential role of vaccination in controlling BJD in cattle (See also S7k)

Vaccination with a killed vaccine may also be a valuable adjunct to current strategies for the control of Johne's disease (BJD or paratuberculosis) in cattle. Government agencies, however, have been reluctant to initiate Johne's disease vaccination programs in cattle. In the past, the most compelling concern was loss of ability to diagnose tuberculosis (usually *M. bovis*) using skin testing [4, 98]. Out of 22 countries with formal BJD control programs, only 7 were reported to have included the option of vaccination [4]. This is despite the evidence that standard pasteurization reliably kills *M. bovis* in bovine milk, in contrast to MAP which is not reliably killed by pasteurization [99, 100].

In Australia, where bovine tuberculosis has been eradicated, vaccination of infected dairy or beef herds against paratuberculosis is considered likely to be cost-effective [4].

We conclude that Government regulatory agencies and the veterinary profession should give serious consideration to progressing further Johne's disease vaccination studies in cattle and to comparing vaccination cost and efficacy with failure to vaccinate [4, 28, 30, 31, 36, 101]. An effective vaccine could make a major contribution to control of the production losses and potential human health risks associated with BJD. The endemic spread of BJD in recent decades has been recently likened by Whittington *et al.* (2019) to a slow moving, uncontrolled long-incubation pandemic [4].

Potential role of abattoir monitoring for BJD in cattle

Similarly, the potential surveillance benefits of implementing abattoir monitoring for BJD in cattle (particularly dairy cattle) should be explored. In a 2019 review, 38 countries out of 48 were reported to have formal tuberculosis control programs [4]. While cattle at slaughter throughout the world are routinely monitored by inspectors for tuberculosis, it is extremely rare for concurrent monitoring to be undertaken for MAP infection [102]. Dahmen et al (2018) recently reported visual detection of 143 cases of

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

BJD during official meat inspection between 2015 and 2017 in an abattoir in Germany [103]. They identified both early and advanced cases of MAP infection (confirmed by histopathology). They also demonstrated the presence of MAP by culture and PCR in skeletal muscle from 39% of the 143 BJD positive cattle.

Deer in New Zealand are routinely monitored at slaughter for both tuberculosis and paratuberculosis in a producer funded program [4].

If MAP is zoonotic, cattle (through milk, milk products and meat) likely present a greater risk than sheep for food-borne infection of humans around the world[18]. In contrast to tuberculosis, only 22 of 48 countries in the same review were identified with formal BJD/paratuberculosis control programs, with major surveillance deficits impacting their ability to implement effective control programs [4]. Routine monitoring of cattle (deer and goats) for paratuberculosis at slaughter, in conjunction with current official monitoring for tuberculosis, would be a cost-effective approach to resolving the surveillance deficit for BJD. As demonstrated in the current study, it would facilitate producer understanding of the extent of infection in their herds and promote more effective control measures including vaccination.

Animal model for mycobacterial diseases in humans

Vaccination of sheep against MAP with Gudair®, a killed whole cell vaccine with oil adjuvant, may also provide an important animal model for vaccination for mycobacterial diseases (tuberculosis, leprosy and possibly Crohn's disease) in humans. While noting the adverse outcome of severe granulomatous reactions typically seen in humans following accidental self-inoculation with Gudair®, alternative adjuvant and delivery mechanisms should be explored.

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Conclusions

Results of the detailed analysis of more than 12 million vaccinations and 7 million carcass examinations presented in this study are consistent with the hypothesis that vaccination against OJD contributed to reversing the previously inexorable increase in animal-level prevalence of OJD in NSW, Australia where more than 35 million sheep were at risk of infection in 2000. The data support the wider use of vaccination to combat OJD. We further conclude that these data support intensive evaluation of the use of vaccination against MAP in other species in which MAP causes or may cause disease. Finally, we propose that disease control to ≤ 1% OJD animal-level prevalence can be achieved by the combination of voluntary vaccination, abattoir monitoring, feedback of surveillance data to sheep producers and the implementation of appropriate disease management strategies on farm.

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Acknowledgements

The program was managed through Animal Health Australia (Lorna Citer, Manager Endemic Diseases) and delivered through NSW DPI (veterinarians, livestock officers and the many committed staff members). The project could not have proceeded without the commitment and diligence of the OJD abattoir inspectors. The support of management at export abattoirs (Dubbo, Goulburn, Deniliquin & Wallangarra), domestic abattoirs (Cowra, Cootamundra, Young, West Wyalong, Junee, Mudgee and Griffith) and interstate abattoirs (Ararat, Brooklyn, Cranbourne, Lobethal, Murray Bridge and Port Pirie), together with the Australian Quarantine Inspection Service (AQIS), was essential.

DVs and Animal Health Officers with RLPBs provided ongoing support notifying producers, undertaking follow-up field investigations and promoting the uptake of vaccination. CSL Limited and Pfizer Animal Health sourced vaccine supplies, provided sales data and strongly promoted safe vaccination procedures. The contributions from the data management team at Wagga Wagga (Tracey Kingham, Jenny Fury, Raylene Heath & James Hamilton), and Orange (Maurie Ryan), and pathologists at the Regional Veterinary Laboratories at Orange and Menangle, are gratefully acknowledged.

We would like to thank the sheep industry representatives who contributed to the development of state and national disease control policies, often under very difficult circumstances, and the many producers who embraced vaccination and implementation of PDMPs for improved control of OJD in their flocks.

The Australian sheep industry owes a great debt of thanks to Dr. Esteban Rodriguez Sanchez (Technical Director) and Dr. Eugenia Puentes (I&D Director) of CZ Veterinaria SA, who developed and evaluated a killed oil-adjuvant vaccine for the control of clinical Johne's disease in sheep and goats in Spain in the early 1990s. This vaccine was subsequently commercialized as Gudair® by CZ Veterinaria SA, who responded with great commitment from 2000 onwards to the unprecedented demand for OJD vaccine from Australian sheep producers.

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

The assistance of Dr. Arieh Bomzon in the final editing of the manuscript is greatly appreciated.

We also acknowledge the on-going behind the scenes support provided by our families during this extended disease control program and the subsequent preparation of this report.

Co-author Marilyn Evers sadly passed away on 28 July 2019. She was a driving force in developing coordinated, practical policies for the control of OJD in NSW and across Australia.

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

References

1. Johne HA, Frothingham L. Ein eigenthumlicher fall von tuberculose beim rind (A particular case of tuberculosis in a cow). Dtsch Zeitschr Tiermed, Vergl Pathol. 1895;21:438-54.

2. USDA. Johne's Disease on U.S. Dairies, 1991–2007 Fort Collins CO, USA: USDA-APHIS-VS-CEAH Agriculture USDo; 2008 #N 521.0408.

3. Waddell L, Rajic A, Stark K, McEwen SA. *Mycobacterium avium* ssp. *paratuberculosis* detection in animals, food,water and other sources or vehicles of human exposure: A scoping review of the existing evidence. Preventive veterinary medicine. 2016;132:32–48.

4. Whittington R, Donat K, Weber MF, Kelton D, Saxmose Nielsen S, al. e. Control of paratuberculosis: who, why and how. A review of 48 countries. BMC Veterinary Research. 2019;15:29.

5. Thompson BR, Clark RG, Mackintosh CG. Intra-uterine transmission of *Mycobacterium avium* subsp *paratuberculosis* in subclinically affected red deer (Cervus elaphus). N Z Vet J. 2007;55(6):308-13. PubMed PMID: 18059649.

6. Cleland PC, Lehmann DR, Phillips PH, Cousins DV, Reddacliff LA, Whittington RJ. A survey to detect the presence of *Mycobacterium avium* subspecies *paratuberculosis* in Kangaroo Island macropods. Veterinary microbiology. 2010;145(3-4):339-46. PubMed PMID: 20400245.

7. AWHN. Kangaroos and *Mycobacterium avium* subspecies *paratuberculosis* - Fact Sheet. Sydney, NSW, Australia: Australian Wildlife Health Network, 2011 21/9/2011. Report No.

8. Whittington R, Marsh I, Choy E, Cousins D. Polymorphisms in IS1311, an Insertion Sequence Common to *Mycobacterium avium* and *M. avium* subsp. *paratuberculosis*, Can be Used to Distinguish Between and Within These Species. Molecular and Cellular Probes. 1998;12:349-58.

9. Whittington RJ, Hope AF, Marshall DJ, Taragel CA, Marsh I. Molecular Epidemiology of *Mycobacterium avium* subsp. *paratuberculosis*: IS900 Restriction Fragment Length Polymorphism and IS1311 Polymorphism Analysis of Isolates from Animals and a Human in Australia. Journal of clinical microbiology. 2000;38:3240-8.

10. Twort F, Ingram GLY. A Method for Isolating and Cultivating the Mycobacterium enteritidis chronicae pseudotuberculosis bovis Johne, and some Experiments on the Preparation of a Diagnostic Vaccine for Pseudotuberculosis

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Enteritis of Bovines. Proceedings of the Royal Society. 1912;Series B 84:517-42.

11. Whittington RJ, Marsh I, McAllister S, Turner MJ, Marshall DJ, Fraser CA. Evaluation of Modified BACTEC 12B Radiometric Medium and Solid Media for Cultrue of *Mycobacterium avium* subsp. *paratuberculosis* from Sheep. Journal of clinical microbiology. 1999;37:1077-83.

12. Ott SL. Johne's Disease on U. S. Dairy Operations. In: USDA, editor. Fort Collins, CO. : APHIS:VS, CEAH, National Animal Health Monitoring System. #N245.1097; 1997. p. 51.

13. Greenstein RJ, Collins MT. Emerging pathogens: is *Mycobacterium avium* subspecies *paratuberculosis* zoonotic? Lancet. 2004;364(9432):396-7. PubMed PMID: 15288721.

14. Waddell LA, Rajic A, Stark KDC, McEwen SA. The potential Public Health Impact of *Mycobacterium avium* ssp. *paratuberculosis*: Global Opinion Survey of Topic Specialists. Zoonoses and Public Health, 2016, , . 2016;63:212–22. doi: doi: 10.1111/zph.12221.

15. Greenstein RJ. Is Crohn's disease caused by a mycobacterium? Comparisons with leprosy, tuberculosis, and Johne's disease. Lancet Infect Dis. 2003;3(8):507-14. PubMed PMID: 12901893.

16. Behr M. Paratuberculosis and Crohn's Disease. In: Behr MA, Collins DM, editors. Paratuberculosis - Organism, Disease, Control. Cambridge, MA 02139, USA: CAB International; 2010. p. 40-9.

17. Naser SA, Ghobrial G, Romero C, Valentine JF. Culture of *Mycobacterium avium* subspecies *paratuberculosis* from the blood of patients with Crohn's disease. Lancet. 2004;364 1039-44.

18. Hermon-Taylor J, El-Zaatari FAK. The *Mycobacterium avium* subspecies *paratuberculosis* problem and its relation to the causation of Crohn disease In: Pedley S, Bartram J, Rees G, Dufour A, Cotruvo J, editors. Pathogenic Mycobacteria in Water: A Guide to Public Health Consequences, Monitoring and Management. London, UK: IWA Publishing, for the World Health Organization; 2004. p. 74-94.

19. Singh A, Singh S, Makharia G, Singh P, Sohal J. Presence and characterization of *Mycobacterium avium* subspecies *paratuberculosis* from clinical and suspected cases of Crohn's disease and in the healthy human population in India. Int J Infect Dis 2008 ; Epub 2008;12(2):190-7. Epub 2007 Oct 2.; PubMed Central PMCID: PMCPMID: 17913536

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

20. Naser S, Sagramsingh S, Naser A, Thanigachalam S. *Mycobacterium avium* subspecies *paratuberculosis* causes Crohn's disease in some inflammatory bowel disease patients. World J Gastroenterol. 2014;20(23):7403-15. Epub Jun 21, 2014. doi: doi: 10.3748/wjg.v20.i23.7403.

21. Collins MT, Lisby G, Moser C, Chicks D, Christensen S, Reichelderfer M, et al. Results of Multiple Diagnostic Tests for *Mycobacterium avium* subsp. *paratuberculosis* in Patients with Inflammatory Bowel Disease and in Controls. J Clin Microbiol 2000;38(12):4373-81. doi: PMCID: PMC87608.

22. Kuenstner JT, Naser S, Chamberlin W, Borody T, Graham DY, McNees A, et al. The Consensus from the *Mycobacterium avium* ssp. *paratuberculosis* (MAP) Conference 2017. Frontiers in Public Health. 2017;5(208). doi: 10.3389/fpubh.2017.00208.

23. Waddell LA, Rajic A, Stark KDC, McEwen SA. The zoonotic potential of *Mycobacterium avium* ssp. *paratuberculosis*: a systematic review and meta-analyses of the evidence. Epidemiol Infect. 2015;143:3135–57. doi: doi:10.1017/S095026881500076X.

24. Begg DJ, Whittington R. Paratuberculosis in Sheep. In: Behr M, Collins DM, editors. Paratuberculosis; Organism, Disease, Control. Cambridge MA USA: CAB International; 2010. p. 157-68.

25. Whittington RJ, Reddacliff LA, Marsh I, McAllister S, V. S. Temporal patterns and quantification of excretion of *Mycobacterium avium* subsp. *paratuberculosis* in sheep with Johne's disease. . Aust Vet J 2000;78(34-37).

26. Benedictus A, Mitchell RM, Linde-Widmann M, Sweeney R, Fyock T, Schukken YH, et al. Transmission parameters of *Mycobacterium avium* subspecies *paratuberculosis* infections in a dairy herd going through a control program. Preventive veterinary medicine. 2008;83(3-4):215-27. Epub 2007/09/18. doi: 10.1016/j.prevetmed.2007.07.008. PubMed PMID: 17868937.

27. Collins MT, Eggleston V, Manning EJ. Successful control of Johne's disease in nine dairy herds: results of a six-year field trial. J Dairy Sci. 2010;93(4):1638-43. PubMed PMID: 20338441.

28. Alonso-Hearn M, Molina E, Geijo M, Vazquez P, Sevilla IA, Garrido JM, et al. Immunization of adult dairy cattle with a new heat-killed vaccine is associated with longer productive life prior to cows being sent to slaughter with suspected paratuberculosis. J Dairy Sci. 2012;95(2):618-29. Epub 2012/01/28. doi: 10.3168/jds.2009-2860. PubMed PMID: 22281327.

29. Hines ME, Stiver S, Giri D, Whittington L, Watson C, Johnson J, et al. Efficacy of spheroplastic and cell-wall competent vaccines for *Mycobacterium*

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

avium subsp. _paratuberculosis_ in experimentally-challenged baby goats. Veterinary microbiology. 2007;120(3-4):261-83. PubMed PMID: 17123751.

30. Juste RA, Perez V. Control of paratuberculosis in sheep and goats. Vet Clin North Am Food Anim Pract. 2011;27(1):127-38. PubMed PMID: 21215897.

31. Kalis CH, Hesselink JW, Barkema HW, Collins MT. Use of long-term vaccination with a killed vaccine to prevent fecal shedding of _Mycobacterium avium_ subsp _paratuberculosis_ in dairy herds. Am J Vet Res. 2001;62(2):270-4. PubMed PMID: 11212038.

32. Reddacliff L, Eppleston J, Windsor P, Whittington R, Jones S. Efficacy of a killed vaccine for the control of paratuberculosis in Australian sheep flocks. Veterinary microbiology. 2006;115(1-3):77-90. PubMed PMID: 16459030.

33. Singh S, Singh P, Singh A, Sohal J, Gupta V, Vihan V. Comparative efficacy of an indigenous 'inactivated vaccine' using highly pathogenic field strain of _Mycobacterium avium_ subspecies _paratuberculosis_ 'Bison type' with a commercial vaccine for the control of Capri-paratuberculosis in India. Vaccine. 2007;25(41):7102-10. . Epub 2007 Aug 15.; PubMed Central PMCID: PMCPMID: 17804124

34. Eppleston J, Reddacliff L, Windsor P, Whittington R, Jones S, editors. Efficacy of a killed _Mycobacterium paratuberculosis_ vaccine for the control of OJD in Australian sheep flocks. Proceedings of the 9th International Colloquium on Paratuberculosis; 2006; Copenhagen, Denmark.

35. Fridriksdottir V, Gunnarsson E, Sigurdarson S, Gudmundsdottir KB. Paratuberculosis in Iceland: Epidemiology and Control Measures, Past and Present. Vet Microbiol 2000;77:263-7.

36. Cho J, Tauer LW, Schukken YH, Gomez MI, Smith RL, Lu Z, et al. Economic analysis of _Mycobacterium avium_ subspecies _paratuberculosis_ vaccines in dairy herds. J Dairy Sci. 2012;95(4):1855-72. Epub 2012/03/31. doi: 10.3168/jds.2011-4787. PubMed PMID: 22459833.

37. Bush RD, Windsor PA, Toribio JA, Webster SR. Financial modelling of the potential cost of ovine Johne's disease and the benefit of vaccinating sheep flocks in southern New South Wales. Australian veterinary journal. 2008;86(10):398-403. PubMed PMID: 18826512.

38. Windsor PA, Eppleston J, Sergeant E. Monitoring the efficacy of Gudair™ OJD vaccine in Australia. Proc Aust Sheep Vet Soc2003. p. 114-22.

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

39. Seaman JT, Gardner IA, Dent CH. Johne's Disease in Sheep. Australian veterinary journal. 1981;57:102-3.

40. Links IJ, Sergeant ES, Molony BJ, Reddacliff LA. Surveillance of Ovine Johne's disease in New South Wales - update to 31 December 1998. Proceedings of the Sixth International Colloquium on Paratuberculosis; 14-18 February 1999; Melbourne, Vic., Australia. Melbourne, Vic. , Australia: International Association for Paratuberculosis and Other Intestinal Mycobacterioses; 1999. p. 132-41.

41. Sergeant ESG. Ovine Johne's disease in Australia - the first 20 years. Australian veterinary journal. 2001;79(7):484-91. Epub 10 MAR 2008.

42. Kennedy DW, Citer L. Paratuberculosis Control Measures in Australia. In: Behr M, Collins DM, editors. Paratuberculosis; Organism, Disease, Control. Cambridge MA USA: CAB International; 2010. p. 330-43.

43. ENRC. Inquiry into the Control of Ovine Johne's Disease in Victoria - Report. Melbourne, Victoria: Parliament of Victoria, Environment and Natural Resources Committee; 2000. p. 355.

44. Erickson A. Understanding ovine Johne's disease [Webpage]. https://www.agric.wa.gov.au/livestock-biosecurity/understanding-ovine-johnes-disease: Department of Agriculture and Food, Government of Western Australia; 2015 [updated 8 June 2015; cited 2015 23 December 2015]. Available from: https://www.agric.wa.gov.au/livestock-biosecurity/understanding-ovine-johnes-disease.

45. Denholm LJ, Ottaway SJ, Corish JA, Merton PW. Control and Eradication of ovine Johne's disease in Australia. . 4th International Congress for Sheep Veterinarians; 6th February 1997; Armidale, New South Wales, Australia1997. p. 158-77.

46. Whittington RJ, Fell S, Walker D, McAllister S, Marsh I, Sergeant E, et al. Use of pooled fecal culture for sensitive and economic detection of *Mycobacterium avium* subsp. *paratuberculosis* infection in flocks of sheep. Journal of clinical microbiology. 2000;38(7):2550-6. PubMed PMID: 20002217728.

47. Links IJ. The NOJDP [National Ovine Johne's Disease Control and Evaluation Program] Business Plan and NSW Agriculture. Wool and Sheepmeat Services Annual Conference 1999; Armidale NSW: Library, NSW Agriculture; 1999. p. 79-89.

48. Links I, Roth I, Denholm L. Control of the Spread of Ovine Johne's Disease (OJD) in NSW. Wool and Sheepmeat Services Program Annual Conference; 2001; Orange NSW: NSW Agriculture; 2001. p. 18-26.

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

49. Kompas T, Che N. An Economic Analysis of the Impact of the National OJD Management Program (OJDMP) in Australia. Canberra, ACT, Australia: Australian Centre for Biosecurity and Environmental Economics (ACBEE), Crawford School of Economics and Government, Australian National University, Canberra., (ACBEE) ACfBaEE; 2011.

50. National Ovine Johne's Disease Control and Evaluation Program, National Workshop on Control Strategies - A Review of the NSW Approach. National Workshop on Control Strategies - A Review of the NSW Approach; 2001 17 - 18 April, 2001; Canberra, Australia: Meat & Livestock Australia; 2001.

51. Links IJ, Evers M, Denholm LJ. Progress with Ovine Johne's Disease Control in New South Wales - update November 2002. Wool and Sheepmeat Services Program Annual Conference, 3-5 December 2002; Tocal Agricultural Centre, Paterson, NSW, Australia: NSW Agriculture; 2002. p. 85-7.

52. Abbott KA. Prevalence of Johne's disease in rabbits and kangaroos TR.050. North Sydney, NSW, Australia: Meat and Livestock Australia Ltd, 2002 February 2002. Report No.: Contract No.: TR.050 Ovine Johne's Disease Research & Development Program.

53. Abbott KA, Whittington RJ. Monte Carlo simulation of flock-level sensitivity of abattoir surveillance for ovine paratuberculosis. Prev Vet Med. 2003;61(4):(Dec 12):309-32.

54. Denholm LJ. Controlling the Spread of Johne's Disease by Compulsory Flock Vaccination: Principal Strategy of the Only Two Successful National Control Programs in Sheep or Goats. . Proceedings of the Wool and Sheepmeat Services Annual Conference 15-16 November 1999; Armidale, New South Wales, Australia: NSW Agriculture; 1999. p. 58-69.

55. Links I, Roth I, Evers M, Denholm L, Ryan M. Ovine Johne's Disease in New South Wales: Update to 31st October 2000. Wool and Sheepmeat Services Program Annual Conference; 2000; Yanco NSW2000. p. 43-51.

56. Links I, Denholm L, Kingham L, Evers M. Abattoir surveillance for Ovine Johnes Disease (OJD) in New South Wales. In: Søren Saxmose Nielsen E-i-C, editor. Proceedings of the 9th International Colloquium on Paratuberculosis; October 28 - November 2, 2007; Tsukuba, Japan: International Association for Paratuberculosis, Inc.; 2008. p. 303-6.

57. Links I, Denholm L, Kingham L, Evers M. A comparison of vaccination for Ovine Johne's Disease (OJD) and prevalence of lesions detected by abattoir monitoring in New South Wales (NSW). In: Søren Saxmose Nielsen E-i-C, editor. Proceedings of the 9th International Colloquium on Paratuberculosis; Tsukuba, Japan: International Association for Paratuberculosis, Inc.; 2008. p. 307-10.

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

58. Links I, Denholm L, Evers M, Kingham L, Greenstein R. Can Johne's Disease be Controlled by Vaccination? Data on 12 Million Ovine Vaccinations and 7.6 Million Carcase Examinations in NSW Australia from 1999-2009 11th International Colloquium on Paratuberculosis (11ICP); 5 - 10 February 2012; Sydney, NSW, Australia. Sydney, NSW, Australia: International Association for Paratuberculosis; 2012. p. 170-1.

59. RLPB. Annual Report. Orange, New South Wales, Australia: State Council of Rural Lands Protection Boards, 2000-2007.

60. LHPA. Rural Lands Protection Boards Annual Report 2008. Orange, New South Wales, Australia: State Council of Livestock Health and Pest Authorities, Orange NSW; 2008. p. 36.

61. LHPA. Livestock Health & Pest Authorities Annual Report - 2009-10. In: Wales NS, Authority LHP, editors. Orange, New South Wales, Australia: NSW Livestock Health & Pest Authority 2009-2010. p. 22.

62. Anon. OJD Policy Manual August 1999. In: Agriculture NSW, editor. August 1999 ed. Orange, NSW, Australia: New South Wales Agriculture; 1999.

63. Anon. OJD Policy Manual November 2001. In: Agriculture NSW, editor. November 2001 ed. Orange, NSW, Australia: New South Wales Agriculture; 2001.

64. Anon. National Johne's Disease Program, Standard Definitions and Rules for Sheep (January 1998). January 1998 ed. Canberra, ACT, Australia: Australian Veterinary Committee; 1998.

65. Anon. National Johne's Disease Program Standard Definitions and Rules for Sheep (August 2001) 3rd Edition ed. August 2001: Animal Health Australia for Australian Veterinary Committee; 2001.

66. Anon. National Ovine Johne's Disease (OJD) Standard Definitions and Rules (SDR's) for Sheep, Goats and Deer, 5th Edition. August 2003. 5th Edition ed. Canberra, ACT, Australia: Animal Health Australia for Animal Health Committee; 2003.

67. Anon. Standard Definitions Rules and Guidelines for the Management of Ovine Johne's Disease in Sheep and Goats to Support the National Ovine Johne's Disease Management Plan 2013-2018. Animal Health Australia (AHA), Canberra, 2013 May 2013. Report No.

68. Gardner I, Nielsen S, Whittington R, Collins M, Bakker D, Harris B, et al. Concensus-based Reporting Standards for Diagnostic Test Accuracy Studies for Paratuberculosis in Ruminants. Prev Vet Med. 2011;101:18-34. Epub 23 May 2011.

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

69. Bull R. Review of the Management of Ovine Johne's disease in NSW - Report to the Minister for Primary Industries. Sydney, Australia: State of New South Wales, Australia., 2008.

70. Eppleston J, Reddacliff L, Windsor P, Links I, Whittington R. Preliminary observations on the prevalence of sheep shedding *Mycobacterium avium* subsp. *paratuberculosis* after 3 years of a vaccination program for ovine Johne's disease. Australian veterinary journal. 2005;83(10):637-8. PubMed PMID: 20053216491.

71. Kingham L, Links IJ. Development of a group approach to facilitate vaccination for OJD in New South Wales. 11th International Colloquium on Paratuberculosis - ICP2012; 5-10 February 2012; Sydney, Australia2012. p. 178.

72. Denholm L, Ryan M, Lugton I. Evaluation and Comparison of Two Methods of Abattoir Surveillance for Detection of Ovine Johne's Disease - OJD.007. North Sydney NSW Australia: Meat & Livestock Australia, 2001 Published February 2002. Report No.

73. Bradley TL. Determination of Individual Animal - Level Sensitivity of Abattoir Surveillance for Ovine Johne's Disease - Project number OJD.029 North Sydney NSW 2059 Meat & Livestock Australia, 2002 September 2002 Report No.: Contract No.: Project number OJD.029

74. Bradley TL, Cannon RM. Determining the sensitivity of abattoir surveillance for ovine Johne's disease. Australian veterinary journal. 2005;83(10):633-6. PubMed PMID: 16255288.

75. Denholm L. Ovine Johne's Disease - Abattoir Surveillance Manual (Version 1b - August 1999). Version 1b ed. Denholm L, editor. Orange, NSW, Australia: NSW Agriculture; 1999 August 1999. 46 p.

76. Links IJ, Ware A, Kingham L. Ovine Johne's Disease, Abattoir Surveillance Manual (Version 2 – November 2004). Links IJ, Ware A, Kingham L, editors. Wagga Wagga, NSW, Australia: NSW Department of Primary Industries; 2004 November 2004. 48 p.

77. Citer L, Links IJ. Ovine Johne's Disease Abattoir Surveillance Manual for Meat Inspectors. Citer L, Links IJ, editors. Canberra, ACT, Australia: Animal Health Australia; 2007. 42 p.

78. Neelsen FCA. Ein casuistischer Bietrag zur Lehre von der Tuberkulose. Zbl med Wiss. 1883;21(497-501).

79. Ziehl F. Zur Farbung des Tuberkelbacillus. Dtsch, med Wschr,. 1882;8:451.

80. Links I, Hum S. Ovine Johne's Disease - Histopathological Findings for

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

13,143 Suspect Lesions from Abattoir Monitoring in NSW Australia from 1999-2009. 11th International Colloquium on Paratuberculosis (11ICP); 5 - 10 February 2012; Sydney, NSW, Australia. Sydney, NSW, Australia: International Association for Paratuberculosis; 2012. p. 153-4.

81. Links IJ. Age at Vaccination and Estimated Vaccination Levels for OJD in the NSW High Prevalence Area 2000-2009. In: Fourichon C, Guatteo R, editors. 13th International Colloquium on Paratuberculosis 2016; 20-24 June 2016; Nantes, France. Nantes, France: International Association for Paratuberculosis; 2016c. p. 1 (p142).

82. Morris CA, Hickey SM, Dobbie JL, Lake DE. A breed difference between Romneys and Merinos in the incidence of Johne's disease on a New Zealand farm. Proc Adv Anim Breed Gen. 2003;15:399.

83. Begg D, Purdie AC, de Silva K, Plain KM, Whittington RJ. Experimental Inoculation of Different Breeds of Sheep with *Mycobacterium avium* sub species *paratuberculosis*; are Breed Susceptibility Differences Evident? In: Fourichon C, Guatteo R, editors. 13th International Colloquium on Paratuberculosis 2016; 20-24 June 2016; Nantes, France. Nantes, France: International Association for Paratuberculosis; 2016. p. 1 (p49).

84. Begg DJ, Purdie AC, de Silva K, Dhand NK, Plain KM, Whittington RJ. Variation in susceptibility of different breeds of sheep to *Mycobacterium avium* subspecies *paratuberculosis* following experimental inoculation. Veterinary Research. 2017;48(1):1-11.

85. Links IJ. Sheep Breeds Confirmed with OJD in NSW– Review of 779 Laboratory Submissions 1981-2006. In: Fourichon C, Guatteo R, editors. 13th International Colloquium on Paratuberculosis 2016; 20-24 June 2016; Nantes, France. Nantes, France: International Association for Paratuberculosis; 2016a. p. 1 (p138).

86. Links IJ. Are Merinos More Susceptible to OJD Than Crossbred Sheep? Analysis of Abattoir Monitoring for 2249 Consignments from the NSW High Prevalence Area - 2003-2007. In: Fourichon C, Guatteo R, editors. 13th International Colloquium on Paratuberculosis 2016; Nantes, France. Nantes, France: International Association for Paratuberculosis; 2016b. p. 1 (p44).

87. Toribio J-A, Bush R, Windsor P. A Study of the Biological and Economic Impact of OJD in Affected Sheep Flocks in NSW - 12 Farm Mortality Study - OJD.023. Technical. North Sydney NSW 2059 Australia: The University of Sydney 2005 September 2005. Report No.: Contract No.: OJD.023

88. Toribio J-A, Bush RD, Windsor PA, Webster SR. Financial modelling of the potential cost of ovine Johne's disease and the benefit of vaccinating sheep flocks

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

in southern New South Wales. Australian veterinary journal. 2008;86(10): · (10):6. doi: DOI: 10.1111/j.1751-0813.2008.00347.x.

89. Eppleston J, Simpson G. Observations of OJD in an endemic area. . NSW Agriculture Wool and Sheepmeat Services Annual Conference; 15-16 November 1999 University of New England, Armidale1999 p. 91-3.

90. Eppleston J, Simpson G, O'Neil l, Thornberry K, Lugton I, Taylor P, et al. Reported Levels of Sheep Mortalities in Flocks Infected with Ovine Johne's Disease in New South Wales Asian-Aus J Anim Sci 2000;13 Supplement C (July 2000):247.

91. Sergeant ES, Baldock FC. The estimated prevalence of Johne's disease infected sheep flocks in Australia. Aust Vet J 2002;80(12 Dec):762-8.

92. Windsor P. A longitudinal study of OJD and the effects of whole flock vaccination with Gudair - Whole flock OJD vaccination at Merrill - OJD.015. Technical Report. North Sydney, NSW, Australia: University of Sydney, 2005 March 2005. Report No.

93. Links I, Templeton R, Nordblom T. Is Whole of Flock Vaccination for Ovine Johne's Disease Justified? . 11th International Colloquium on Paratuberculosis (11ICP); 5 - 10 February 2012; Sydney, NSW, Australia. Sydney, NSW, Australia: International Association for Paratuberculosis; 2012. p. 172-3.

94. Windsor PA, Eppleston J, Dhand NK, Whittington RJ. Effectiveness of Gudair™ vaccine for the control of ovine Johne's disease in flocks vaccinating for at least 5 years. Australian veterinary journal. 2014;92(7):263-8. doi: 10.1111/avj.12194.

95. Links IJ, Evers M, Wright T, Barwell R. Evaluation of 108 Abattoir Traces for Ovine Johne's Disease to the New South Wales Low Prevalence Area - A Standardised Approach 11th International Colloquium on Paratuberculosis (11ICP); 5 - 10 February 2012; Sydney, NSW, Australia. Sydney, NSW, Australia: International Association for Paratuberculosis; 2012. p. 176-7.

96. Richardson GD, Links IJ, Windsor PA. Gudair (OJD) vaccine self-inoculation: a case for early debridement. The Medical journal of Australia. 2005;183(3):151-2. Epub 2005/08/02. PubMed PMID: 16053420.

97. Windsor PA, Bush R, Links I, Eppleston J. Injury caused by self-inoculation with a vaccine of a Freund's complete adjuvant nature (Gudair (TM)) used for control of ovine paratuberculosis. Australian veterinary journal. 2005;83(4):216-20. PubMed PMID: WOS:000228418300017.

98. Nedrow AJ, Gavalchin J, Smith MC, Stehman SM, Maul JK, McDonough

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

SP, et al. Antibody and skin-test responses of sheep vaccinated against Johne's Disease. Vet Immunol Immunopathol. 2007;116(1-2):109-12. PubMed PMID: 17275919.

99. Grant IR, Ball HJ, Rowe MT. Incidence of *Mycobacterium paratuberculosis* in bulk raw and commercially pasteurized cows' milk from approved dairy processing establishments in the United Kingdom. Applied and environmental microbiology. 2002;68(5):2428-35. Epub 2002/04/27. PubMed PMID: 11976118; PubMed Central PMCID: PMC127574.

100. Ellingson JL, Anderson JL, Koziczkowski JJ, Radcliff RP, Sloan SJ, Allen SE, et al. Detection of viable *Mycobacterium avium* subsp. *paratuberculosis* in retail pasteurized whole milk by two culture methods and PCR. J Food Prot. 2005;68(5):966-72. PubMed PMID: 15895728.

101. van Schaik G, Kalis CH, Benedictus G, Dijkhuizen AA, Huirne RB. Cost-benefit analysis of vaccination against paratuberculosis in dairy cattle. The Veterinary record. 1996;139(25):624-7. Epub 1996/12/21. PubMed PMID: 9123788.

102. Vecerek V, Kozak A, Malena M, Tremlova B, Chloupek P. Veterinary meat inspection of bovine carcasses in the Czech Republic during the period of 1995–2002. Vet Med Czech 2003;48(7):183–9.

103. Dahmen H, Duenner M, Zimmer K, Steinhauer D, Hermon-Taylor J, Rayment N. Detection and Isolation of MAP from Skeletal Muscle tissue of 39% of 143 Cattle at a German Abattoir. Proceedings of the 14th International Colloquium on Paratuberculosis; 4-8 June 2018; Riviera Maya, Mexico: International Association for Paratuberculosis, Inc.; 2018. p. p162.

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Supplementary text

S1 - Introduction

S2 - Vaccine regulation

S3 - Vaccine procedures

S4 - Property disease management plans (PDMPs), financial assistance and producer workshops

S5 - Inspection, sampling & data collection

S6 - Feedback to producers

S7 - Discussion

S7a - Importance for the Merino wool and sheepmeat industry

S7b - Economic and social impact of OJD

S7c – Factors in disease spread

S7d - Cost of vaccination

S7e - Vaccinating infected sheep – concerns regarding exacerbation disease

S7f - Vaccine – preventative or therapeutic?

S7g - "Herd" immunity

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

S7h - Sensitivity and specificity

S7i - Saleyard consignments

S7j - Vaccine abscesses

S7k - Potential role of vaccination in controlling BJD in cattle

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Abbreviations

AFO – Acid-fast organism

BJD - Bovine Johne's disease (paratuberculosis)

"C" Strain – "Cattle" strain of MAP

DPI – Department of Primary Industries

DV – District Veterinarian

H&E – Haematoxylin and Eosin stain

HPA – High Prevalence Area

INC – Inconclusive

LPA – Low Prevalence Area

MPA – Medium Prevalence Area

MAP – Mycobacterium avium subsp. paratuberculosis

NEG - Negative

NLIS – National Livestock Identification System

NSW – New South Wales

OJD – Ovine Johne's disease

PIC – Property Identification Code

PDMP – Property Disease Management Plan

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

PFC – Pooled Fecal Culture

POS - Positive

RLPB – Rural Lands Protection Board

"S" Strain – "Sheep" strain of MAP

ZN – Ziehl-Neelsen stain

END

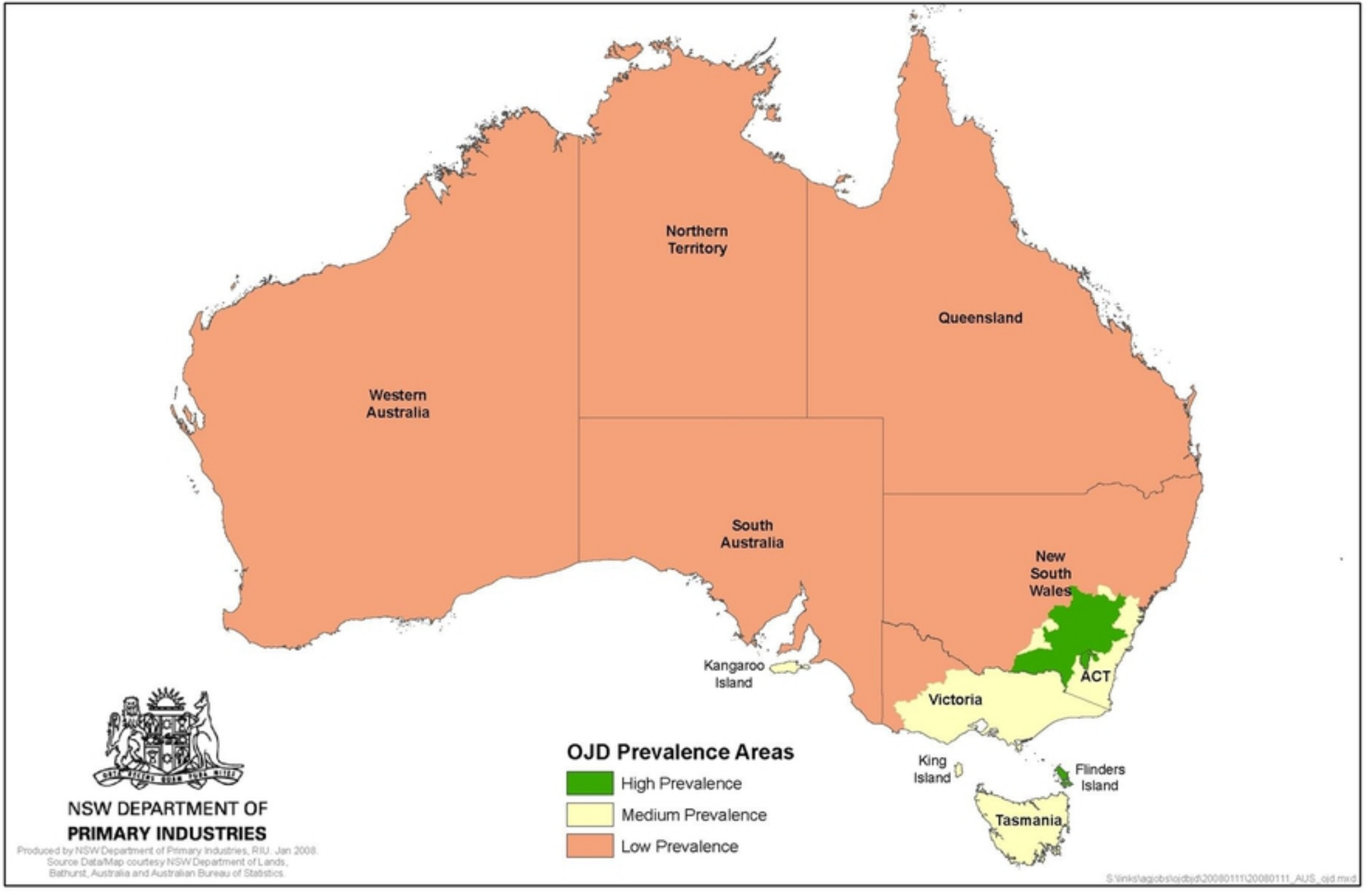

Northern Territory
Queensland
Western Australia
South Australia
New South Wales
ACT
Victoria
Kangaroo Island
King Island
Flinders Island
Tasmania
OJD Prevalence Areas
High Prevalence
Medium Prevalence
Low Prevalence
NSW DEPARTMENT OF
PRIMARY INDUSTRIES
Produced by NSW Department of Primary Industries, RIU. Jan 2008.
Source Data/Map courtesy NSW Department of Lands,
Bathurst, Australia and Australian Bureau of Statistics.

Figure 1

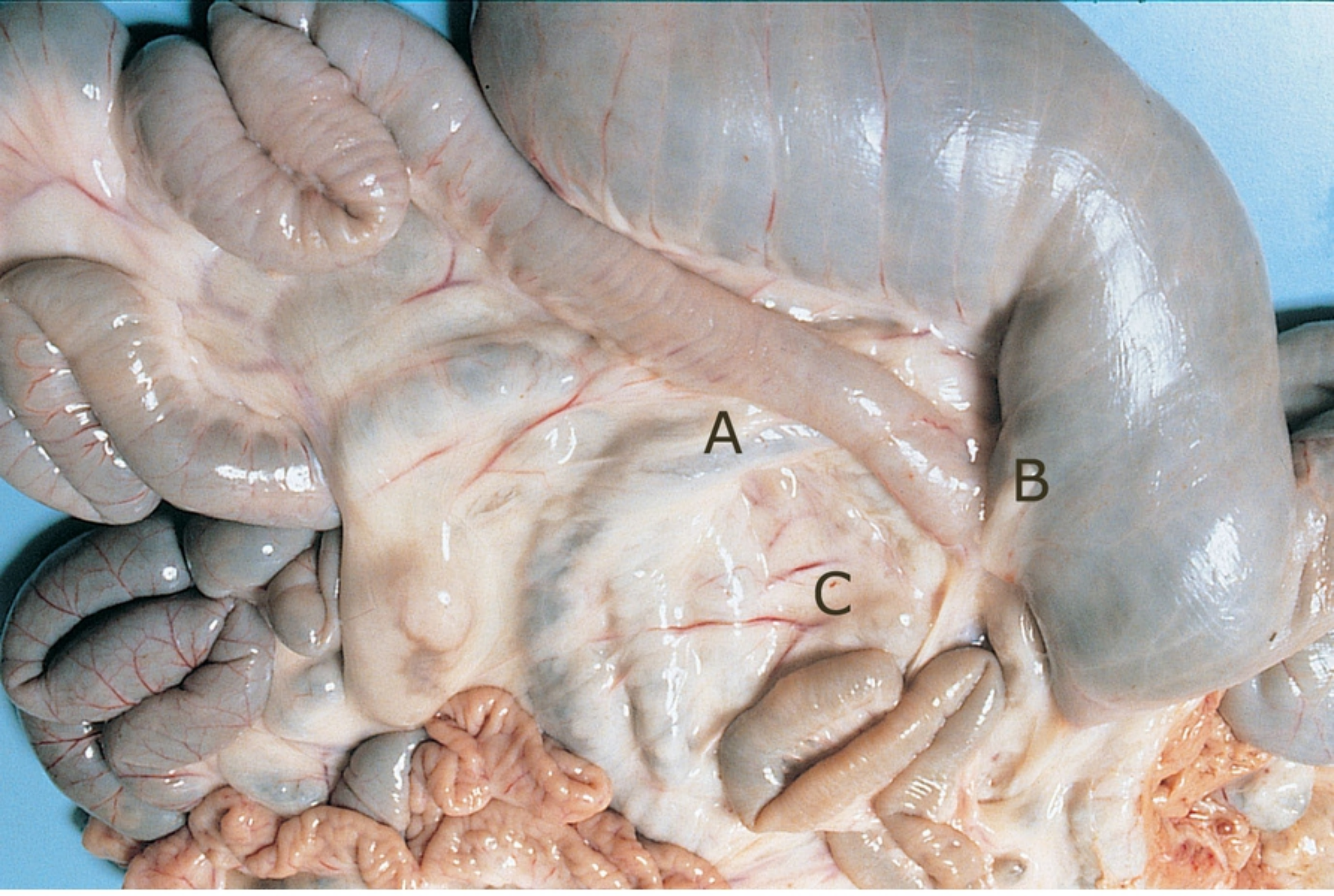


Figure 2

Figure 3

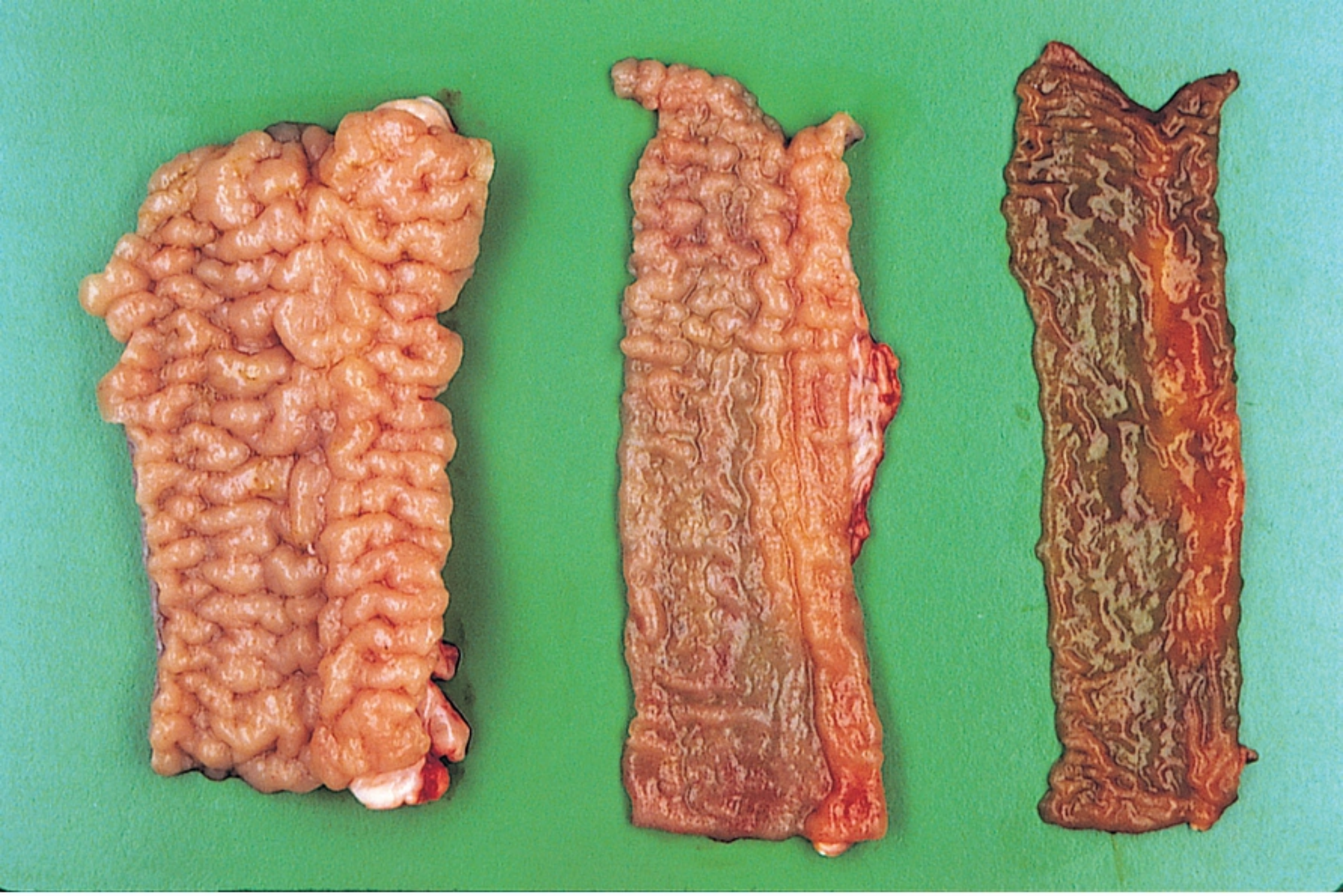


Figure 4

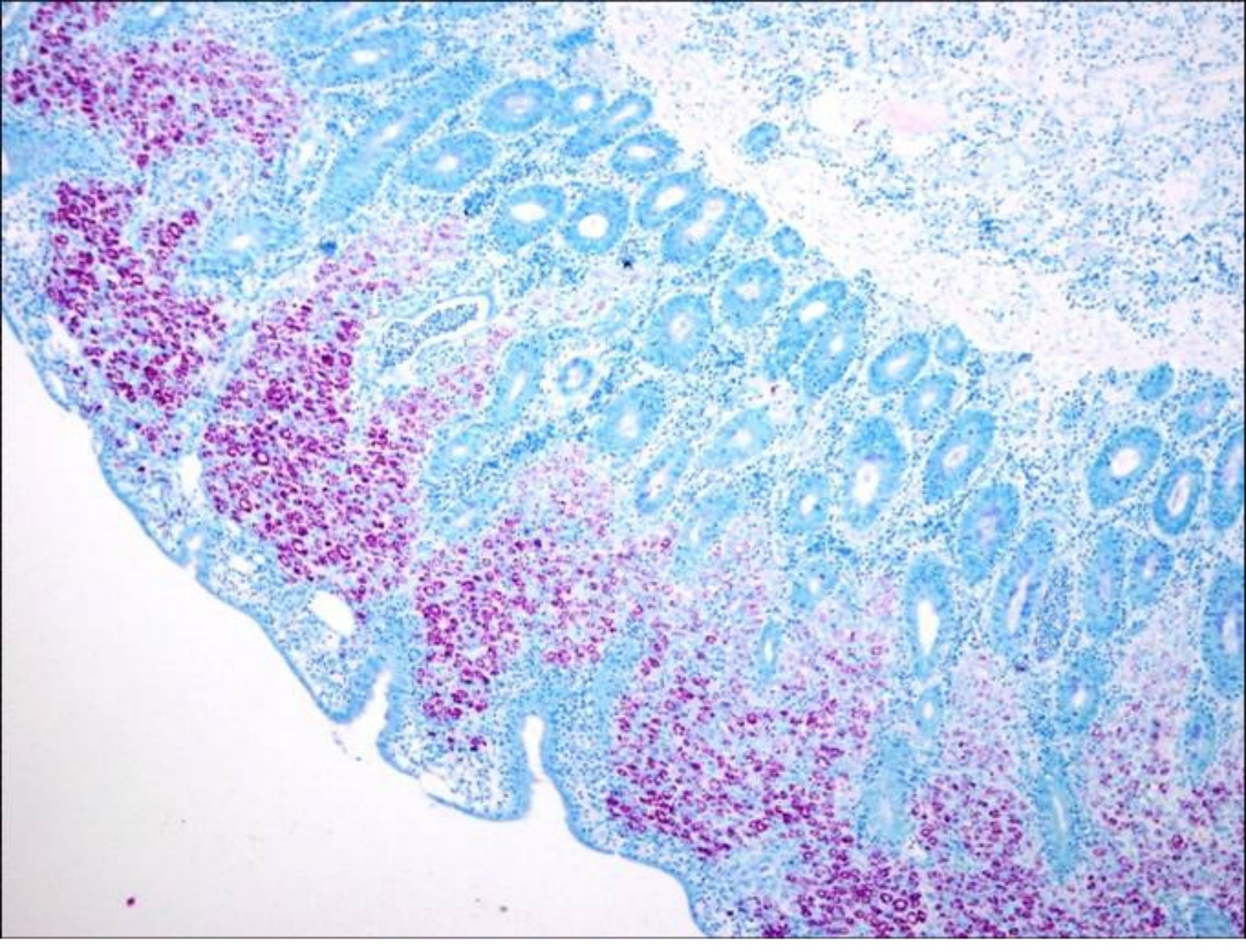


Figure 5a

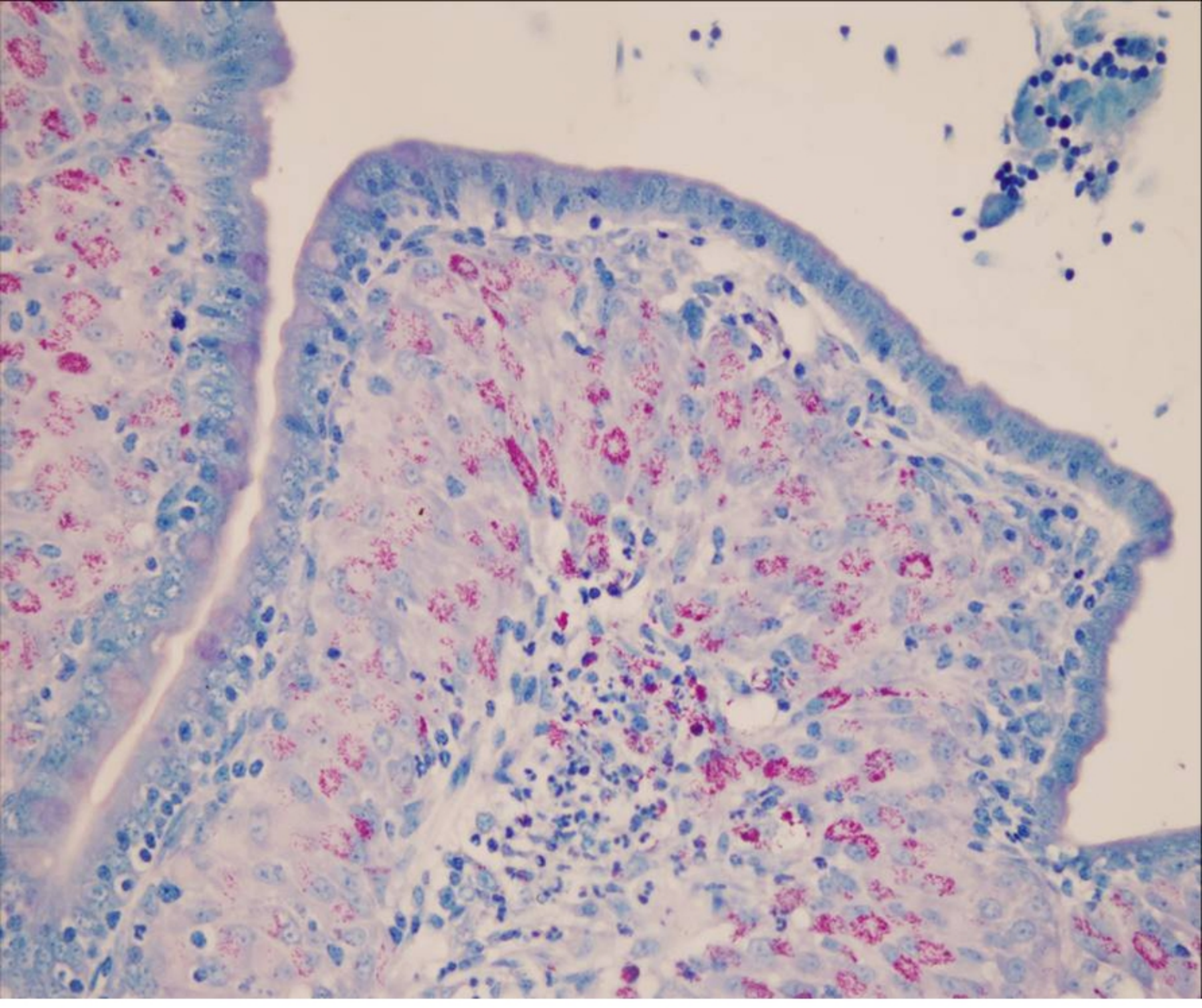


Figure 5b

preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

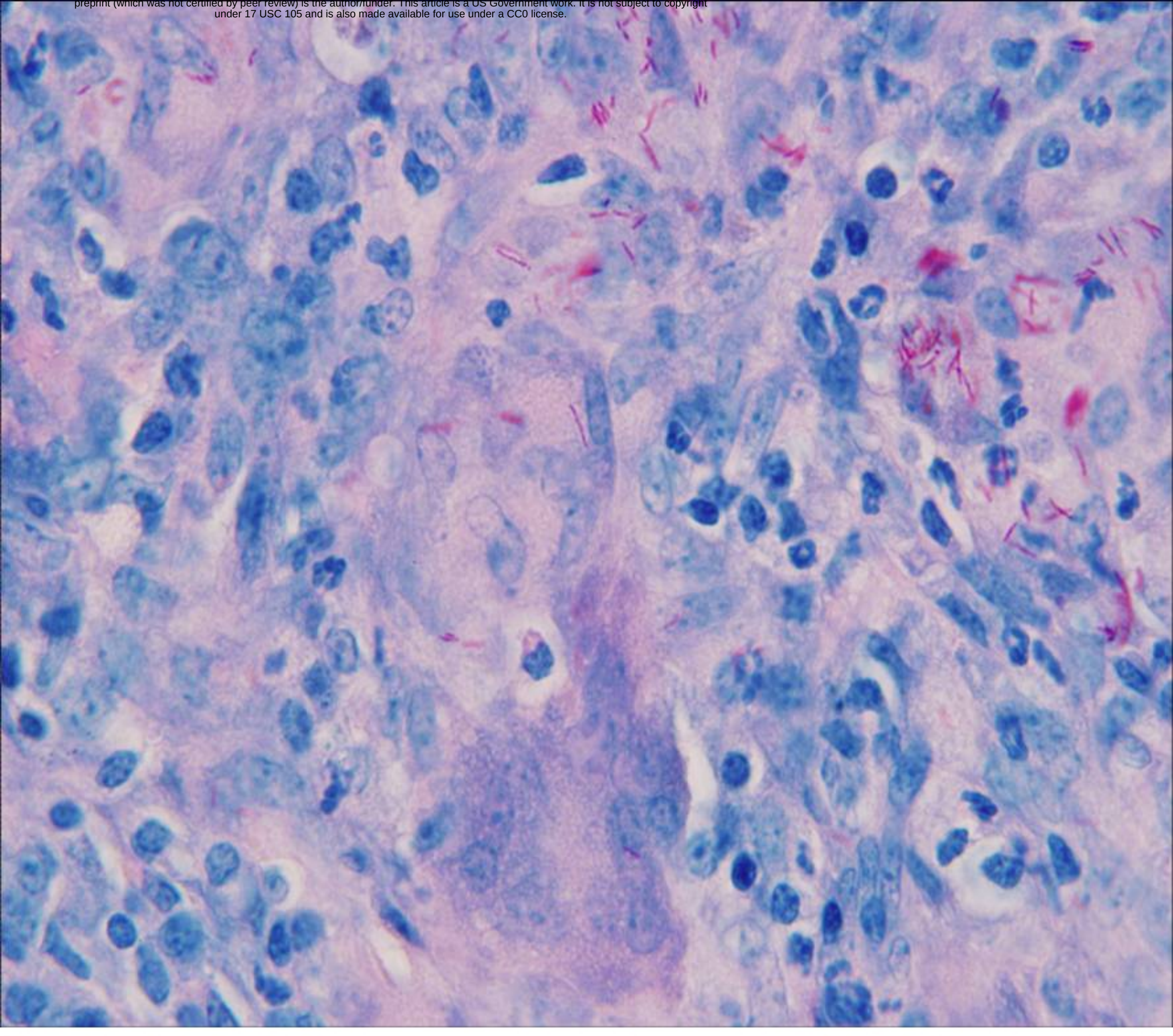


Figure 5c

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

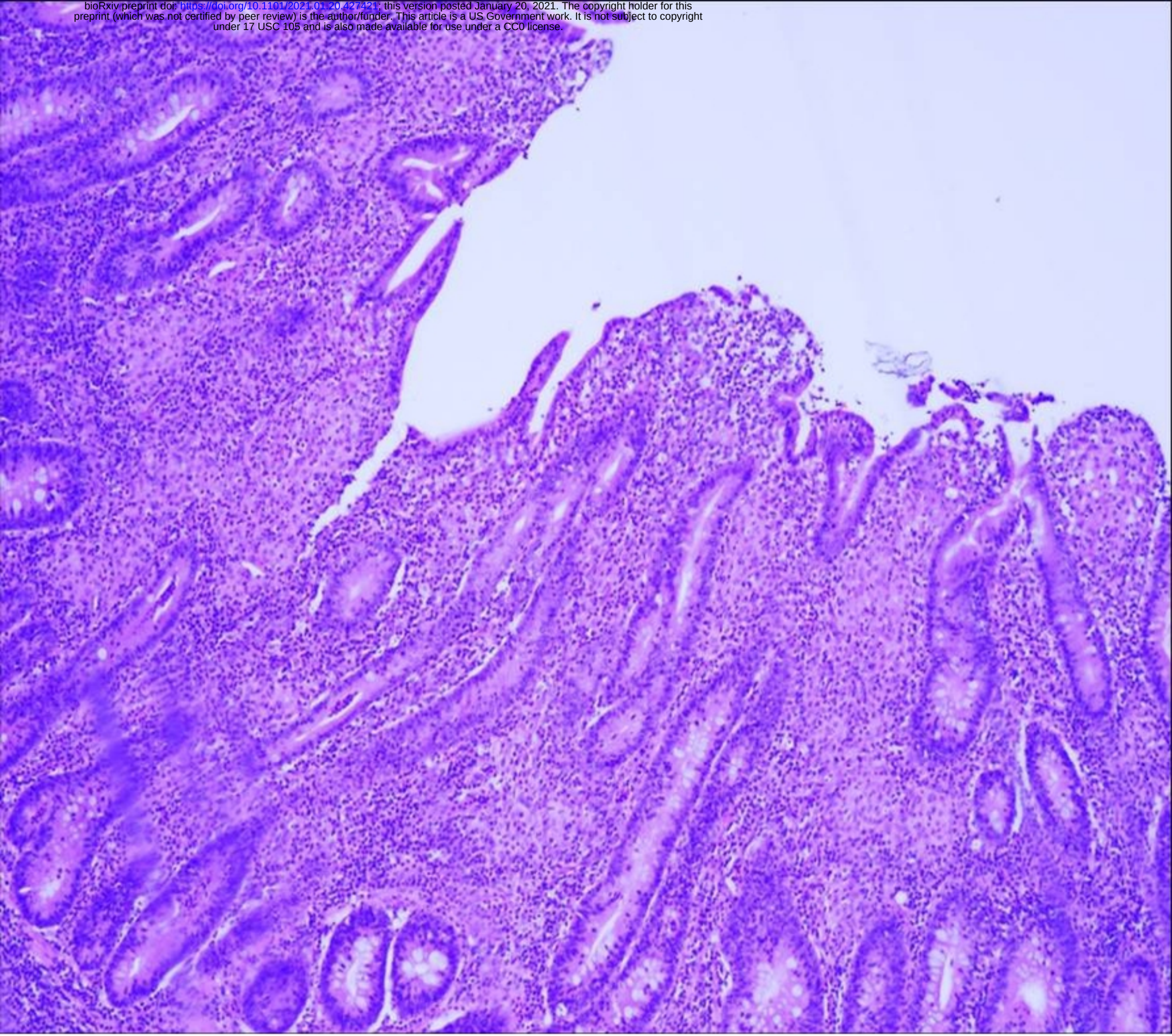


Figure 5d

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

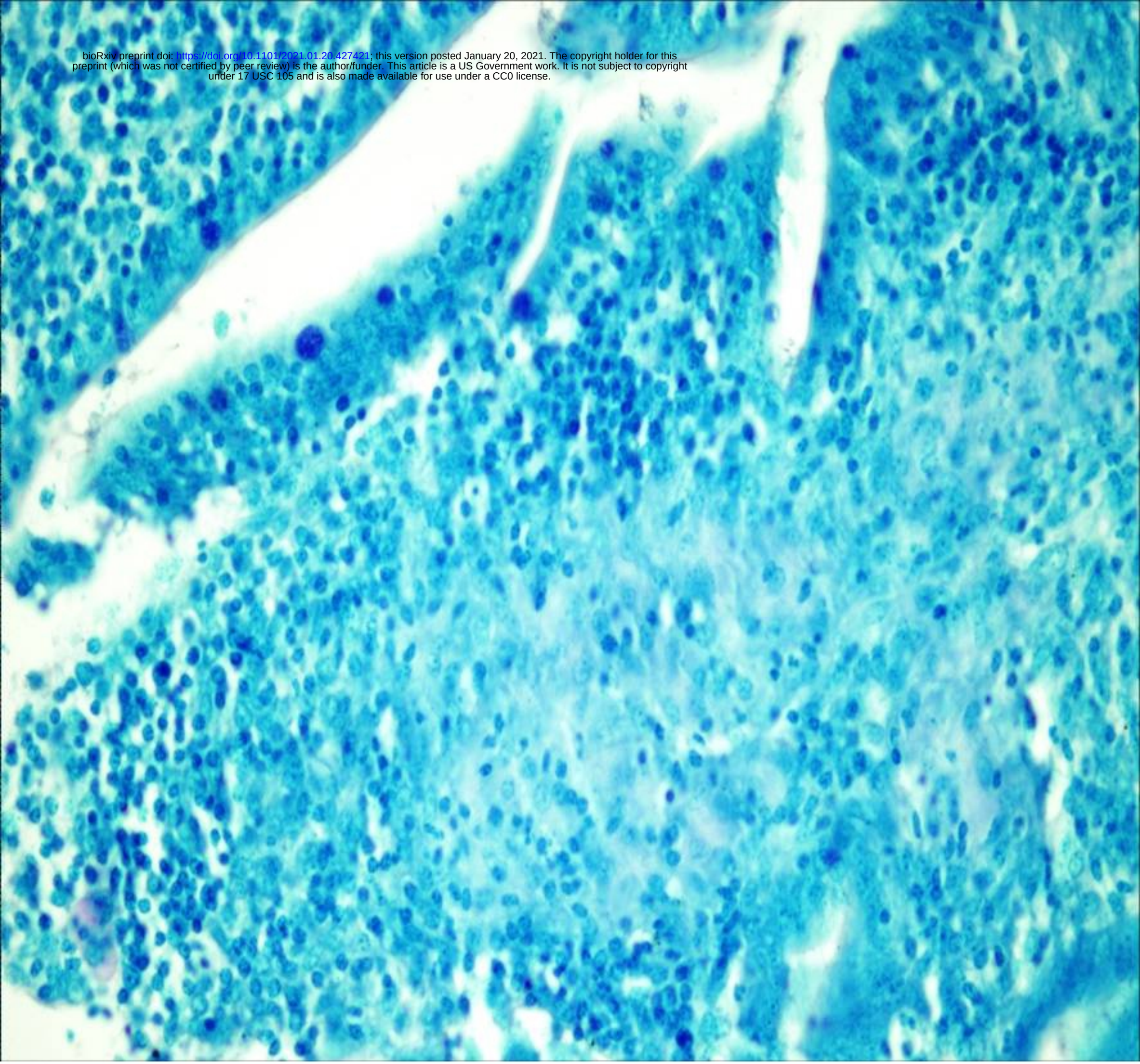


Figure 5e

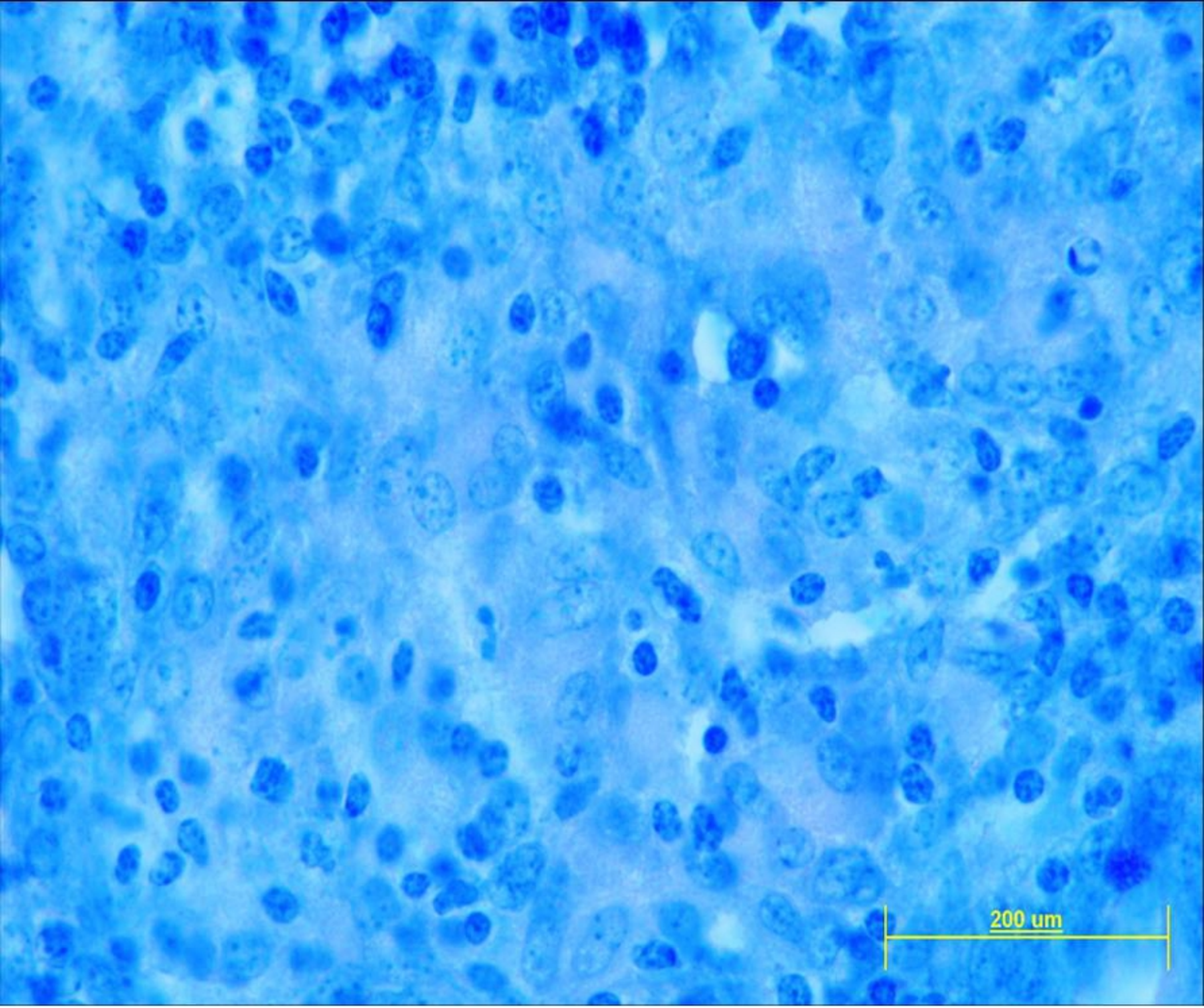


Figure 5f

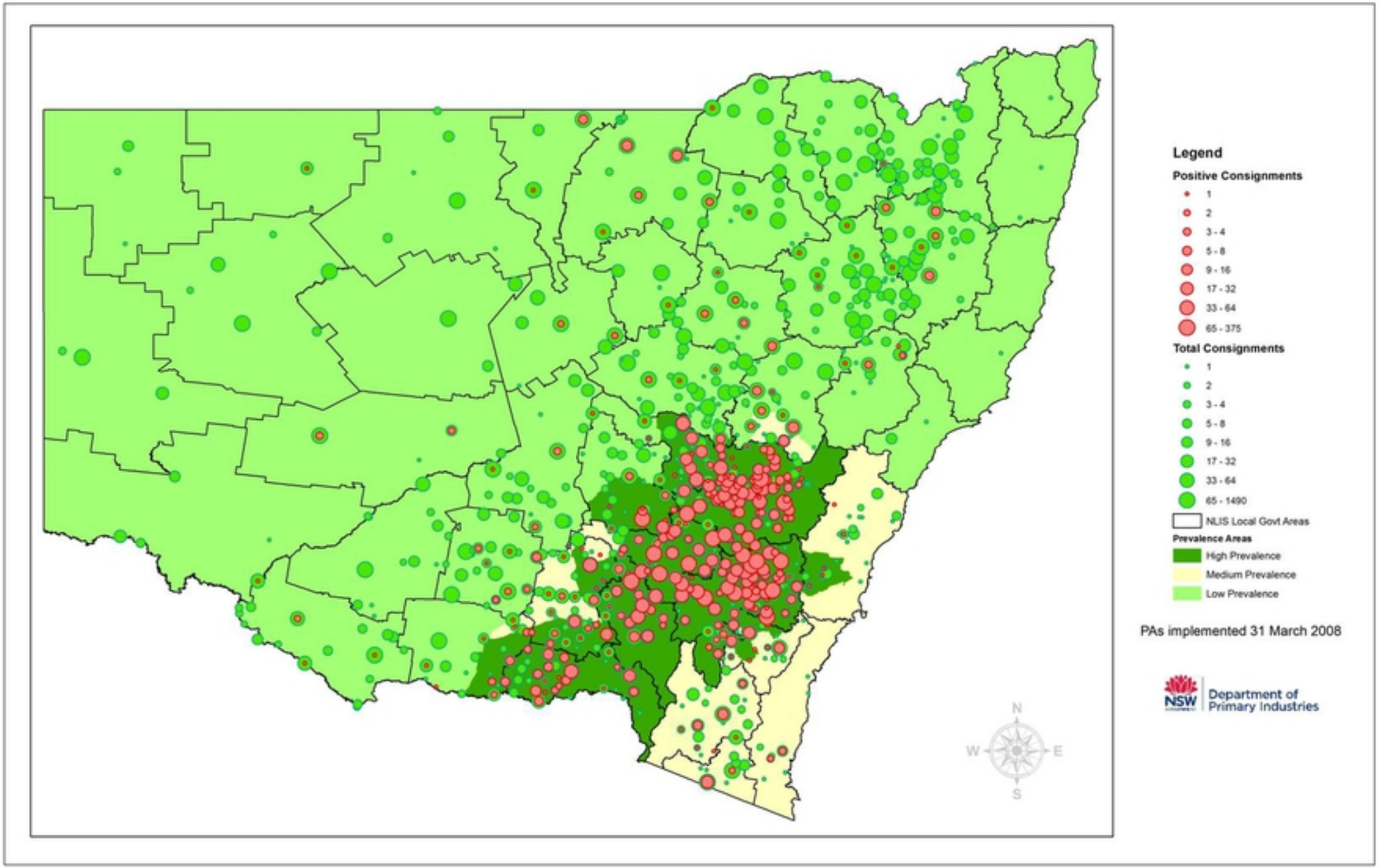
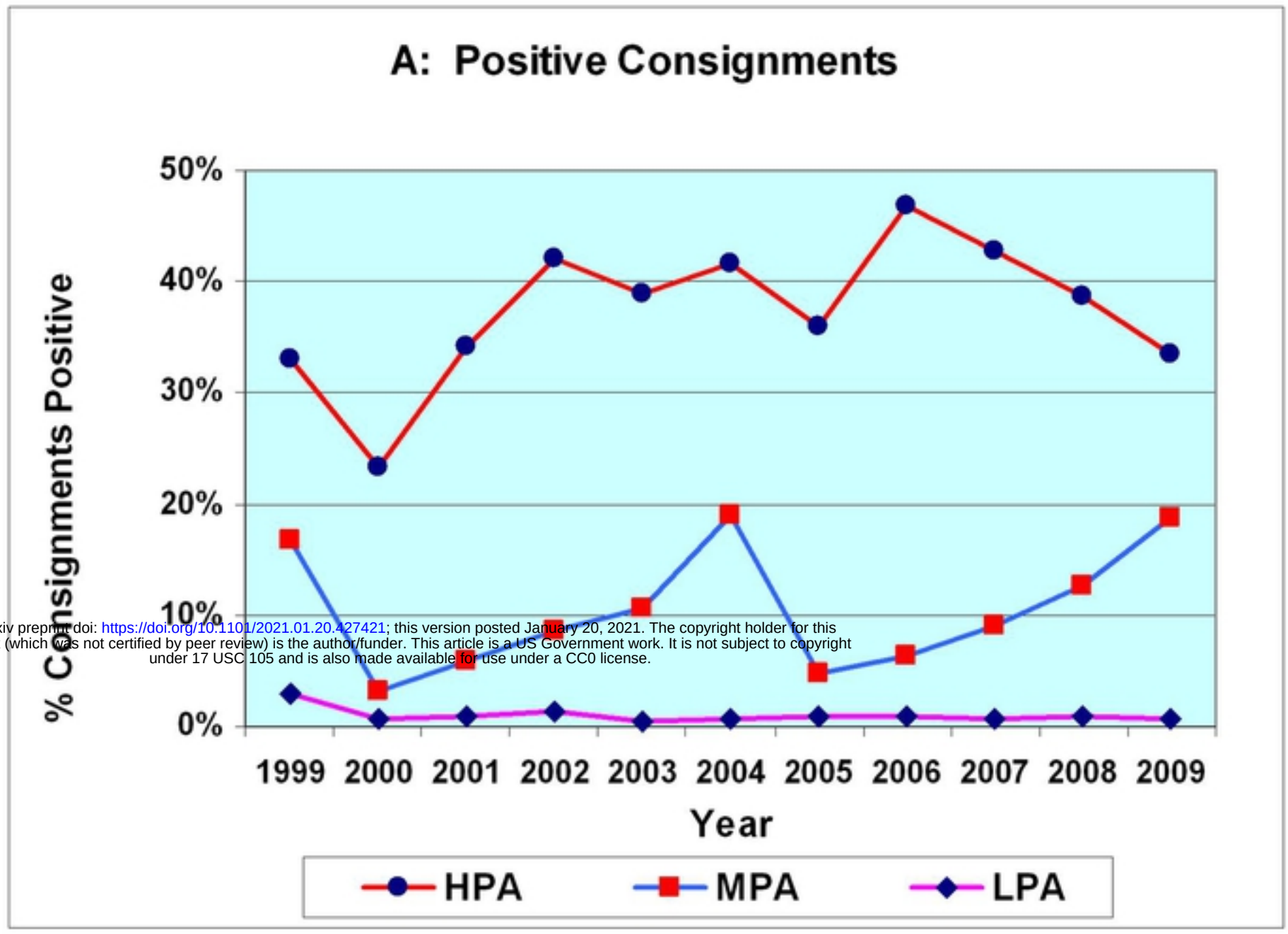

Legend
Positive Consignments
1
2
3 - 4
5 - 8
9 - 16
17 - 32
33 - 64
65 - 375
Total Consignments
1
2
3 - 4
5 - 8
9 - 16
17 - 32
33 - 64
65 - 1490
NLIS Local Govt Areas
Prevalence Areas
High Prevalence
Medium Prevalence
Low Prevalence
PAs implemented 31 March 2008
NSW
Department of Primary Industries
N
W
E
S

Figure 6



bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

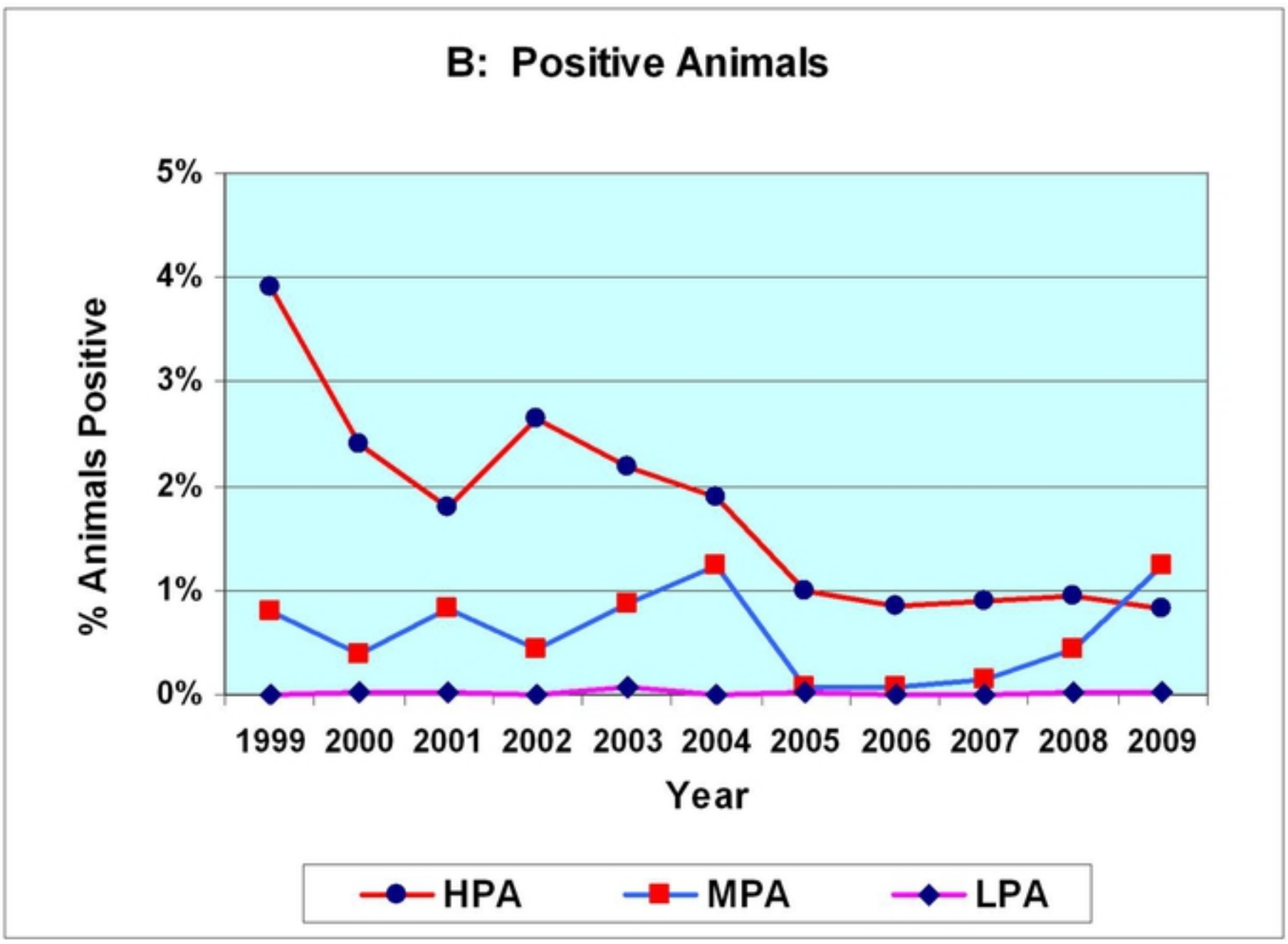
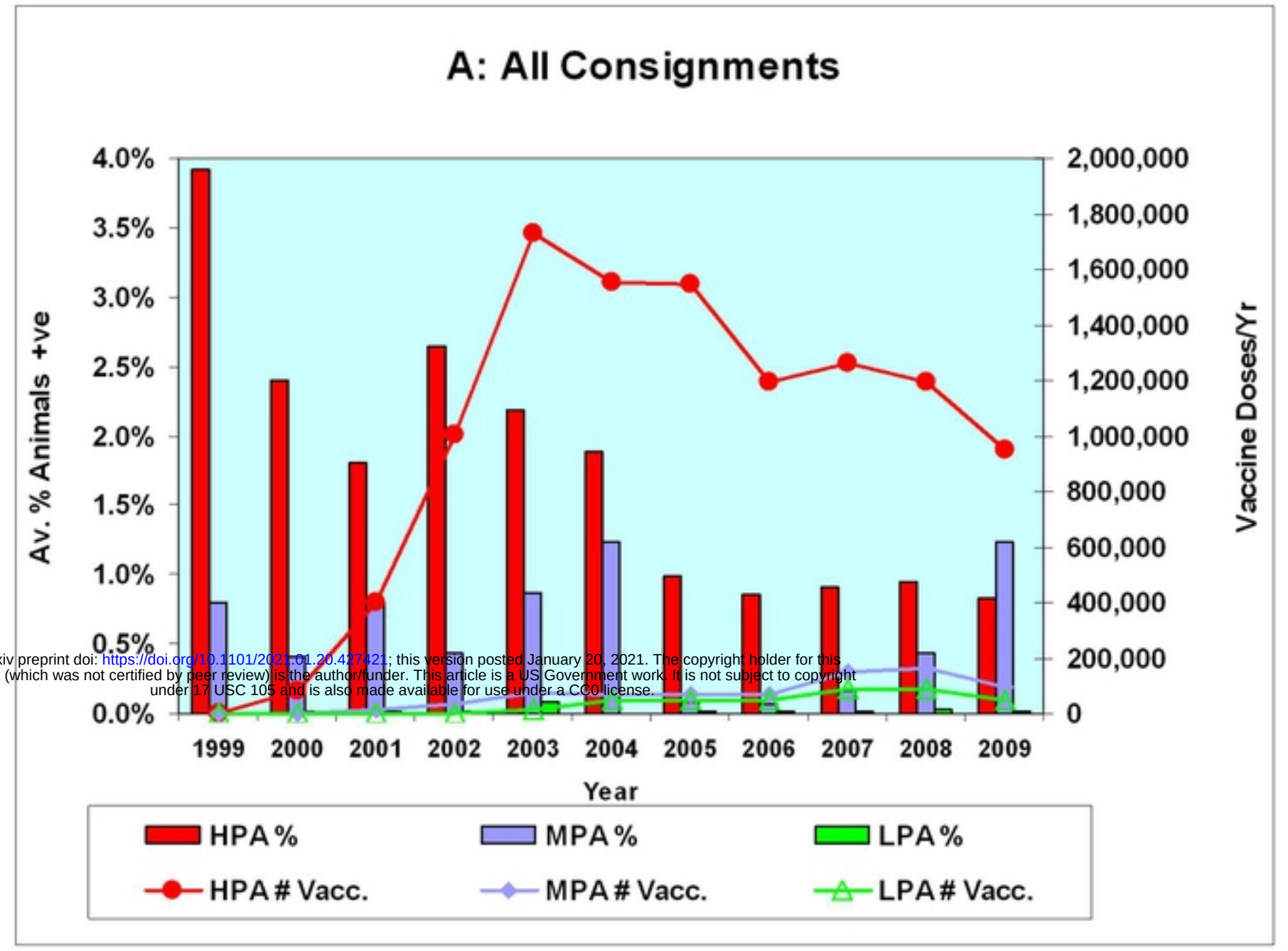


Figure 7



bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

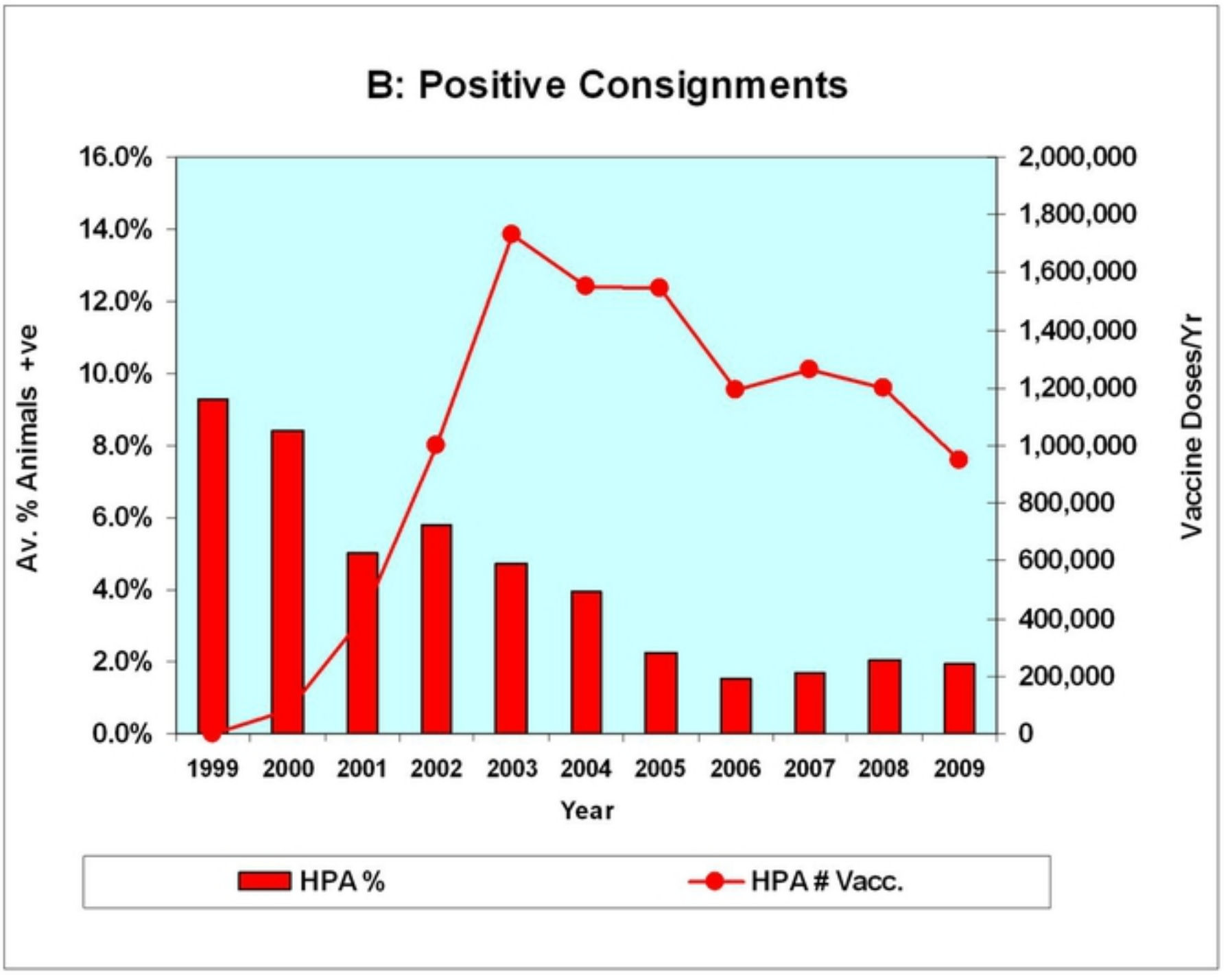
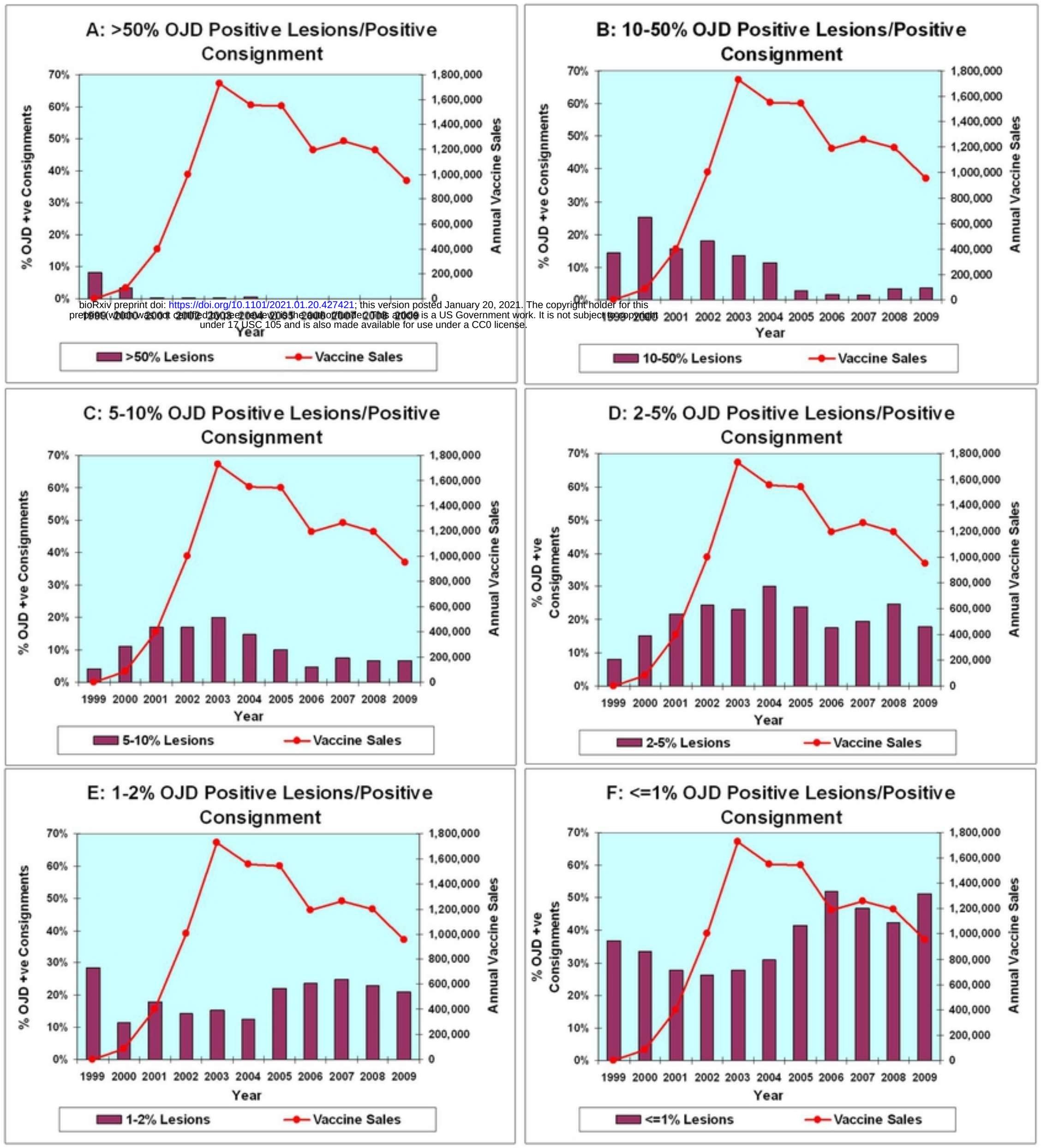


Figure 8



bioRxiv preprint doi: https://doi.org/10.1101/2021.01.20.427421; this version posted January 20, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. This article is a US Government work. It is not subject to copyright under 17 USC 105 and is also made available for use under a CC0 license.

Figure 9