Serologic monitoring of herds with and without bacterin vaccination for *Actinobacillus pleuropneumoniae*

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Summary

Objective: Investigate diagnostic serology for *Actinobacillus pleuropneumoniae* (APP) infections in naturally infected and vaccinated pigs.

Materials and methods: The APP status of 12 farms (A-L) was established by lung cultures and isolate serotyping. Screening enzyme-linked immunosorbent assay (ELISA) detected antibodies to ApxIV antigen or multiple APP serotypes. Serotype-specific ELISAs were conducted for serotypes 5 and 7.

Seven groups of farm F pigs (serotype 7) were moved to farm K (serotype 5). Autogenous vaccines (V1/V2) prepared from APP serotype 5 cultures from farm K and a commercial, killed APP vaccine (V3) containing serotypes 1, 7, and 15 were used to vaccinate pigs in each group twice or thrice at 3-week intervals, commencing at 10 weeks of age. Blood samples were analyzed with ELISAs specific for serotype 5 and ApxI and ApxII toxins. Serum titers were compared using an analysis of variance.

Results: Serotypes 5, 7, 12, or 15 were present in lung cultures. The ApxIV screening ELISA and mix-serotype ELISA regularly detected serotypes 5, 7, and 15. Serotype 12 infections were detected in the mix-serotype ELISA, but not in the ApxIV assays. The serotype 5 or 7 specific ELISA regularly detected herd infections with the relevant serotype.

Serotype 5 titers of pigs vaccinated with V1/V2 thrice were higher than those dosed twice with the equivalent volume ($P < .05$). Pigs receiving V3 showed no serotype 5 antibody response. The ApxI and II titers in V1/V2-vaccinated pigs were higher than controls.

Implications: Screening and serotype-specific ELISAs verified APP status. Repeated serotype-specific autogenous APP vaccine doses provided a strong antibody response.

Key words: swine, *Actinobacillus pleuropneumoniae*, ELISA serology, autogenous vaccination

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Resumen - Seguimiento serológico de piaras con y sin vacunación con bacterina para *Actinobacillus pleuropneumoniae*

Objetivo: Investigar la serología diagnóstica de las infecciones por *Actinobacillus pleuropneumoniae* (APP) en cerdos infectados naturalmente y vacunados.

Materiales y métodos: El estado de APP de 12 granjas (A-L) se estableció mediante cultivos de pulmón y serotipificación de aislamientos. El monitoreo mediante el ensayo de inmunoadsorción ligado a enzimas (ELISA) detectó anticuerpos contra el antígeno ApxIV o múltiples serotipos de APP. Se realizaron ELISA de serotipos específicos para los serotipos 5 y 7.

Siete grupos de cerdos de la granja F (serotipo 7) se trasladaron a la granja K (serotipo 5). Se utilizaron vacunas autógenas (V1/V2) preparadas a partir de cultivos de APP de serotipo 5 de la granja K y una vacuna comercial de APP muerta (V3) que contenía los serotipos 1, 7, y 15 para vacunar a los cerdos de cada grupo dos o tres veces a intervalos de 3 semanas, comenzando a las 10 semanas de edad. Las muestras de sangre se analizaron con ELISA específicas para el serotipo 5 y las toxinas ApxI y ApxII. Los títulos de suero se compararon mediante un análisis de varianza.

Resultados: Los serotipos 5, 7, 12, o 15 estaban presentes en cultivos pulmonares. La ELISA de monitoreo de ApxIV y la ELISA de serotipo mixto detectaron consistentemente los serotipos 5, 7, y 15. Se detectaron infecciones por el serotipo 12 en la ELISA de serotipo mixto, pero no en las pruebas de ApxIV. La ELISA específica de serotipo 5 o 7 detectó regularmente infecciones en la piara con el serotipo relevante.

Los títulos del serotipo 5 de los cerdos vacunados tres veces con V1/V2 fueron más altos que los que recibieron dos dosis con el volumen equivalente ($P < .05$). Los cerdos que recibieron V3 no mostraron respuesta de anticuerpos del serotipo 5. Los títulos de ApxI y II en cerdos vacunados con V1/V2 fueron más altos que los controles.

Implicaciones: El monitoreo y las ELISA específicas de serotipo confirmaron el estatus de APP. Las dosis repetidas de vacuna de APP autógena específica de serotipo proporcionaron una fuerte respuesta de anticuerpos.

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Résumé - Suivi sérologique pour *Actinobacillus pleuropneumoniae* de troupeaux avec et sans vaccination avec une bactérine

Objectif: Examiner le diagnostic sérologique pour l'infection par *Actinobacillus pleuropneumoniae* (APP) chez des porcs naturellement infectés et des porcs vaccinés.

Matériels et méthodes: Le statut pour APP de 12 fermes (A-L) fut établi à la suite de cultures de poumons et de sérotypage des isolats. Une épreuve immuno-enzymatique (ELISA) de tamisage détecta des anticorps contre l'antigène ApxIV ou de multiples sérotypes d'APP. Une épreuve ELISA spécifique de sérotype fut effectuée pour les sérotypes 5 et 7.

Sept groupes de porcs de la ferme F (sérotype 7) furent déménagés à la ferme K (sérotype 5). Des vaccins autogènes (V1/V2) préparés à partir d'APP sérotype 5 obtenu de la ferme K et un vaccin tué commercial (V3) contenant les sérotypes 1, 7, et 15 furent utilisés pour vacciner les porcs dans chaque groupe deux ou trois fois à 3 semaines d'intervalle, commençant à 10 semaines d'âge. Des échantillons sanguins furent analysés par ELISA spécifique pour le sérotype 5 et les toxines ApxI et ApxII. Les titres sériques furent comparés en utilisant une analyse de variance.

Résultats: Les sérotypes 5, 7, 12, ou 15 étaient présents dans les cultures effectuées à partir des poumons. Le tamisage par ELISA pour ApxIV et ELISA pour sérotypes multiples permit de détecter régulièrement les sérotypes 5, 7, et 15. Les infections par le sérotype 12 furent détectées par ELISA pour sérotypes multiples, mais pas par le test pour ApxIV. L'ELISA spécifique pour les sérotypes 5 ou 7 détecta régulièrement des infections dans les troupeaux avec le sérotype approprié.

Les titres envers le sérotype 5 chez les porcs vaccinés avec V1/V2 trois fois étaient plus élevés que ceux chez les animaux vaccinés deux fois avec un volume équivalent ($P < .05$). Les porcs recevant V3 n'ont présenté aucune réponse en anticorps contre le sérotype 5. Les titres envers ApxI et II chez les porcs vaccinés avec V1/V2 étaient plus élevés que chez les témoins.

Implications: Les épreuves ELISA de tamisage et spécifique de sérotype ont vérifié le statut pour APP. Des doses répétées de vaccin APP autogène spécifique de sérotype ont conféré une forte réponse en anticorps.

Acute porcine pleuropneumonia caused by *Actinobacillus pleuropneumoniae* (APP) remains a major clinical problem, particularly in European, Latin American, and Australasian herds; subclinical infections are also common globally. There are 18 recognised APP serotypes, based on their capsule polysaccharide composition.[1,2] These serotype-specific capsule antigens are key factors in the host immune reaction, but without providing significant heterogenous cross-protection.[1] The prevalence of various APP serotypes varies globally, with serotypes 2 and 9 more common in Europe[3] and serotypes 5, 7, and 12 more common in North America and Australia.[4,5] Isolates of serotypes 1, 5, and 9 are considered more pathogenic than others due to their greater expression of the tissue-destroying ApxI and ApxII exotoxins.[6] However, infections with serotype 1 are now less common due to its eradication from many breeding company herds.[4] Infections with APP serotype 5 and 9 are therefore considered of greatest current concern.

Serology is the preferred method for APP surveillance and detection of subclinical infections in pig herds, with established commercial assays available globally. Currently, there are three established antigen formats for APP serology tests. First, a serotype-specific test based on the individual serotype's long-chain lipopolysaccharide (LPS) "O" antigen[7] and second, a test based on the ApxIV exotoxin antigen,[8] which is APP-specific but cannot differentiate between serotypes. The second test is therefore aimed at screening pigs for their APP status. A further screening test has also been established commercially in the first format, by using a pool of long-chain LPS antigens from various serotypes. A third type of test format based on the ApxI or ApxII toxin antigens has also been established,[9] but is considered less specific for APP infection, and therefore less useful for herd screening. Previous studies have used these serologic tests for exploration of APP epidemiology, individual herd status, and response to vaccination.[10-12] Blood samples taken from late-stage finisher pigs (such as 16 to 20 weeks of age) are considered the most sensitive for detection of APP antibody responses representative of herd status.[4,10]

The pathogenesis of APP consists of the separate stages of colonization, resistance to clearance and damage to lungs.[13] Current vaccination programs for APP disease fall into different categories.[14] With knowledge of the APP serotype status of the herd, relevant serotype-specific bacterins can be supplied commercially or prepared as an autogenous vaccination program. These aim to prevent colonization. Subunit vaccines based on the major Apx exotoxin antigens have also been commercially developed and utilised. These aim to prevent tissue damage and can provide protection across APP serotypes. Other vaccine strategies for APP have been developed, such as live attenuated vaccines, but are yet to find wide acceptance.[14] One analysis suggested that 90% of global APP vaccination still occurs via the specific bacterin programs.[15]

In this study, we aimed to further characterize the serologic response for pig herds infected with major serotypes of APP and the response of pigs within a vaccination program for APP serotype 5.

Materials and methods

The Animal Ethics Care Committee of the State Government of Victoria approved the animal use and sampling protocols used in this study.

Case farms and APP status

Twelve separate pig finisher units A to L located across eastern Australia were selected for APP testing. These grower-finisher herds were characterised by their intake of 10-week-old grower pigs per week (Table 1). These herds were each derived from separate breeding/nursery herds either on the same site or under the same management system. The herds A to K had all suffered occasional outbreaks of clinical APP disease, but mortality in these herds was consistently below 2% in the intake to slaughter interval over the study period. All herds incorporated routine vaccination programs for *Mycoplasma hyopneumoniae* infection, but no APP vaccination. The herds had been free of clinical signs associated with porcine reproductive and respiratory syndrome virus and pathogenic porcine circovirus type 2 for 5 years preceding and throughout this study, as

Table 1: Description of study farm *Actinobacillus pleuropneumoniae* (APP) status and APP ELISA results

Farm	APP culture & serotype*	Finisher herd size†	No. pigs examined‡	Pleurisy exam§	ApxIV serology§	APP 5 serology§	APP 7 serology§	APP mix serology§
A	12	800	36	5/36	0/36	0/36	0/36	5/36
B	12	200	27	1/27	0/27	1/27	0/26	5/26
C	15	480	25	0/21	5/25	0/25	0/25	7/25
D	7,15	800	28	2/28	28/28	1/28	8/25	25/28
E	7	240	29	1/29	8/29	0/29	22/29	16/29
F	7	800	20	0/20	12/20	1/20	3/20	19/20
G	7	400	16	3/16	11/14	0/16	16/16	12/15
H	5	400	30	11/30	23/30	28/29	1/29	28/30
I	5	600	16	5/16	11/15	6/14	0/14	2/16
J	5	800	13	6/13	12/13	12/13	0/13	5/9
K	5	180	20	2/20	17/20	16/20	0/20	20/20
L	ND	800	30	0/30	1/30	0/30	1/30	1/30

* APP culture and serotype identification derived from infected lungs harvested immediately prior to study period.

† Herd size expressed as the average number of pigs in each weekly intake batch of 10-week-old pigs.

‡ Pigs examined and blood collection for ELISA at scheduled slaughter at 23 weeks of age.

§ Results expressed as number of positive/number examined.

ND = none detected.

monitored by on-going necropsy, specific serology, and immunohistochemistry studies.

In addition to historical diagnostic results data, five fresh lung samples, some with noticeable lesions of pleuropneumonia, were collected from each farm either at on-farm necropsy or at scheduled slaughter (23 weeks of age). Bacteriologic culture, biochemical identification, and capsule serotyping for APP were performed on each lung sample using established methods [5] to confirm each herd's APP status immediately prior to the study period.

Blood samples were then collected from at least 13 pigs from each farm at scheduled slaughter (Table 1). Lungs from each pig were also examined visually for pleurisy lesions. Serum derived from each blood sample was stored in aliquots at -20°C then thawed and subjected to 4 separate commercial APP serologic assays: 1) indirect ELISA based on recombinant ApxIV antigen (Idexx APP ApxIV ab test; Idexx Laboratories Inc); 2) indirect ELISA based on extract of long-chain LPS antigen for APP serotypes 5 (Swinecheck APP 5a, 5b; Biovet Inc), 4, or 7 (Swinecheck APP 4, 7; Biovet Inc); 3) indirect ELISA based on a mix set of long-chain LPS antigens derived from pools of APP serotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 15 (Swinecheck Mix APP 1-9-11, 2, 3-6-8-15, 4-7, 5, and 10-12; Biovet Inc).

The ELISA procedures were performed according to the manufacturer's instructions and were similar to those described previously.[7,8] Briefly, the appropriate dilution of the LPS or ApxIV antigen was determined by checkerboard titration in microtiter plates using 0.5M carbonate buffer. Plates coated with each antigen (50 µL/well) were incubated overnight at 4°C. Plates were then washed with phosphate buffered saline-0.05% Tween 20 (PBST) and blocked with PBST-1% bovine serum albumin (BSA) for 1 hour at 20°C. After washing with PBST, serum samples (diluted 1:100 with PBST-1% BSA) were added; plates were then incubated for 30 minutes at 37°C. After washing with PBST, peroxidase-conjugated rabbit anti-swine IgG (1:10,000; Rockland Immunochemicals Inc) was added and allowed to react for 30 minutes at 37°C. The plates were washed twice with PBST and a chromogenic solution was allowed to react for 30 minutes at 30°C. The optical density at 490 nm was measured with 650 nm as the reference. The ELISA titer = (sample value absorbance − negative reference absorbance) ÷ (positive reference absorbance − negative reference absorbance). Typical absorbances of the negative and positive reference sera were 0.00 to 0.07 and 1.0, respectively. Each ELISA was performed in batches incorporating identical reagents.

Vaccination programs and monitoring

Growing pigs selected for gilt development at 10 weeks of age (n = 164) were assembled and individually tagged on farm F (APP serotype 7 positive; Table 1) for premovement isolation and were later moved to farm K (APP serotype 5 positive; Table 1).

Seven groups, each with 20 to 30 pigs, within the farm F cohort of pigs were enrolled to assess serologic response to two serotype 5 bacterin vaccines and a commercial APP vaccine. The inoculation protocol for groups 1 to 6 (vaccinated) and group 7 (non-vaccinated controls) is outlined in Table 2. All 164 pigs remained in pens in one large barn enclosure, with *ad libitum* feed, water, and bedding. No antibiotics were administered to any pigs during the study period. While pigs remained healthy throughout, occasional pigs were removed during the study for non-study purposes.

Table 2: Inoculation protocol for each *Actinobacillus pleuropneumoniae* (APP) vaccination group*

	No. of pigs				
Vaccine group	**D0**	**D64**	**Dose, mL**	**No. of doses**	**Period between doses, wk**
1) Control	30	27	0	0	-
2) APP serotype 5 V1†	20	16	1	2	3
3) APP serotype 5 V1†	20	18	1	3	3
4) APP serotype 5 V1†	20	19	2	2	3
5) APP serotype 5 V1†	20	17	2	3	3
6) APP serotype 5 V2†	24	19	2	3	3
7) Commercial APP V3‡	30	27	2	3	3

* Seven groups of cohort pigs, each of individually tagged pigs. Test pigs were given vaccine doses on days 0 and 23; and on day 42 for groups 3, 5, 6 and 7.

† Each dose of V1 and V2 had 1×10^9 *A pleuropneumoniae*/mL.

‡ Each dose of V3 had $> 5 \times 10^8$ *A pleuropneumoniae*/mL.

V1, V2 = separate bacterin vaccines derived from APP serotype 5 isolates from farm K; V3 = commercial APP bacterin vaccine containing serotypes 1, 7 and 15 (Porcilis APPvac; Intervet Co).

For autogenous bacterin production, two APP serotype 5 cultures (V1 and V2) derived from farm K had been expanded and finally grown separately for 6 hours in 2 L culture vessels containing Tryptone yeast extract broth, with added nicotinamide adenine dinucleotide (10 µg/mL) and 5% vol/vol inactivated bovine serum. Each batch was then tested for potency (colony forming units/mL) and purity by cultures titrated onto routine aerobic and anaerobic plates. Each batch of pure culture was then inactivated with a final 0.2% vol/vol formalin, blended into the final vaccine strain and a commercial aluminum hydroxide gel adjuvant added at 500 µg/mL. Aliquots from each selected final batch were decanted into 100 mL bottles for use as specific autogenous bacterin vaccines in the assembled pigs.

A commercial, whole-cell, killed APP vaccine (V3; Porcilis APPvac; Intervet Co), stated to contain APP serotypes 1, 7, and 15 and commercial adjuvant, was purchased and used according to the manufacturer's recommendations.

Pigs in each group were dosed intramuscularly behind the ear with 2 mL of V3 vaccine ($> 5 \times 10^8$ APP/mL) or either 1 mL (single dose) or 2 mL (double dose) of V1 or V2 vaccine (approximately 1×10^9 APP/mL). Doses were given either twice or thrice at 3-week intervals, commencing at 10 weeks of age (Day 0 of the study period; Table 2).

Serologic evaluation of the vaccine study was conducted via response to APP serotype 5 LPS, and also to Apx toxins, as APP serotypes 1 and 5 are known to contain ApxI and II, whereas serotype 7 only contains ApxII.[5] Blood samples were collected from each pig at 10, 13, 16, and 19 weeks of age (Days 0, 23, 42, and 64). Serum from each sample was stored in aliquots at -20°C and incorporated into: 1) the indirect ELISA based on extract of long-chain LPS for APP serotype 5, 2) the indirect ELISA based on ApxI toxin antigen, and 3) the indirect ELISA based on ApxII toxin antigen. The procedures for the ApxI and II ELISAs were performed according to methods described previously.[9,16] Recombinant Apx toxin antigens were kindly provided by Dr Han Sang Yoo, of Seoul National University. Briefly, the plates were coated with respective antigen at 4°C overnight. Preliminary checkerboard titration results indicated that the optimal concentration of recombinant ApxI and ApxII antigen was 625 and 100 ng/well, respectively. Plates were washed with PBST after antigen coating and blocked with 10% horse serum for 2 hours at 37°C. After washing with PBST, serum samples (diluted 1:100 with PBST-1% BSA) were added, incubated for 2 hours at 37°C, followed by washing and incubation with peroxidase-conjugated goat anti-pig IgG (Rockland Immunochemicals Inc) for 30 minutes at 37°C. The plates were washed twice with PBST and a chromogenic solution was allowed to react for 30 minutes at 30°C. The optical density at 405 nm was measured with 650 nm as the reference. The largest differences between positive and negative controls were found with anti-pig IgG at a 1:1000 dilution (ApxI) or a 1:2000 dilution (ApxII). Each ELISA titer = (sample value absorbance − negative reference absorbance) ÷ (positive reference absorbance − negative reference absorbance). Typical absorbances of the negative and positive reference sera were 0.05 to 0.1 and 0.1 to 0.15, respectively. Each ELISA was performed in batches incorporating identical reagents.

Differences between serum titers at each blood collection point for each group of vaccinate or control pigs were compared using an analysis of variance.

Results

Serologic analysis of APP herds

The results of lung culture for APP and identification of APP serotypes identified in pigs on farms A to K are shown in Table 1, with the on-farm presence of APP serotypes 5, 7, 12, and 15 identified. These herd designations confirmed historical diagnostic sample results (data not shown). Only one herd (D) had an apparent dual serotype infection with serotypes 7 and 15. One herd (L) was apparently free of APP infection during the study period.

The results of testing with four separate APP ELISAs of blood samples collected from slaughter pigs from farms A to L are shown in Table 1. The results of lung examinations for visible lesions of pleurisy in these sampled pigs are also shown in Table 1. A greater proportion of sampled pigs with pleurisy was

noted in herds positive for APP serotype 5 compared to those infected with other serotypes.

Both the ApxIV ELISA and the APP mix-LPS ELISA regularly detected all apparent herd infections with 5, 7, and 15 serotypes, albeit with a variable ratio of 14% to 100% of blood samples analyzed. However, while the 2 farms identified as having APP serotype 12 infections were detected in the mix-LPS ELISA screen, these reactions were not detected in the ApxIV screen assays performed on the same sets of sera (Table 1). The ELISA employing LPS antigen specific to serotypes 5 or 7 regularly detected herd infections with the relevant serotype, albeit with a ratio of 15% to 100% of blood samples analyzed. Occasional single cross-reactions were detected with these assays in samples taken from pigs in uninfected herds or those herds infected with other serotypes (Table 1).

Vaccination monitoring

A summary of the results of ELISA testing for APP serotype 5 status and Apx toxin antibody status of each group of vaccinated pigs and control pigs from day 0 through day 64 is shown in Figures 1 and 2, respectively. The specific LPS-antigen titers of pigs vaccinated three times with autogenous APP serotype 5 were noticeably higher at day 64 than those dosed only twice with the equivalent 1 mL or 2 mL dose sizes (Figure 1). The analysis of variance indicated a significant difference at day 64 between the groups vaccinated three times with either autogenous serotype 5 strain V1 or V2 (groups 3, 5, and 6) and their titers at day 0 and the control group at day 64 ($P < .05$). The analyses of other vaccinated groups at day 64 and of all groups at other blood collection points indicated no significant difference in the ELISA titers detected from those at day 0 or from the control group.

The ApxI toxin antibody analysis indicated a noticeable anamnestic response within each group of the serotype 5 bacterin vaccine program (Figure 2; groups 2-6). The ApxII toxin antibody analysis indicated a limited response to all bacterin vaccines (Figure 2). Following subsequent movement of pigs to farm K, APP was not detected clinically or at post-mortem examinations of exposed vaccinates for 6 months.

Discussion

The current diagnosis of individual and herd status for APP is established via culture of lungs and serotyping of APP cultures and ELISA serology. Our study confirms the generally good diagnostic relationship between pleurisy lesions, lung cultures, and current ELISA serology techniques for a range of on-farm APP serotype infections. The long-chain LPS antigen ELISA methodology allowed accurate identification of herd status in all 12 farms examined. Analysis of sufficient samples is indicated to account for occasional cross-reactions. We established that the ApxIV antigen ELISA methodology also accurately predicted herd status, except for the two herds known to be infected with APP serotype 12. In contrast, a previous herd study[12] found some false positives with the ApxIV assay (case No. 7). Use of both the LPS and ApxIV antigen assays may be required to fully clarify the status of herds with APP serotype 12. While some APP serology studies have failed to accurately determine herd status with limited sample numbers,[10] other studies found that the combined use of LPS and ApxIV assays on sufficient sample sets was associated with accurate investigations of herd status.[4,12]

The pathogenesis of APP consists of the three separate stages of colonization, resistance to clearance, and damage to lungs.[13] Although all serotypes of APP are considered pathogenic, the lung examinations for pleurisy lesions supplemented the diagnostic information from culture and ELISA results confirming the greater extent of lesions likely to be seen with APP serotype 5 infections. This is considered to be due to greater expression of the Apx exotoxins,

Figure 1: Results of ELISA for *Actinobacillus pleuropneumoniae* (APP) serotype 5 antibodies in pig sera collected in APP vaccine study. The average and mid 50 percentile optical density readings are presented for pigs in each group at each time point following vaccination. Results for day 23 showed no significant differences to day 0 and are not presented. Group 1) Control non-vaccinated pigs; Group 2) Pigs received 1 mL doses of V1 twice; Group 3) Pigs receiving 1 mL doses of V1 thrice; Group 4) Pigs received 2mL doses of V1 twice; Group 5) Pigs received 2 mL doses of V1 thrice; Group 6) Pigs received 2 mL doses of V2 thrice; Group 7) Pigs received 2mL doses of V3 thrice. See Table 2 for group size and designation. V1 and V2 = bacterin vaccines derived from APP serotype 5 isolates from farm K; V3 = commercial APP bacterin vaccine (Porcilis APPvac; Intervet Co).

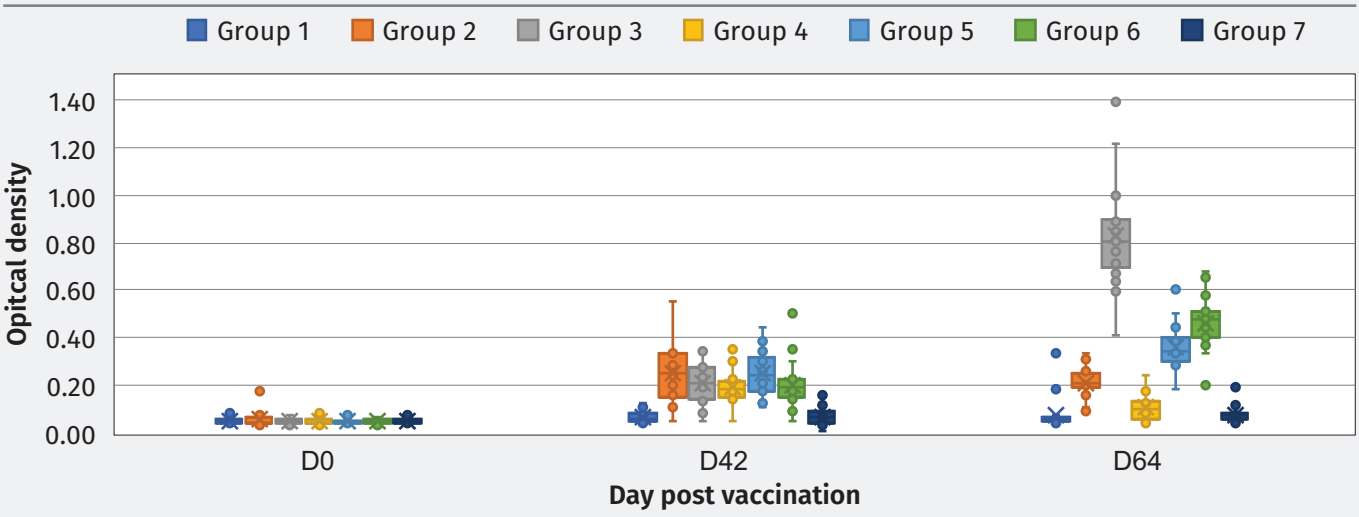
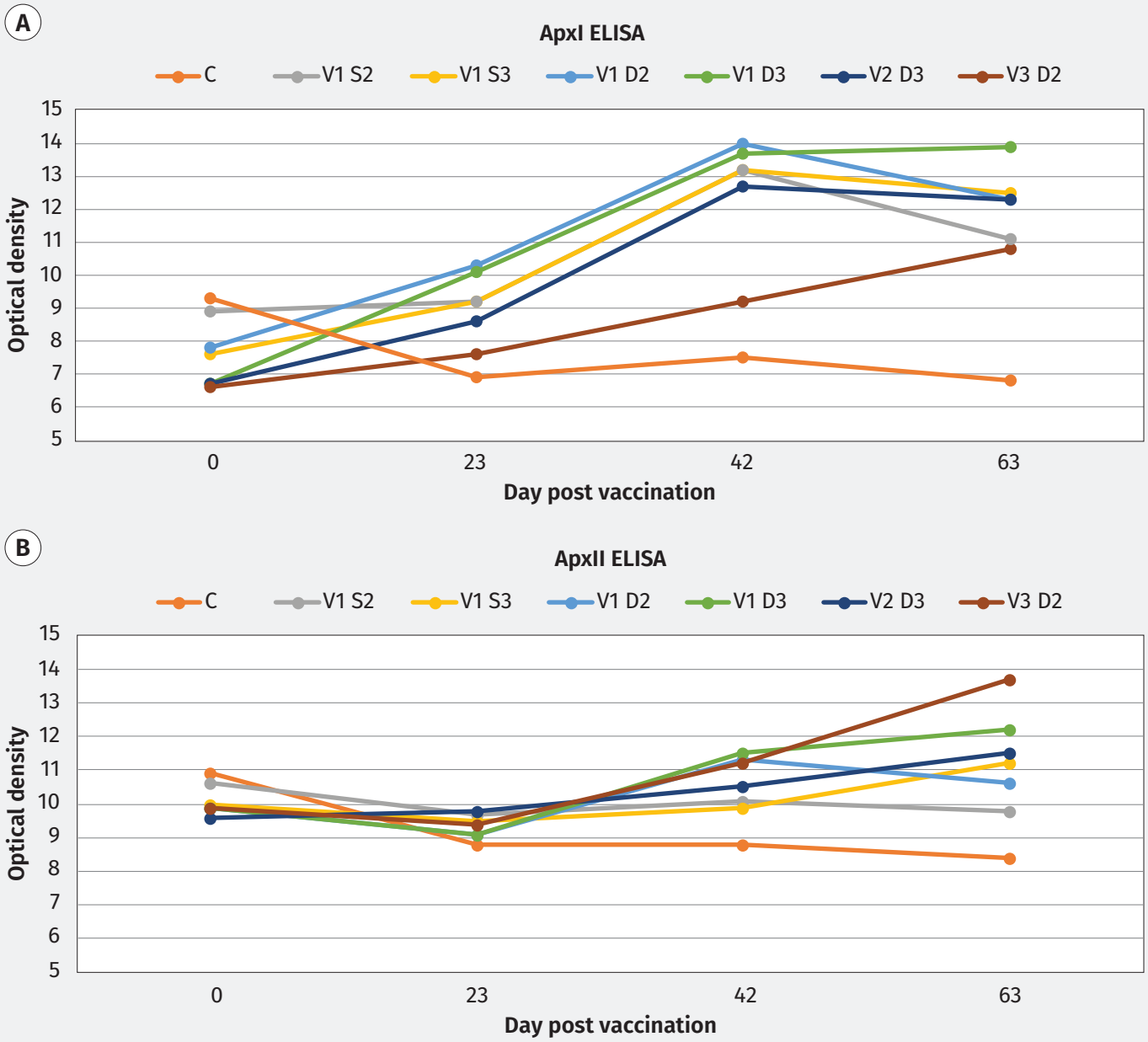


Figure 2: Results of ELISA for A) ApxI and B) ApxII antibodies in pig sera collected in *Actinobacillus pleuropneumoniae* (APP) vaccine study. The average optical density readings are presented for pigs (n = 20-30) in each group at each time point following vaccination. Standard deviations for each assay point were consistently less than 10% of the average. Pigs were vaccinated twice (S2, D2) or three times (S3, D3). See Table 2 for group size and designation. C = control non-vaccinated pigs; V1 and V2 = bacterin vaccines derived from APP serotype 5 isolates from farm K; V3 = commercial APP bacterin vaccine (Porcilis APPvac; Intervet Co); S = 1 mL dose; D = 2 mL dose.



particularly ApxI and ApxII.[6] While the pathogenicity of APP in terms of tissue damage varies according to serotype and Apx content, the number of animals infected within any particular herd may also vary according to serotype. Our study confirms that serologic monitoring of APP herd status is best achieved via approximately 30 samples, of which 3 or more clear positives leads to an accurate indication of APP status, as also suggested previously.[4]

Prevention or eradication of APP infection across serotypes has remained elusive, with novel strategies, such as live attenuated vaccines or outer membrane vesicle subunit toxoids, found to be ineffective.[14] Commercial subunit vaccines based on the Apx toxoids are effective at reducing the tissue damage phase, but do not appear to prevent the initial colonization stage. Their use is therefore considered ineffective at controlling initial APP infections leading to the possible presence of carrier pigs among infected herds.[14,17] In our study, serologic evaluation of bacterin vaccines was conducted via response to APP serotype 5 LPS and Apx toxins, as APP serotypes 1 and 5 are known to contain ApxI and II, whereas serotype 7 only contains ApxII.[6] We confirmed that noticeable antibody production to the ApxI and II toxins occurred in vaccinated pigs, particularly those given APP serotype 5 bacterin. In our study and others,[4] the interpretation

of Apx ELISAs was considered more problematic due to less certain cut-off values and its limited availability. Although our results indicated that repeated dosage of APP serotype-specific vaccines can be monitored successfully by LPS ELISA serology, these assays based on APP LPS antigen are aimed at detection of infection status rather than vaccine responses. It is possible that vaccinated pigs may develop reactions to other protective antigens, unrelated to any detectable LPS response.

The long-standing use of serotype-specific bacterin vaccines remains the most popular form of APP vaccination, despite the considerable time and effort required for their autogenous preparation. It is possible that the use of bacterin vaccines may confer both some protection against colonization and some protection against Apx related tissue damage. Our results indicated that repeated dosage of APP serotype-specific vaccines can be monitored successfully by LPS ELISA serology, with an anamnestic response to LPS antigen (note titers at days 0, 42, and 64 presented in Figure 1) similar to vaccinated pigs in previous studies of the administration of APP serotype 5 bacterin vaccines.[18] It is possible that the preparation and use of whole-cell, unwashed bacterial material for production of the autogenous vaccines in our study may have conferred some protective advantage in comparison to other vaccine substrates. These previous studies also confirmed that repeated doses of APP bacterins are required for a measurable response and that there is little difference in the measured LPS antigen titer response to pigs given either 1 mL or 2 mL doses.[18] In our study, we found that a greater response occurred with three doses of APP serotype 5 bacterin compared to only two doses. Whether this may be useful for all APP bacterins, or merely this example of pathogenic APP serotype 5 infection, is not clear.

Implications

Under the conditions of this study:

- Commercial and serotype-specific ELISAs were used to identify herd APP status.
- Repeated autogenous APP serotype 5 vaccine doses provided strong antibody responses.

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Conflict of interest

None reported.

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Conversion tables

Weights and measures conversions

Common (US)	Metric	To convert	Multiply by
1 oz	28.35 g	oz to g	28.35
1 lb (16 oz)	0.45 kg	lb to kg	0.45
2.2 lb	1 kg	kg to lb	2.2
1 in	2.54 cm	in to cm	2.54
0.39 in	1 cm	cm to in	0.39
1 ft (12 in)	0.3 m	ft to m	0.3
3.28 ft	1 m	m to ft	3.28
1 mi	1.6 km	mi to km	1.6
0.62 mi	1 km	km to mi	0.62
1 in^2	6.45 cm^2	in^2 to cm^2	6.45
0.16 in^2	1 cm^2	cm^2 to in^2	0.16
1 ft^2	0.09 m^2	ft^2 to m^2	0.09
10.76 ft^2	1 m^2	m^2 to ft^2	10.8
1 ft^3	0.03 m^3	ft^3 to m^3	0.03
35.3 ft^3	1 m^3	m^3 to ft^3	35.3
1 gal (128 fl oz)	3.8 L	gal to L	3.8
0.26 gal	1 L	L to gal	0.26
1 qt (32 fl oz)	0.95 L	qt to L	0.95
1.06 qt	1 L	L to qt	1.06

Temperature equivalents (approx)

°F	°C
32	0
50	10.0
60	15.5
61	16.1
65	18.3
70	21.1
75	23.8
80	26.6
82	27.7
85	29.4
90	32.2
102	38.8
103	39.4
104	40.0
105	40.5
106	41.1
212	100.0

°F = (°C × 9/5) + 32

°C = (°F - 32) × 5/9

Conversion calculator available at: amamanualofstyle.com/page/si-conversion-calculator

Conversion chart, kg to lb (approx)

Pig size	Lb	Kg
Birth	3.3-4.4	1.5-2.0
Weaning	7.7	3.5
	11	5
	22	10
Nursery	33	15
	44	20
	55	25
	66	30
Grower	99	45
	110	50
	132	60
Finisher	198	90
	220	100
	231	105
	242	110
	253	115
Sow	300	136
	661	300
Boar	794	360
	800	363

1 tonne = 1000 kg

1 ppm = 0.0001% = 1 mg/kg = 1 g/tonne

1 ppm = 1 mg/L