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

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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,

52 the percentage of animals with lesions attributable to OJD at slaughter fell progressively
53 from 2.4% (10,406/432,860) at commencement of vaccination in 2000 to 0.8%
54 (1,573/189,564) by 2009. Herd immunity from vaccination in the HPA was estimated
55 at 70% by 2009. This coincided with a progressive decrease in reports of clinical disease
56 and mortalities in vaccinated flocks.

57 Significance

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

63 Introduction (See also S1 Text)

64 Johne's disease [1] is a chronic, incurable, fatal wasting disease of animals, particularly
65 ruminant animals, caused by *Mycobacterium avium* subspecies *paratuberculosis*
66 (MAP). Granulomatous enteritis, usually characterized by the presence of acid-fast
67 staining MAP, is a consistent feature of the disease. Johne's disease occurs on all
68 continents and infects domesticated [2-4] and wild animals [3-7]. Sheep with OJD in
69 Australia are infected by the S or "sheep" strain of MAP [8] which is slow growing and
70 fastidious in culture requirements on artificial media compared with the "cattle" or C
71 strain isolated from bovine Johne's disease (BJD) [9]. While the C strain was cultured
72 in Britain as early as 1912 [10], in contrast, reliable isolation of the S strain was not
73 reported until the late-1990's, with cultures requiring incubation for up to 16 weeks
74 [11]. Bovine Johne's disease is characterized by intractable diarrhea, leading to wasting
75 and death. It is a major concern for the dairy industry worldwide, with disease control
76 programs in many countries. In the USA for example, in 1997 when dairy herd MAP
77 infection herd-level prevalence was 22% [12], the estimated cost of BJD was in excess
78 of US$200 million /yr. By 2007 the USA herd-level prevalence had increased to 68%
79 of dairy herds (95% of herds with >500 cattle) [2], with a probable commensurate

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

83 While diarrhea is not a feature of OJD, the slowly progressive granulomatous enteritis
84 leads to malabsorption of nutrients and chronic weight loss. before the animal succumbs
85 2-6 months later. In some flocks, where lambs are exposed to heavy contamination,
86 deaths may be evident as early as 2 years of age. More often sheep succumb at 3-5 years
87 of age [24]. Spread of infection is by the feco-oral route, with late-stage animals
88 excreting as many as 10^8 organisms/gm of feces [25].

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

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

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

103 In the other Australian states, disease had not been detected in Victoria until 1989 in a
104 single ewe introduced from Tasmania and was not confirmed in home-bred sheep until
105 1995 [43]. Infection was confirmed on mainland South Australia in 1997 while the first
106 detection in Western Australia occurred in 2000 [41, 44].

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

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

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

154 This observational report documents the progressive changes in OJD prevalence
155 following the sale of 12,021,963 vaccine doses and 7,635,513 carcass examinations in
156 NSW, Australia from 1999 to 2009. During this period the sheep population of NSW
157 decreased by 50% from approximately 35.4 million in 2000 to 17.7 million in 2009
158 [59-61].

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

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

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

213 **Vaccination procedures** (See also S3 Text)

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

216 Standard Operating Procedures were developed to minimize the risk of operator self-
217 inoculation and a national register of accidental self-inoculation was maintained by
218 Pfizer Animal Health. To optimize the advantage of vaccination before significant
219 exposure to MAP, it was recommended as a single dose in lambs from 4-16 weeks of
220 age in NSW. Vaccination of older sheep was initially only permitted with approval of
221 NSW DPI. Some producers vaccinated older lambs, hoggets (12-24 months-old) and/or
222 2- year-old ewes, while whole of flock vaccination was undertaken in a number of
223 infected flocks.

224 Property disease management plans (PDMPs), financial 225 assistance and producer workshops (See also S4 Text)

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

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

233 Abattoir monitoring

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

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].

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

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

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

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

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

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

291 Data were recorded in a standardized format on inspection sheets and transmitted daily
292 by facsimile to a central facility at Orange (1999-2003), then Wagga Wagga NSW
293 (2003-2009). Inspection details and laboratory results were entered into a Microsoft
294 Access database maintained by NSW DPI.

295 Histopathology

296 Histopathology is considered the "Gold Standard" diagnostic test for OJD. Sensitivity,
297 however, was not assumed to be 100% because of the possibility that sites selected for
298 histological examination were not representative of the sites observed with gross lesions
299 [53]. Initially, samples from the terminal ileum, ileo-cecal valve and adjacent
300 mesenteric lymph nodes were submitted for confirmatory histopathology (Fig 2). From
301 2002, however, only the terminal ileum was examined after a formal research trial
302 demonstrated a minimal loss of sensitivity with this modification to the procedure
303 (unpublished report to Sub-Committee on Animal Health Laboratory Standards - Sept.
304 2002). This change to methodology permitted the terminal ileum from three carcasses
305 to be examined on a single microscope slide, thereby reducing laboratory costs by up
306 to two thirds.

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

310 Case Definition

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

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

316 Classification of samples and consignments

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

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

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

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

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

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

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

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

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

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

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

349 **"Corrected"** data were utilized for all analyses.

350 Specificity of visual inspection

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

354 Feedback to producers (See also S6 Text)

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

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

365 Flock and sheep numbers

366 Annual adult sheep and flock numbers (containing ≥50 adult sheep) in NSW (2000-
367 2009) were obtained from the RLPB (later Livestock Health and Pest Authority) Annual
368 Land & Stock Returns [59-61]. Flocks were allocated to prevalence areas based either
369 on RLPB or by property locality in the case of RLPBs which were split by prevalence
370 area boundaries.

371 Age at vaccination and cumulative (3 year) percentage of adult sheep 372 vaccinated

373 The age categories vaccinated and number of doses were recorded for 2000-2002 and
374 2007-2009. For the purposes of analysis, data for 2003-2006 were assumed to be similar
375 to 2007-2009. The vaccine doses sold annually as a percentage of adult sheep provide
376 a raw estimate of annual vaccination usage. However, the vaccine is "one dose for life"
377 and sheep may be vaccinated as lambs (<12-months-old, but recommended at <4-
378 months-old), hoggets (12-24-months-old) or adults (≥ 2-years-old). Hence, the
379 percentage of adult sheep currently vaccinated was determined by the cumulative
380 number of adults remaining in the flock that were vaccinated over the preceding years.
381 For the purposes of estimating the "cumulative" (rolling 3 year) percentage of
382 vaccinated adult sheep (corrected for age at vaccination), it was assumed that: a) all
383 vaccinated sheep were culled when they were nearing 5 years-old (representing 3 years
384 of productive adult life); b) those vaccinated as lambs and hoggets contributed to the
385 vaccinated adult numbers when 2 years-old, and c) all sheep vaccinated as adults were
386 vaccinated when they were 2 years-old [81]. Vaccine orders per flock per year were
387 determined from 2000-2008 (RLPB records) and 2007-2012 (Pfizer Database).

388 Sheep Breeds Affected

389 On-Farm Investigations 1982-2006

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

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.).

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

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

428

429 **Table 1. Number of Sheep Consignments Monitored at Abattoirs in NSW from**
430 **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

431 *The total number of sheep consignments monitored at abattoirs in NSW from 1999-*
432 *2009. Stratification is by OJD prevalence area. Total number per year (right-hand*
433 *column) and cumulative number per Prevalence Area from 1999-2009 (bottom row).*

434

435 **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

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

439

440 **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

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

444 Of the 1,379,819 carcasses which were inspected in the "Positive" consignments, 94%
445 (1,300,022) originated from the HPA (Table 4). Using our established criteria (see Case
446 Definition), 50,719 animals had OJD of which 96% (48,514) originated from the HPA
447 (Table 5). Of the 1,300,022 carcasses in OJD "Positive" consignments which
448 originated from the HPA, 3.7% (48,514) had OJD lesions (Tables 4 and 5).

450 **Table 4. Total Number of Sheep Inspected in "Positive" Consignments (1999-2009)**
451 **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

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

455

456 **Table 5. Total Number of Sheep with OJD Lesions in "Positive" Consignments by**
457 **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

458 *The numbers of sheep detected with OJD lesions in "Positive" consignments from*
459 *1999-2009. Stratification is by NSW Prevalence Area. Total number per year (right-*
460 *hand column) and cumulative number per Prevalence Area from 1999-2009 (bottom*
461 *row).*

462

463 During the course of the study, despite some variation between years, the percentage of
464 consignments that were "Positive" in the HPA leveled off between 33% and 47% (Table
465 3 & Fig 7A) confirming that flock-level prevalence remained consistently high.

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

473 Overall, the percentage of animals that were positive for OJD in **all** consignments
474 originating from the HPA fell progressively from 2.4% (10,406/432,860) of the
475 carcasses inspected at commencement of vaccination in 2000, to 0.8% (1,573/189,564)
476 of the carcasses inspected in 2009 (Tables 2 and 5; Figs 7B and 8A). However, during
477 this period, the percentage of animals that were positive for OJD in **"Positive"**
478 consignments from the HPA fell markedly from 8.5% (11,689/137,236) of the carcasses
479 inspected in 1999-2000 to 2.0% (3,849/190,399) of the carcasses inspected in 2008-
480 2009 (Tables 4 and 5; Fig 8B).

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

490 # Vaccination

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

494 infected flocks whose annual mortality attributable to OJD was greater than 5%. A total
495 of 63,000 doses had been ordered for 51 flocks by the end of September 2000. By April
496 2002, when the Gudair® OJD vaccine was approved and registered for use in all states
497 of Australia and restrictions eased considerably, there were 237 heavily infected flocks
498 (>5% annual mortality) enrolled in NSW allocated 574,150 doses.

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

508

509

510 **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

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

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

519 consignment (Fig 8B). A more detailed analysis revealed a time-dependent positive
520 correlation between the increasing number of vaccinated sheep (as vaccination of the
521 HPA population proceeded) and a declining number of "Positive" consignments with a
522 high percentage of infected animals (Figs 9A-F). This decrease was most pronounced
523 for OJD "Positive" consignments categorized with >10% of positive animals. There
524 were 28.5% of consignments in this category in 2000 falling to ~2.3% of consignments
525 from 2005-2009 (Figs 9A and B). Furthermore, there was a progressive increase in
526 consignments with a low percentage of infected animals as vaccination proceeded.
527 Positive consignments with <1% of animals infected comprised ~34% in 2000 rising to
528 ~50% by 2009 (See Figs 9C-F for trends).

529 **Fig 9. Percentage of Lesions Attributed to OJD in Positive Consignments from**
530 **the HPA Following Institution of Vaccination in 2000.** (A-F) - For clarity, the
531 Results from the HPA only are displayed. A comparison of the annual number of
532 vaccine doses sold with the percentage of positive animals per positive consignment. In
533 each graph, the total doses of vaccine sold are repetitively represented by a line (scale
534 on the right-hand side vertical axis) while the percentage of positive consignments in
535 each percentile category is shown by vertical bars (scale on the left-hand side vertical
536 axis). (A) shows data where the percentage of animals positive per positive
537 consignment exceeds 50%. This only occurs in 1999 and 2000, when vaccine sales were
538 negligible. (B) and (C) show a progressive fall in the percentage of consignments where
539 5%-50% of animals were positive per positive consignment. In contrast, by (F) (≤1%)
540 an increasing percentage of consignments have a low percentage of animals positive as
541 vaccination progresses. We interpret these results as indicating improvement in the
542 control of OJD by vaccination.

543 Specificity of visual inspection assessed by histopathology

544 Review of the histopathology results for 11,687 individual sheep in consignments
545 derived from the HPA confirmed 9,570 as OJD positive (either multibacillary or
546 paucibacillary), an individual animal specificity of 82%. In the MPA the specificity was
547 63% (271/428) and in the LPA, where inspectors were deliberately encouraged to

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

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

554 Flock and sheep numbers

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

562

563 **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%

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

568 Age at vaccination and percentage of adult sheep vaccinated

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

573 23% at 7-12 months. In contrast, vaccine sales in the HPA from 2007-2009 were
574 categorized as: 94% lambs (<12 months), 2% hoggets and 4% adults. Corresponding
575 figures for sheep in the MPA (2007-2009) were 87%, 3% and 10% and e 90%, 1% and
576 9% for sheep in the LPA.

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

582 **Table 8. Estimated % of Sheep Vaccinated in NSW (1999-2009) by Prevalence**
583 **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%

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

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

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.

604 **Table 9a. Number of Flocks Vaccinated in the High Prevalence Area 2000-2008**
605 **(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]

606

607

608

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

611

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]

612

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

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

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

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

621 were vaccinated at least once between 2000 and 2008 (400-600 flocks per year from
622 2002 onwards) and 2483 flocks were vaccinated at least once from 2007-2012 (~1300
623 flocks annually). The average number of sheep vaccinated per flock annually rose from
624 1522 in 2000 to 2095 in 2001 before declining to 1205 in 2002 and levelling off at
625 ~600-800/year from 2003-2012. The percentage of these flocks with more than 2000
626 sheep vaccinated annually ranged from 10-18% from 2000 to 2002, reduced to ~4-6%
627 of from 2003-2006 then rose to 6-8% from 2007-2012 after sales were permitted
628 through rural merchandisers.

629 Sheep Breeds Affected

630 On-Farm Investigations 1982-2006

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

634 Abattoir Monitoring NVDs 2003-2007

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

638 Merinos comprised 89.3% of consignments, 93.8% of 616,500 sheep inspected, 94.7%
639 of 1036 positive consignments, 96.1% of 340,940 sheep in positive consignments and
640 97.5% of 6904 sheep with lesions attributable to OJD. Merinos also showed a higher
641 proportion of heavily infected consignments with 14.6% (143/981) of consignments
642 detected with ≥5% lesions, 4.5% (44/981) with ≥10% lesions and 0.2% (2/981)
643 exceeding 30% lesions. This compared with positive consignments involving
644 Crossbred sheep where 3.6% (2/55) were detected with ≥5% lesions while none
645 exceeded 10% lesions.

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

674 94.7% of OJD positive consignments and 97.5% of sheep with lesions attributable to
675 OJD were of the Merino breed [86]. Merinos also showed a higher proportion of heavily
676 infected consignments with 14.6% of positive consignments detected with ≥5%
677 lesions,compared with 3.6% of consignments involving Crossbred sheep. However,
678 these findings could be confounded by differences in flock sizes between breeds,
679 regional differences in breed distribution or repeat investigation of individual flocks.

680 Economic and social impact of OJD (See also S7b)

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

687 At the same time, many infected flocks had a high annual mortality rate (≥5%) [55, 87-
688 90] evidenced by the demand for vaccination between January 2000 and April 2002
689 prior to registration of the vaccine for widespread use [55].

690 Factors in disease spread (See also S7c)

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

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

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

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

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

730 Concurrently, the impact of drought in NSW generally, and the HPA in particular, from
731 2002 onwards led to accelerated culling of non-breeding stock (wethers for wool
732 production) and older age groups of ewes (frequently unvaccinated). The selective
733 culling of more heavily OJD infected mobs showing clinical disease (wasting or
734 mortalities) may have contributed to a surge in detection from 1999-2004, particularly
735 consignments with 10% or more lesions attributable to OJD (Figs 9A & B). At the same
736 time, culling high risk mobs would have reduced (at least temporarily) the average
737 animal-level prevalence within these flocks.

738 The initial shortage of vaccine and restricted usage pending final registration in April
739 2002 [55] would also have delayed the overall impact of vaccination in the HPA.
740 Financial stringency due to the drought, balanced by financial assistance to purchase
741 vaccine, was also a significant factor in the uptake of vaccination. Thereafter, however,
742 there was a progressive area-wide decrease in animal level disease prevalence in the
743 HPA, as would be expected in an effective vaccination campaign. This was despite the
744 flock-level prevalence continuing to remain very high, with more than 50% of HPA
745 flocks estimated to be infected in 2008 based on abattoir monitoring (median 51.8%;
746 95% upper confidence limit – 95.6%) [49].

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

753 Vaccinating infected sheep – concerns regarding 754 exacerbation of disease (See also S7e)

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

764 Vaccine – preventative or therapeutic? (See also S7f)

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

777 Age groups vaccinated

778 The primary message was to vaccinate as lambs (75% of vaccine usage 2000-2002 and
779 94% from 2007-2009) and preferably <4 months of age ("approved vaccinates" – 41%
780 from 2000-2002). However, many producers throughout the period of this study,
781 particularly those suffering heavy losses, were found to have vaccinated their whole
782 flock or selected older age groups (17% hoggets and 8% adults from 2000-2002 falling

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

789 "Herd" immunity (See also S7g)

790 Vaccination of older lambs, hoggets and adult sheep undoubtedly contributed to a more
791 rapid increase in "Flock/Herd Immunity", both within individual flocks and the general
792 sheep population of the HPA. This outcome is evidenced by the "cumulative"
793 vaccination status in the HPA reaching an estimated 16% by 2004, 45% by 2006 and
794 70% in 2009 (Table 8) [81]. This result is consistent with a previous estimate that ~70%
795 of replacement sheep were being vaccinated annually in the HPA by 2007 [57].
796 Vaccination of older sheep is highly likely to have contributed to a more rapid decline
797 in animal-level prevalence of OJD than might otherwise have been observed [93].

798 Vaccinating flocks in the HPA

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

805 Persistent flock infection/excretion

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

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

810 These results are consistent with the findings of Windsor (2014) who reported that 81%
811 (30/37) of known infected commercial flocks monitored in a research trial using PFC
812 in NSW and Victoria continued to shed MAP. This was despite significant declines in
813 estimated OJD prevalence following vaccination as lambs for ≥5 years [94]. They
814 considered these flocks at risk of spreading disease or suffering recrudescence of losses
815 if vaccination were to cease and they advised flock managers to persist with
816 vaccination. They also reported a decline in animal-level prevalence from a pre-
817 vaccination median of 2.7% (95% confidence interval: 1.4 - 6.9) to a post-vaccination
818 median of 0.7% (0.4 - 1.3).

819 Factors contributing to decrease in regional animal-level 820 prevalence

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

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

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

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

853 Prevalence areas – role of abattoir monitoring

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

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

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

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

875 NLIS and traceability to property of origin

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

890 Sensitivity and specificity (See also S7h)

891 The sensitivity and specificity of abattoir monitoring was unknown at the

892 commencement of monitoring in 1999. However it was clear within weeks that the
893 procedure was capable of detecting sub-clinical OJD (often in a high proportion of
894 animals) in many consignments from the HPA, while consignments from the LPA were
895 routinely negative. A research trial was incorporated into the routine abattoir
896 monitoring in 1999-2000, to determine the sensitivity and specificity of visual/tactile
897 inspection of viscera for lesions suggestive of OJD, with follow-up histopathology on
898 3 animals [72]. This study estimated consignment sensitivity of 97%, and a practical
899 working sensitivity of 90%, in consignments of >300 adult sheep, for any sheep
900 population in which OJD has been recognized for many years (see S7h for details).

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

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

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

917 There remains no clear scientific explanation as to why the majority of sheep sampled
918 (83%) exhibit multibacillary pathology while others in the same consignment exhibit
919 paucibacillary pathology. It is clear that inspectors are capable of detecting both forms

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

922 Limitations

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

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

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

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

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

964 Support by abattoir management

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

972 Producer acceptance (trust) of abattoir monitoring results

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

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

981 Saleyard consignments (See also S7i)

982 Concerns were raised about producers avoiding monitoring by selling through saleyards
983 and their sheep being slaughtered in mixed consignments from multiple vendors [41,
984 50]. This was considered a factor in missed detection of some smaller individual
985 infected flocks, however the impact was minimized with the cooperation of the saleyard
986 and transport operators. The integrity of the majority of larger individual property
987 consignments sold through saleyards was able to be carefully maintained up to the time
988 of slaughter. This segregation enabled them to be classified as "direct" single property
989 consignments and included in the study. This approach was particularly important for
990 regions distant from an abattoir where it was more cost effective for producers to sell
991 through saleyards.

992 Regional prevalence data and surveillance

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

999 Ongoing and effective disease surveillance is necessary to be able to determine the
1000 efficacy, or lack thereof, of control interventions such as vaccination. More importantly,
1001 ongoing and effective disease surveillance is necessary to be able to rapidly provide

1002 sheep producers with evidence of recent infection in their flocks. It is likely that the lag
1003 phase in detection, due to the long incubation period of OJD, has most impact in regions
1004 where OJD is newly emerging. However, the comprehensive centralized monitoring
1005 afforded by abattoir surveillance, offers the best opportunity for early and more cost
1006 effective detection compared with more targeted, labor intensive, on-farm surveillance
1007 procedures. This evidence also provides the best motivation for a producer to initiate
1008 vaccination – well before OJD mortality rises to a level where there is high level and
1009 widespread environmental contamination on the property due to increased infection
1010 rates.

1011 Accidental operator self-inoculation

1012 Minimizing the risk of accidental self-inoculation with Gudair® was also a primary aim
1013 of the vaccination program. The risk was mitigated by a concerted effort by Pfizer,
1014 NSW DPI, RLPBs and the sheep industry to communicate the nature of the risk through
1015 training programs for producers and vaccine merchandisers, and through standardized
1016 vaccine handling and vaccination procedures. Development by Pfizer of the novel
1017 "Sekurus"® vaccinator syringe with a protective shroud and double locking
1018 mechanism, and increasing the awareness within the rural medical community were
1019 critical initiatives. Many producers minimized their personal risk by employing contract
1020 vaccinators. Nonetheless small numbers of producers suffered severe impact from
1021 accidental self-inoculation [96, 97] and fear of self-inoculation caused some producers
1022 to avoid vaccinating their flocks.

1023 Vaccine abscesses (See also S7j)

1024 The risk of vaccine abscesses [32] was also of serious concern to the sheep industry
1025 both from an animal welfare perspective and the potential for abscesses to impact on
1026 abattoir processing. Producer workshops and a standardized vaccination procedure
1027 requiring sub-cutaneous injection high on the neck (at the base of the ear) were effective
1028 in minimizing untoward outcomes. From commencement of monitoring in 1999,
1029 producers were notified directly by the abattoir if any abscesses were detected in neck

1030 muscle at slaughter.

1031 Potential role of vaccination in controlling BJD in cattle (See also S7k)

1032 Vaccination with a killed vaccine may also be a valuable adjunct to current strategies
1033 for the control of Johne's disease (BJD or paratuberculosis) in cattle. Government
1034 agencies, however, have been reluctant to initiate Johne's disease vaccination programs
1035 in cattle. In the past, the most compelling concern was loss of ability to diagnose
1036 tuberculosis (usually *M. bovis*) using skin testing [4, 98]. Out of 22 countries with
1037 formal BJD control programs, only 7 were reported to have included the option of
1038 vaccination [4]. This is despite the evidence that standard pasteurization reliably kills
1039 *M. bovis* in bovine milk, in contrast to MAP which is not reliably killed by
1040 pasteurization [99, 100].

1041 In Australia, where bovine tuberculosis has been eradicated, vaccination of infected
1042 dairy or beef herds against paratuberculosis is considered likely to be cost-effective [4].

1043 We conclude that Government regulatory agencies and the veterinary profession should
1044 give serious consideration to progressing further Johne's disease vaccination studies in
1045 cattle and to comparing vaccination cost and efficacy with failure to vaccinate [4, 28,
1046 30, 31, 36, 101]. An effective vaccine could make a major contribution to control of the
1047 production losses and potential human health risks associated with BJD. The endemic
1048 spread of BJD in recent decades has been recently likened by Whittington *et al.* (2019)
1049 to a slow moving, uncontrolled long-incubation pandemic [4].

1050 Potential role of abattoir monitoring for BJD in cattle

1051 Similarly, the potential surveillance benefits of implementing abattoir monitoring for
1052 BJD in cattle (particularly dairy cattle) should be explored. In a 2019 review, 38
1053 countries out of 48 were reported to have formal tuberculosis control programs [4].
1054 While cattle at slaughter throughout the world are routinely monitored by inspectors for
1055 tuberculosis, it is extremely rare for concurrent monitoring to be undertaken for MAP
1056 infection [102]. Dahmen et al (2018) recently reported visual detection of 143 cases of

1057 BJD during official meat inspection between 2015 and 2017 in an abattoir in Germany
1058 [103]. They identified both early and advanced cases of MAP infection (confirmed by
1059 histopathology). They also demonstrated the presence of MAP by culture and PCR in
1060 skeletal muscle from 39% of the 143 BJD positive cattle.

1061 Deer in New Zealand are routinely monitored at slaughter for both tuberculosis and
1062 paratuberculosis in a producer funded program [4].

1063 If MAP is zoonotic, cattle (through milk, milk products and meat) likely present a
1064 greater risk than sheep for food-borne infection of humans around the world[18]. In
1065 contrast to tuberculosis, only 22 of 48 countries in the same review were identified
1066 with formal BJD/paratuberculosis control programs, with major surveillance deficits
1067 impacting their ability to implement effective control programs [4]. Routine monitoring
1068 of cattle (deer and goats) for paratuberculosis at slaughter, in conjunction with current
1069 official monitoring for tuberculosis, would be a cost-effective approach to resolving the
1070 surveillance deficit for BJD. As demonstrated in the current study, it would facilitate
1071 producer understanding of the extent of infection in their herds and promote more
1072 effective control measures including vaccination.

1073 Animal model for mycobacterial diseases in humans

1074 Vaccination of sheep against MAP with Gudair®, a killed whole cell vaccine with oil
1075 adjuvant, may also provide an important animal model for vaccination for
1076 mycobacterial diseases (tuberculosis, leprosy and possibly Crohn's disease) in humans.
1077 While noting the adverse outcome of severe granulomatous reactions typically seen in
1078 humans following accidental self-inoculation with Gudair®, alternative adjuvant and
1079 delivery mechanisms should be explored.

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.

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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

1532 **S7h - Sensitivity and specificity**

1533 **S7i - Saleyard consignments**

1534 **S7j - Vaccine abscesses**

1535 **S7k - Potential role of vaccination in controlling BJD in cattle**

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

1554 PFC – Pooled Fecal Culture

1555 POS - Positive

1556 RLPB – Rural Lands Protection Board

1557 “S” Strain – “Sheep” strain of MAP

1558 ZN – Ziehl-Neelsen stain

1559 END

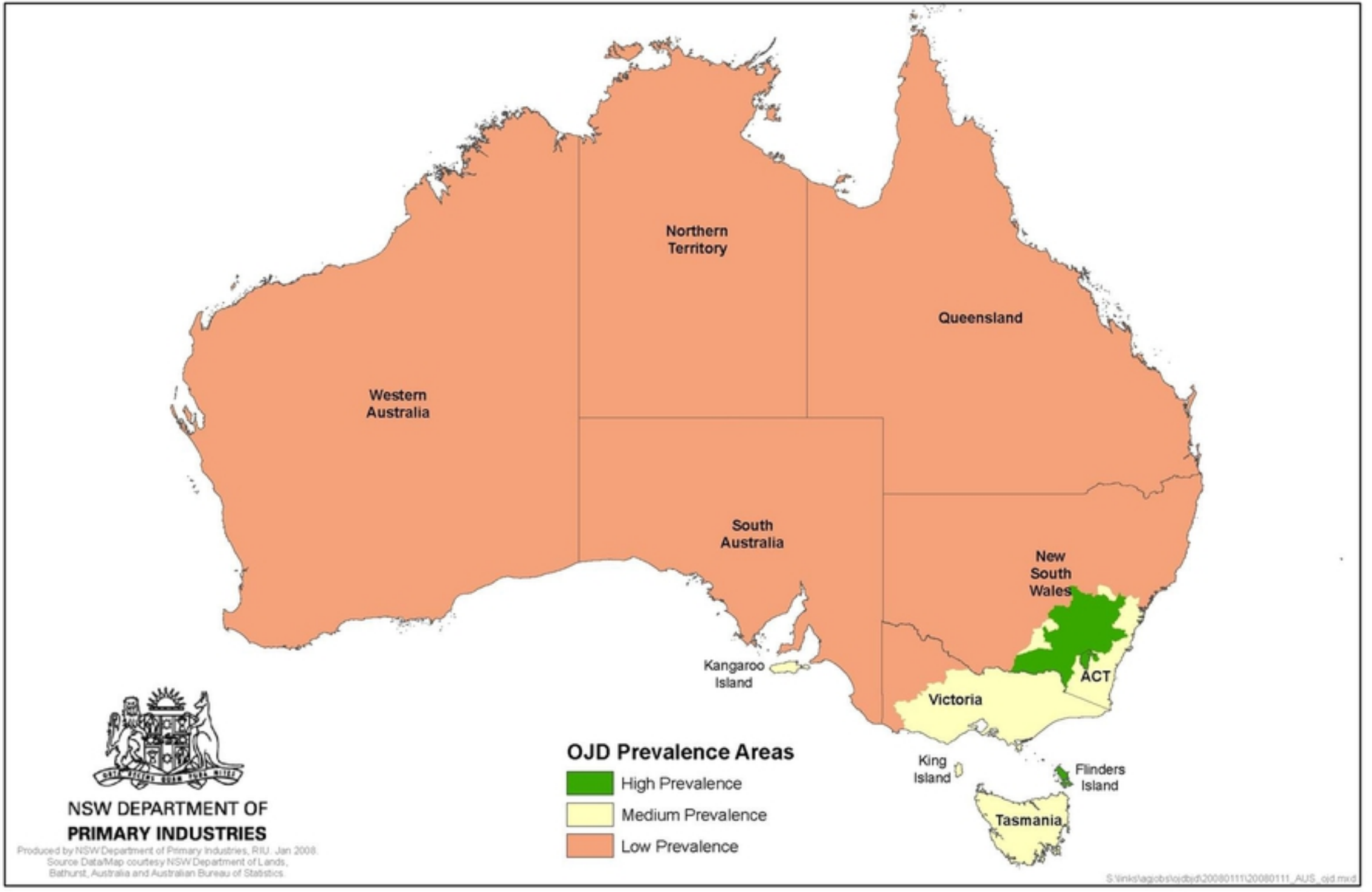

Northern Territory
Queensland
Western Australia
South Australia
New South Wales
ACT
Victoria
Kangaroo Island
King Island
Flinders Island
Tasmania
OJD Prevalence Areas
High Prevalence
Medium Prevalence
Low Prevalence
NSW DEPARTMENT OF
PRIMARY INDUSTRIES
Produced by NSW Department of Primary Industries,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

Figure 1

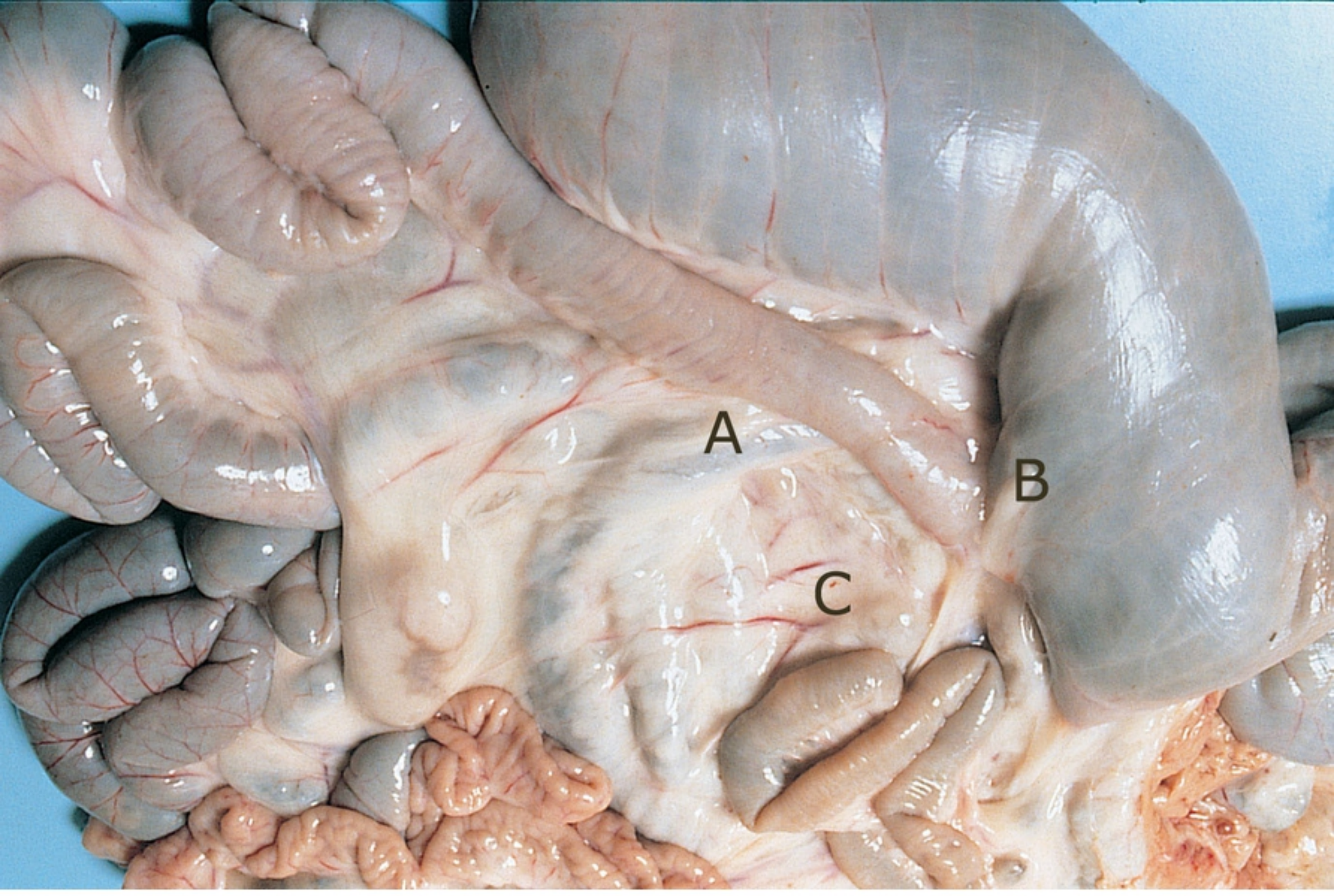


Figure 2

Figure 3

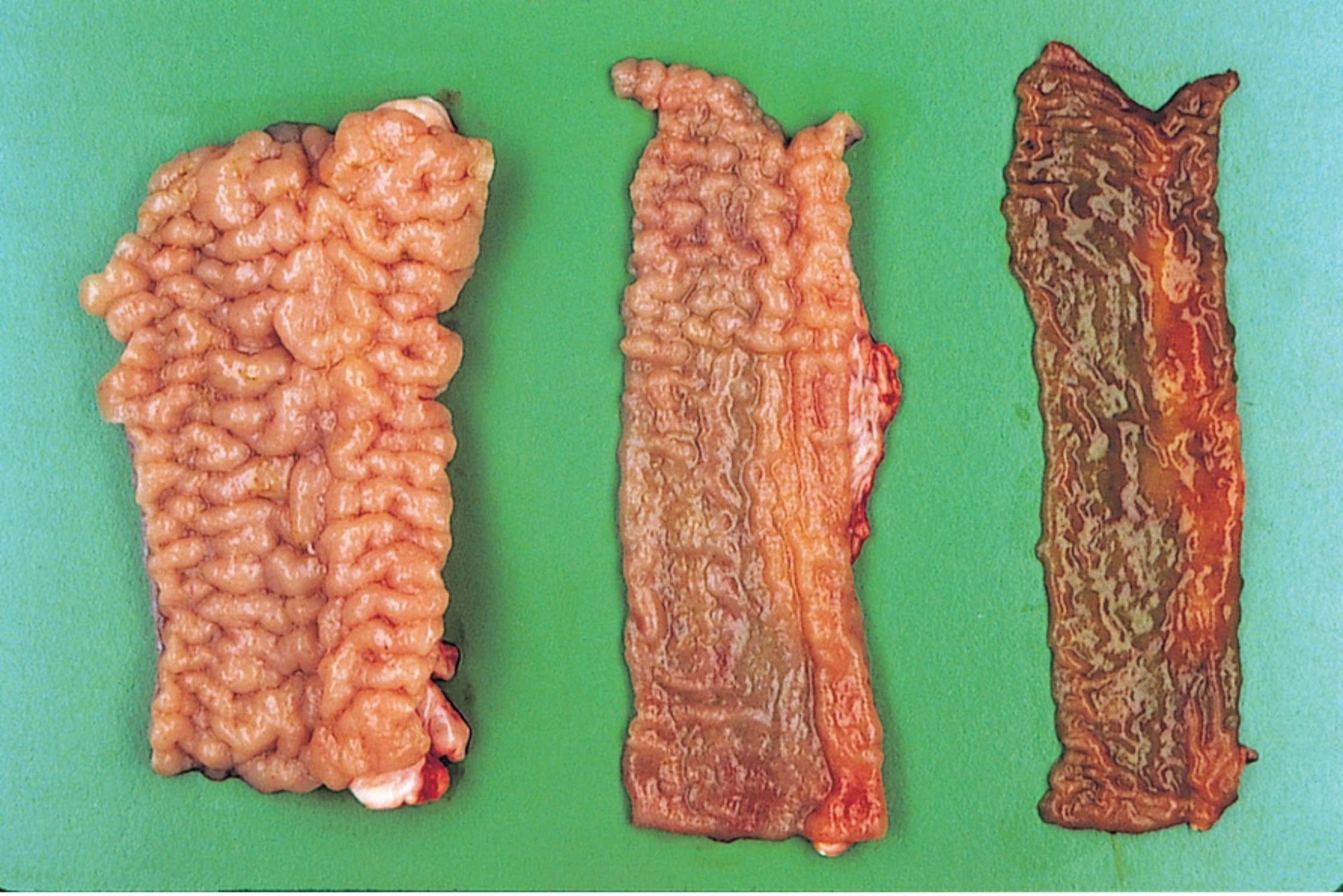


Figure 4

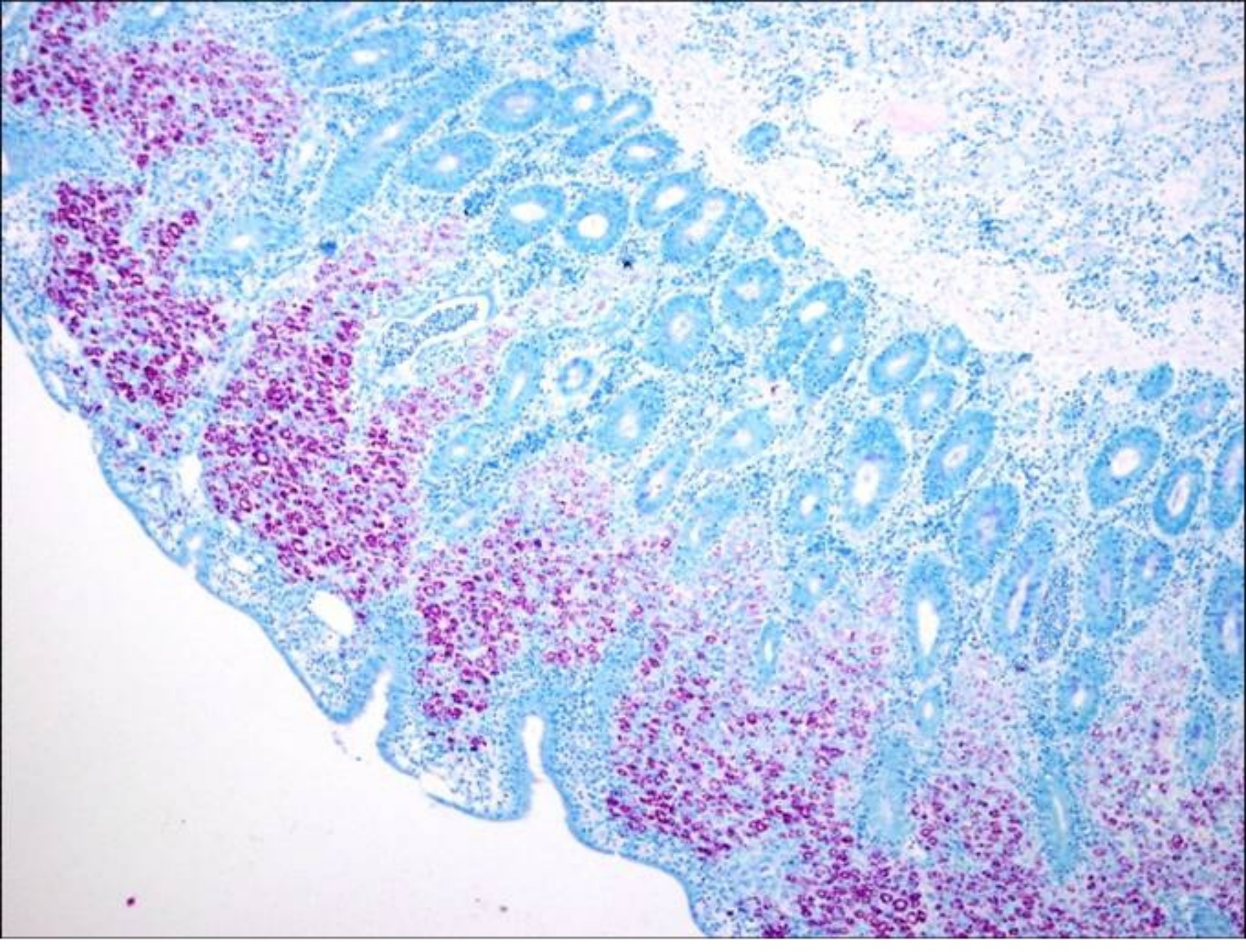


Figure 5a

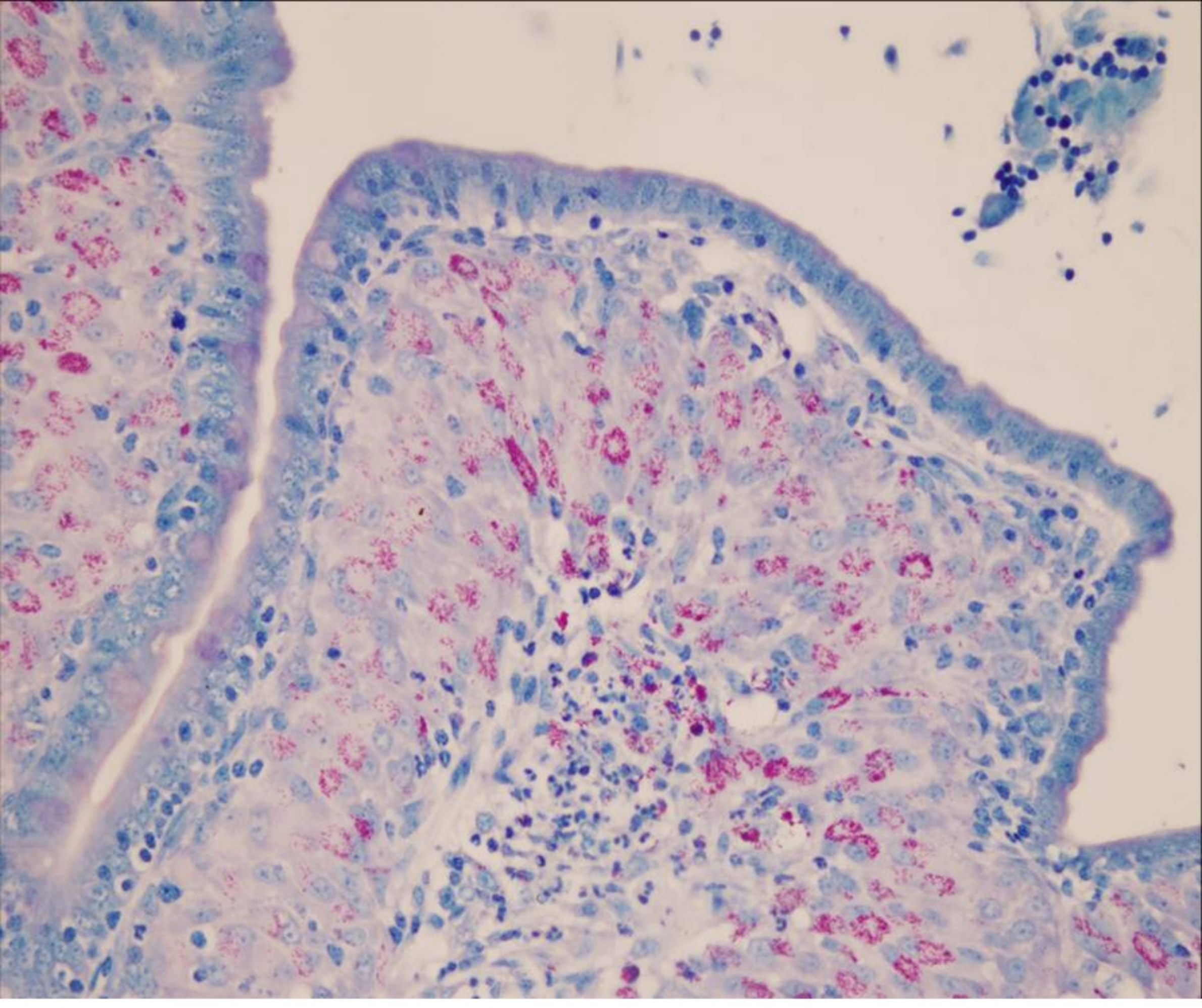


Figure 5b

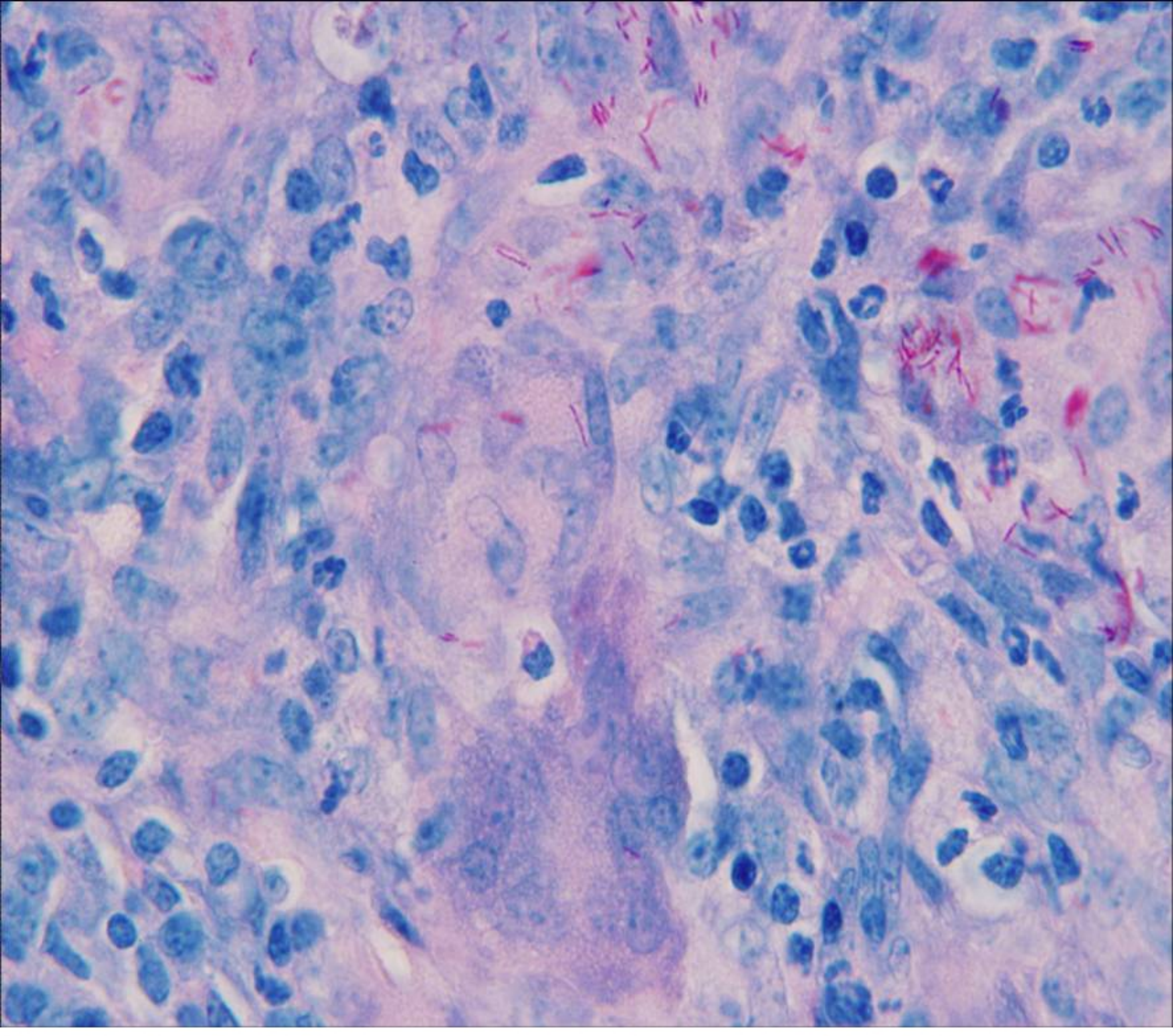


Figure 5c

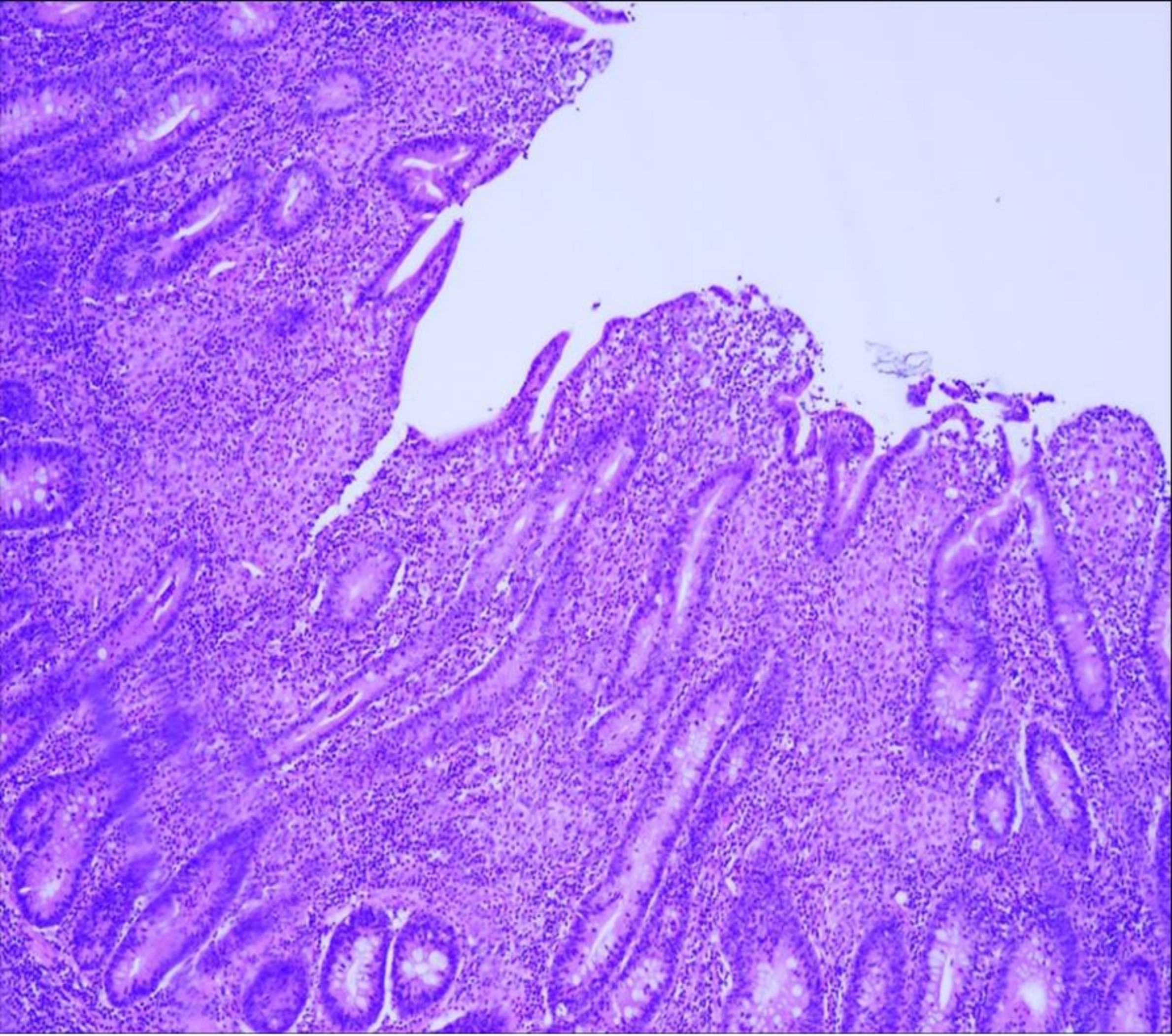


Figure 5d

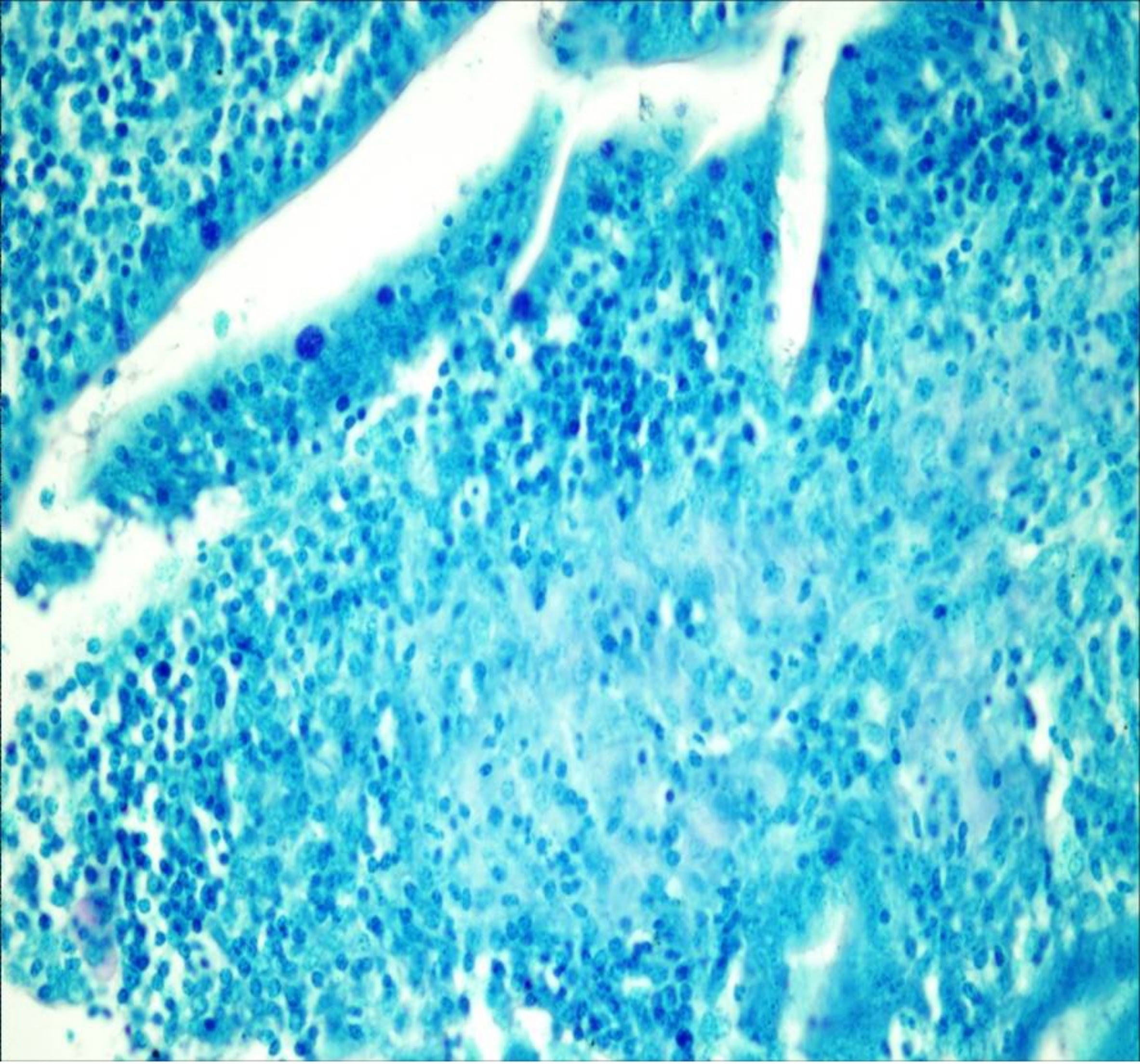


Figure 5e

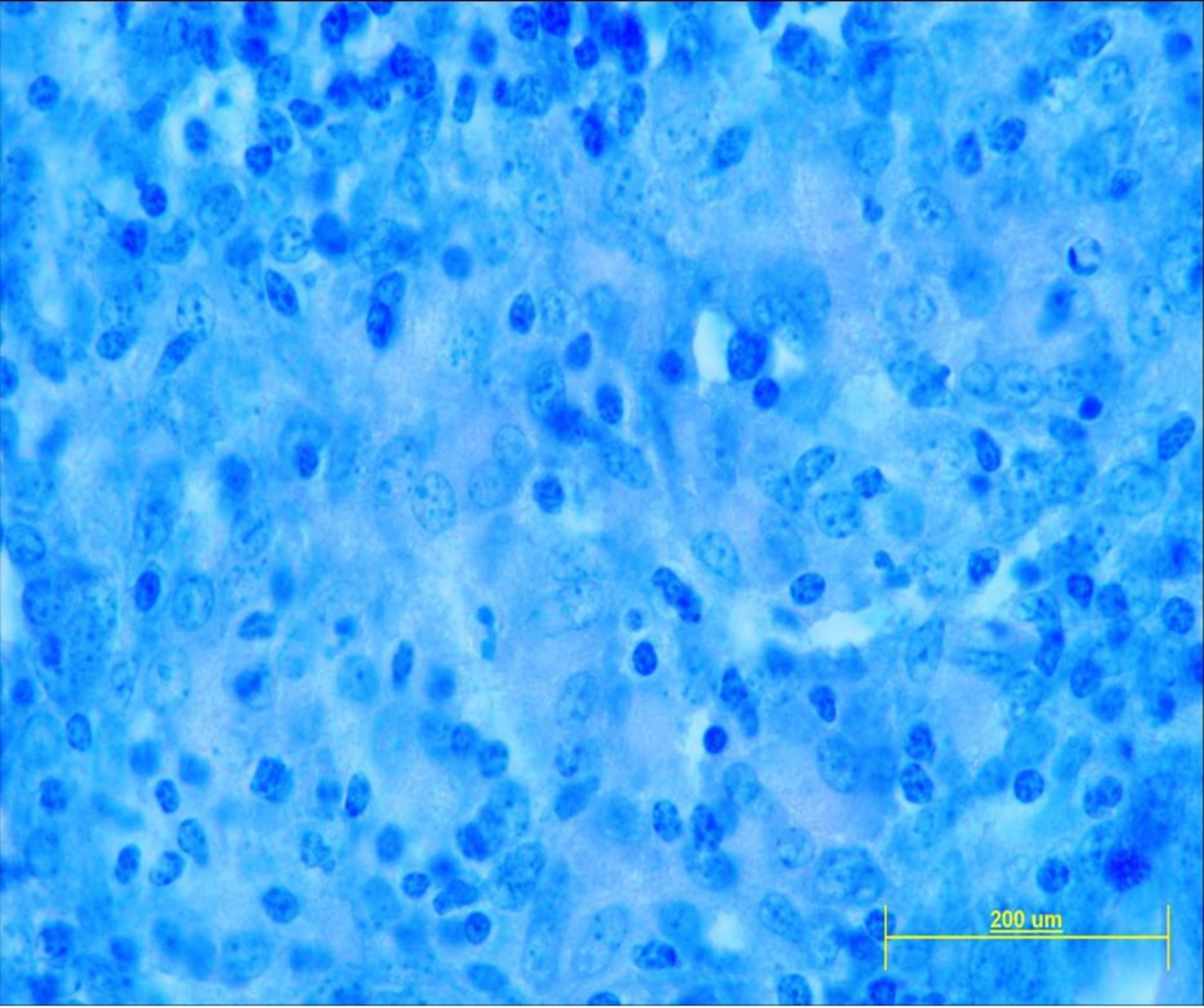


Figure 5f

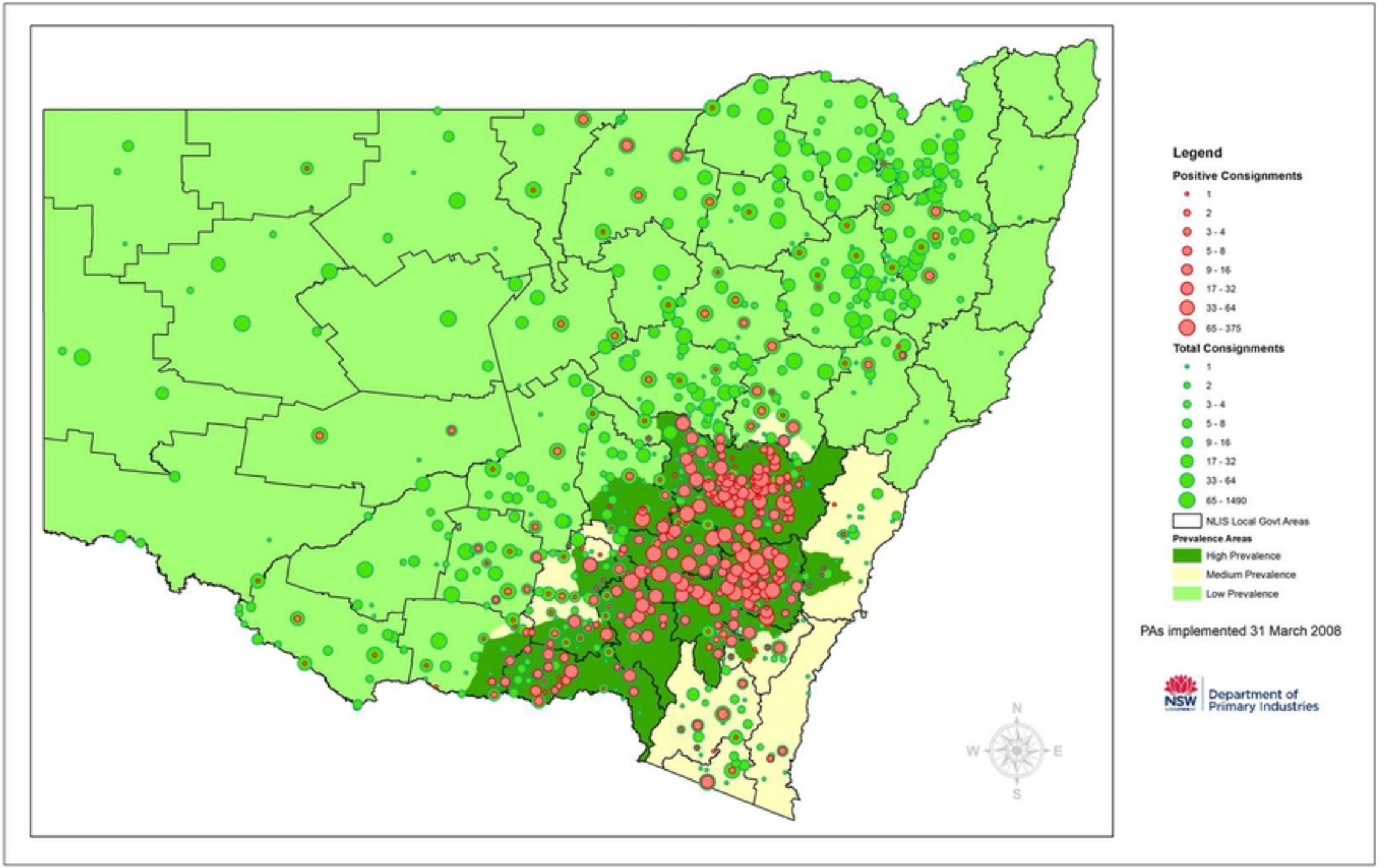
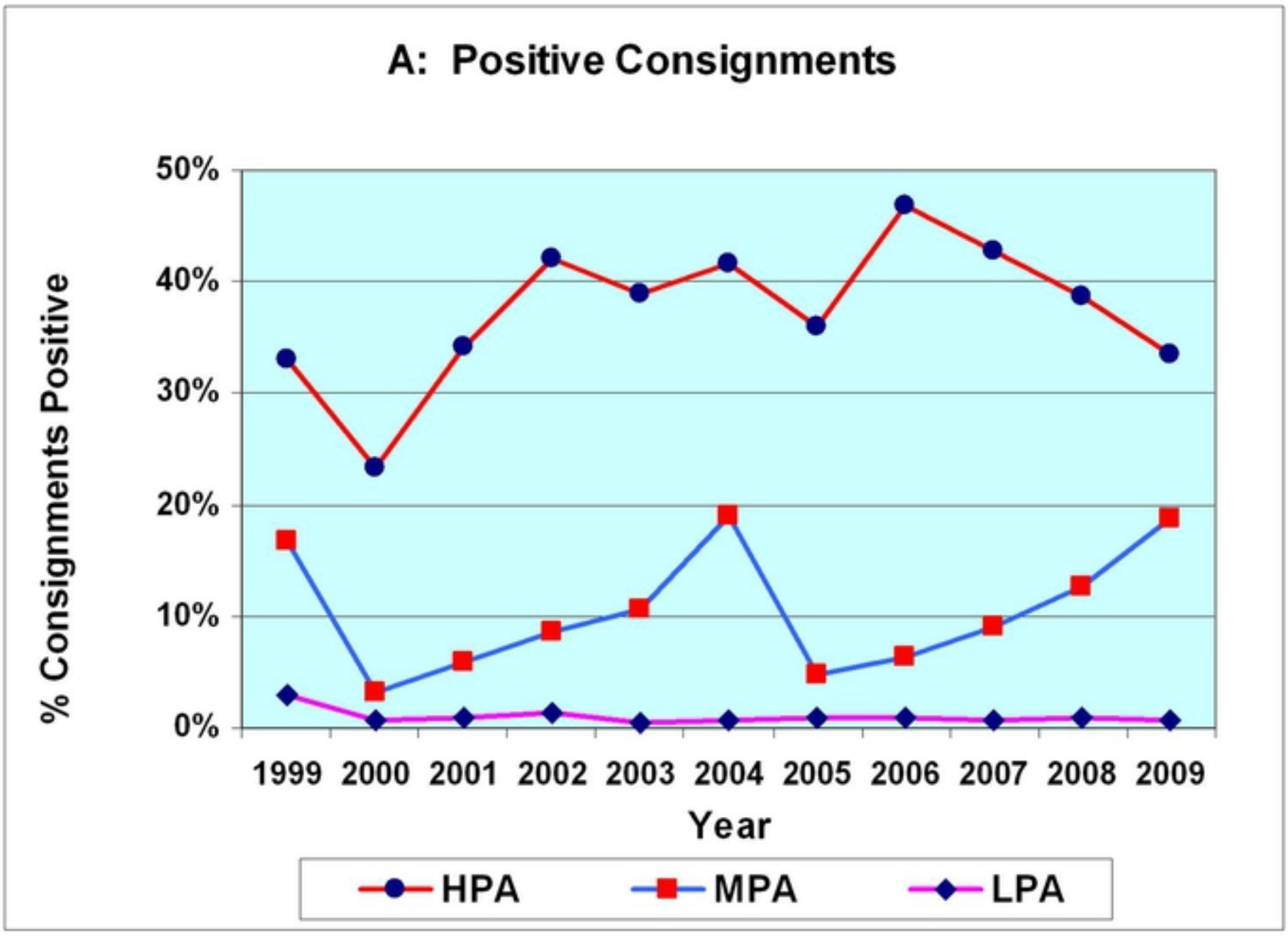

Legend
Positive Consignments
1
2
3 - 4
5 - 8
9 - 16
17 - 32
33 - 64
65 - 375
Total Consignments
1
2
3 - 4
5 - 8
9 - 16
17 - 32
33 - 64
65 - 1490
NLIS Local Govt Areas
Prevalence Areas
High Prevalence
Medium Prevalence
Low Prevalence
PAs implemented 31 March 2008
NSW
Department of Primary Industries
N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

Figure 6


A: Positive Consignments
% Consignments Positive
50%
40%
30%
20%
10%
0%
1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009
Year
HPA
MPA
LPA

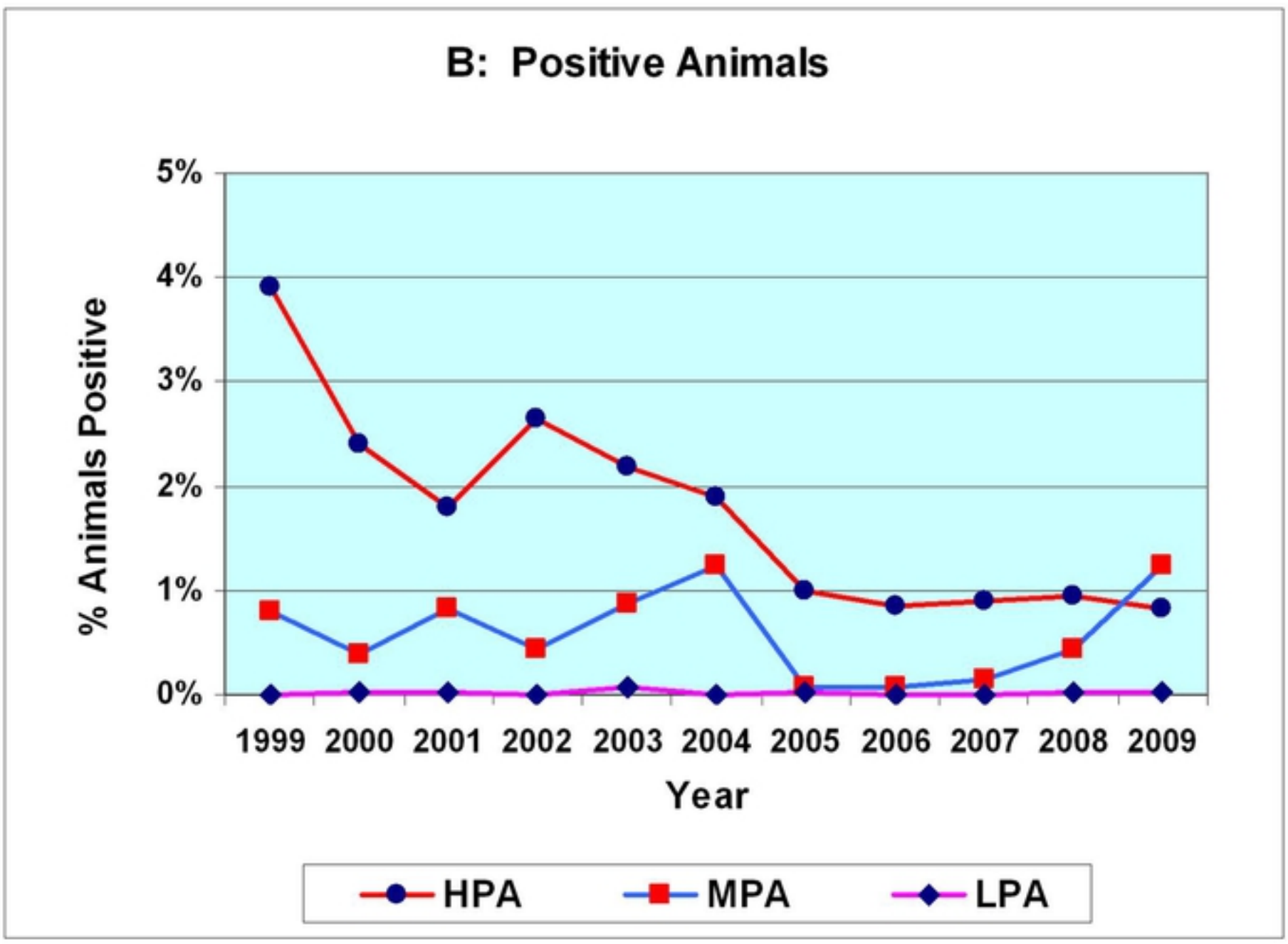
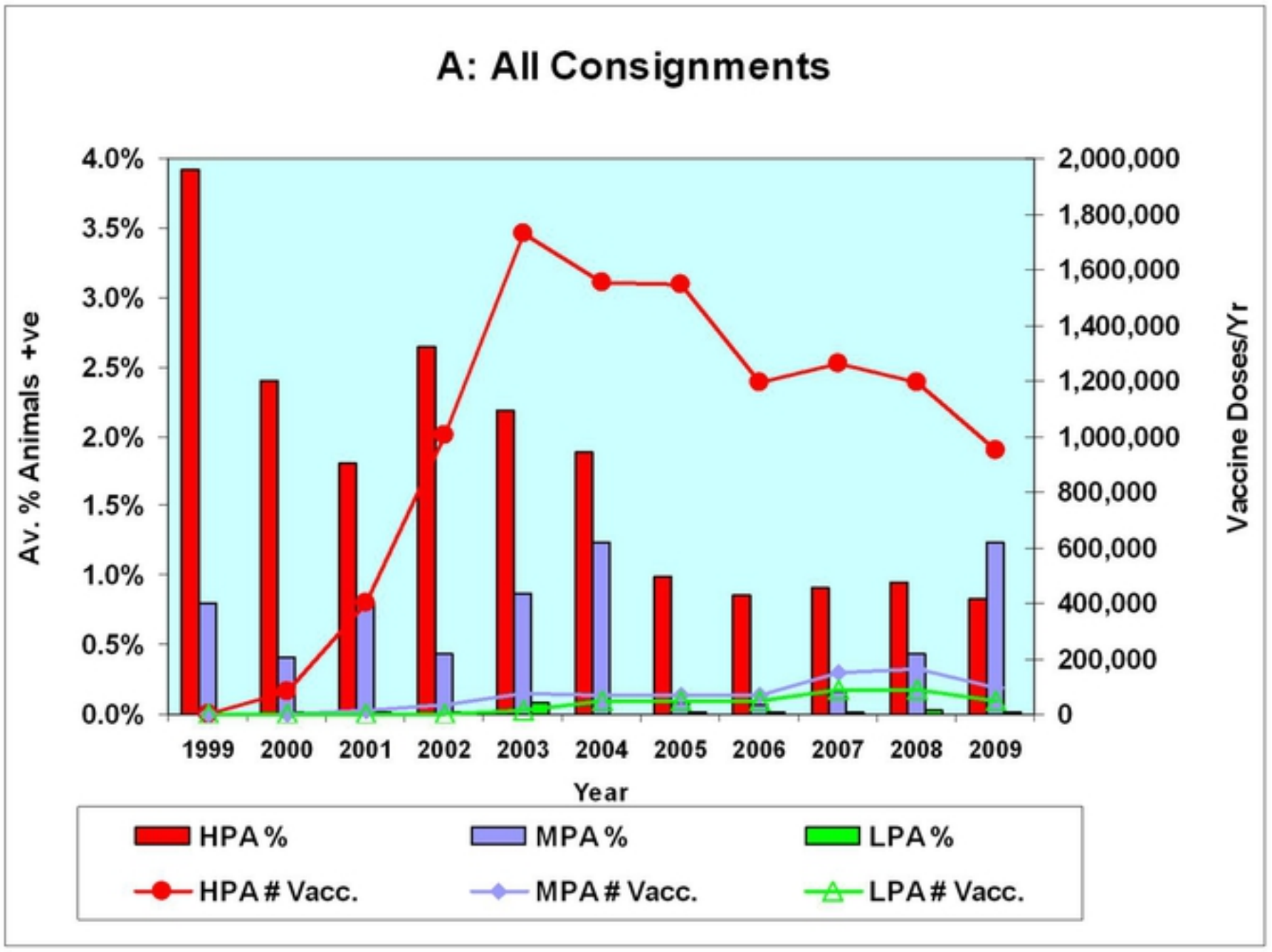

B: Positive Animals
% Animals Positive
5%
4%
3%
2%
1%
0%
1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009
Year
HPA
MPA
LPA

Figure 7


A: All Consignments
Av. % Animals +ve
Vaccine Doses/Yr
4.0%
3.5%
3.0%
2.5%
2.0%
1.5%
1.0%
0.5%
0.0%
2,000,000
1,800,000
1,600,000
1,400,000
1,200,000
1,000,000
800,000
600,000
400,000
200,000
0
1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009
Year
HPA %
MPA %
LPA %
HPA # Vacc.
MPA # Vacc.
LPA # Vacc.

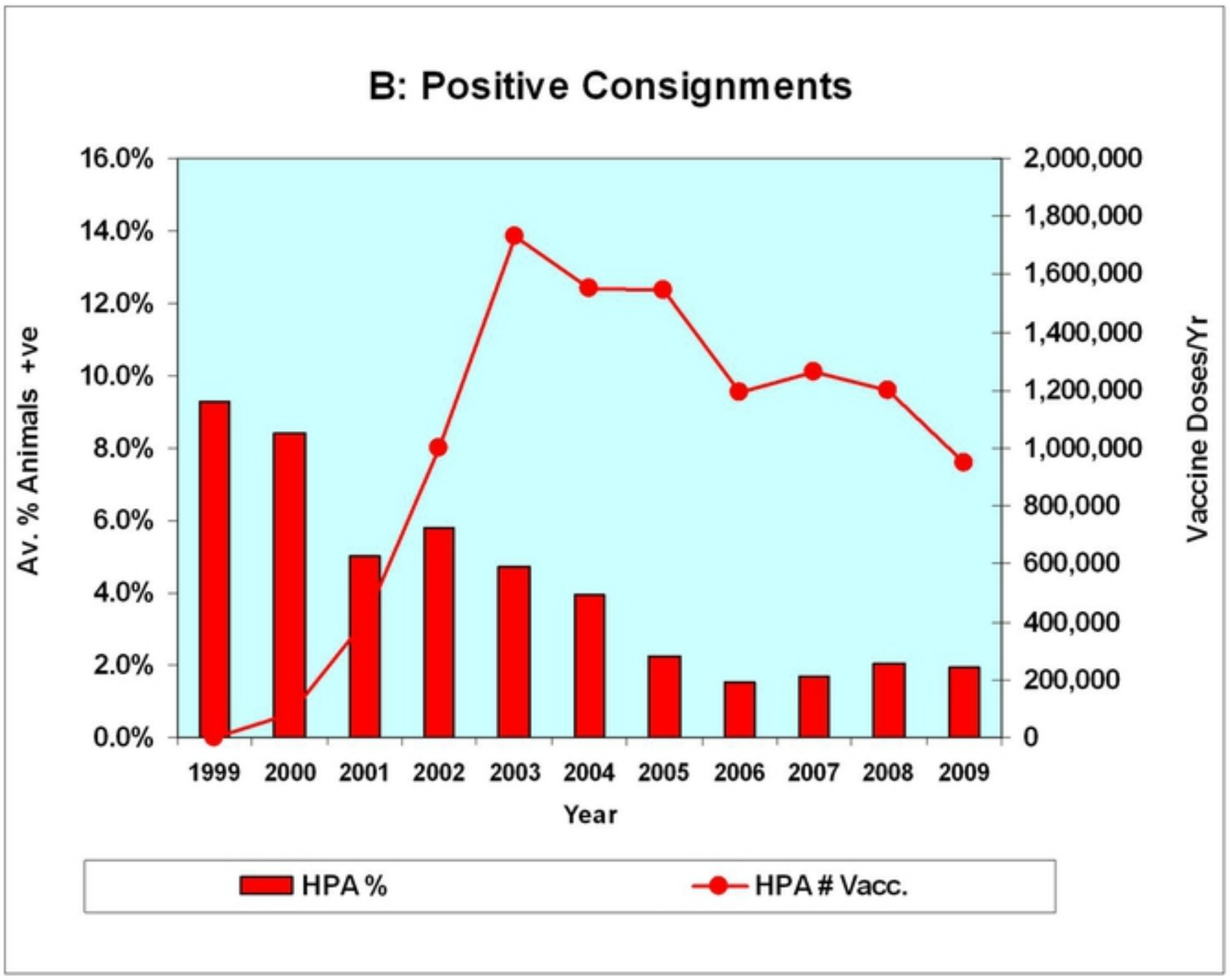

B: Positive Consignments
Av. % Animals +ve
Vaccine Doses/Yr
16.0%
14.0%
12.0%
10.0%
8.0%
6.0%
4.0%
2.0%
0.0%
2,000,000
1,800,000
1,600,000
1,400,000
1,200,000
1,000,000
800,000
600,000
400,000
200,000
0
1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009
Year
HPA %
HPA # Vacc.

Figure 8

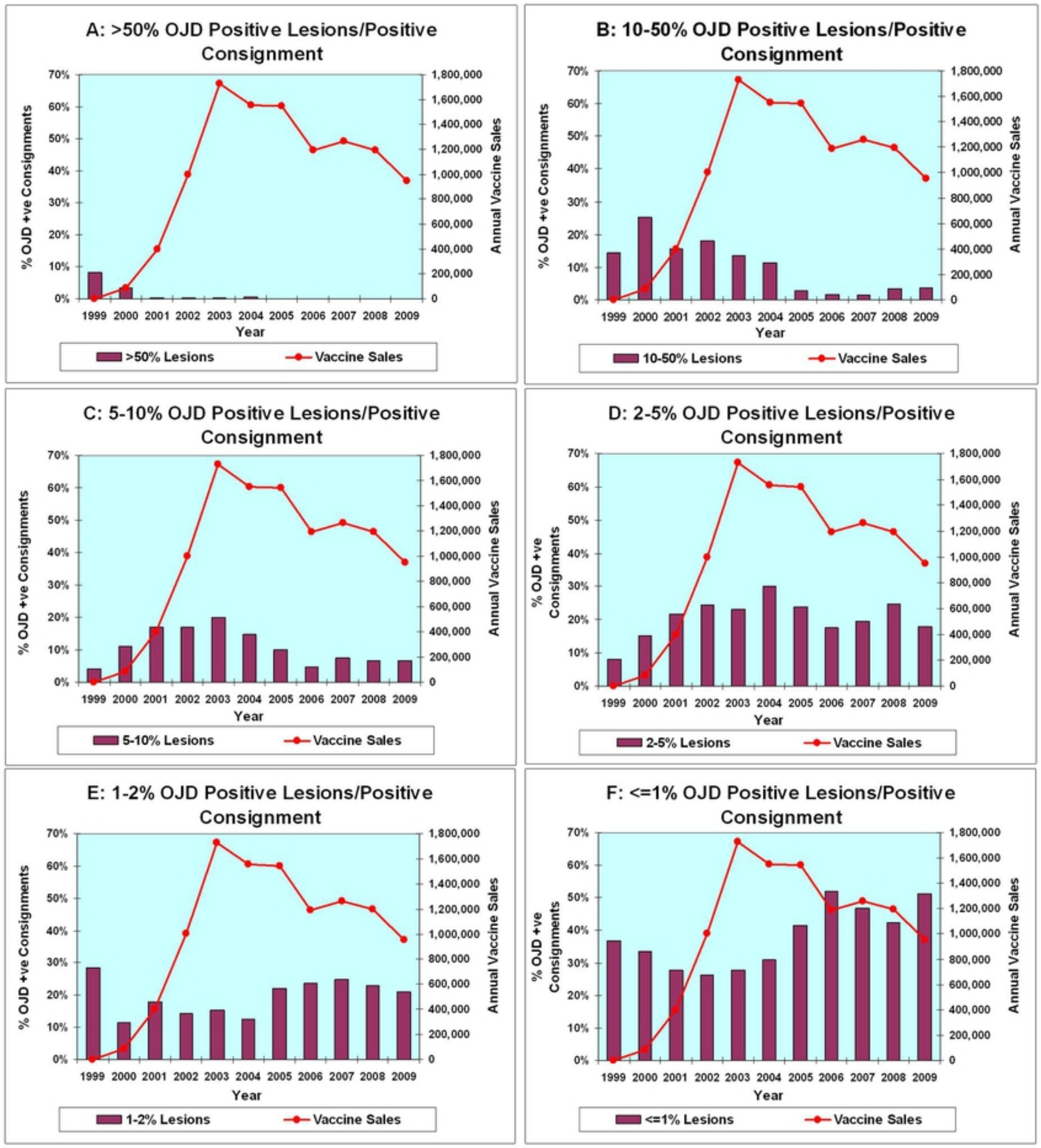

A: >50% OJD Positive Lesions/Positive Consignment
% OJD +ve Consignments
Annual Vaccine Sales
Year
>50% Lesions
Vaccine Sales
B: 10-50% OJD Positive Lesions/Positive Consignment
10-50% Lesions
C: 5-10% OJD Positive Lesions/Positive Consignment
5-10% Lesions
D: 2-5% OJD Positive Lesions/Positive Consignment
2-5% Lesions
E: 1-2% OJD Positive Lesions/Positive Consignment
1-2% Lesions
F: <=1% OJD Positive Lesions/Positive Consignment
<=1% Lesions
70%
60%
50%
40%
30%
20%
10%
0%
1,800,000
1,600,000
1,400,000
1,200,000
1,000,000
800,000
600,000
400,000
200,000
0
1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009

Figure 9