Emerging Trends in Veterinary Diagnostic Submissions in Canada and Understanding New Technology

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Gallant Custom Laboratories operations include a veterinary diagnostic division that receives swine samples from across Canada. The primary function of the diagnostic laboratory is to use adapted technology to evaluate bacterial and viral pathogenic organisms implicated in a given clinical case. Classical bacteriology has now been paired with Polymerase Chain Reaction (PCR) to identify, genotype and virotype micro organisms, providing invaluable information on the virulence potential of strains found in the lab. Diagnostic samples submitted for respiratory cases typically include an entire pluck, but if septicemia is suspected, the submission may also include a brain or meningeal swab, spleen, liver, joint (also for arthritis) or kidney; enteric cases submissions may include intact tied-off gut loops or rectal swabs, depending on the suspected organism. Laboratory findings in submitted clinical cases may include swine pathogens such as *Pasteurella multocida, Staphylococcus hyicus* and *Escherichia coli,* which are typically associated with atrophic rhinitis, greasy pig and diarrhea disease, respectively. However, a review of our diagnostic results reveals that these organisms are emerging as a causative agent in other clinical signs, as well, such as septicemia, polyserositis and arthritis, and they are more frequently presenting themselves as a primary pathogen. Their presence in the brain, heart, joint and kidney in our clinical cases reveal correlations to reduced performance and increased mortality, resulting in expensive treatments or economic losses. Gallant has routinely isolated these organisms from these atypical sites and proposes that they should be considered when developing treatment and prevention programmes. Knowing the significance of the lab finding will enable an informed and more targeted approach to disease treatment and prevention.

Pasteurella multocida

Pasteurella multocida is a gram negative bacterium that is associated with disease in many species such as poultry, swine, cattle, rabbits and humans. Along with *Mycoplasma hyopneumoniae,* it is the cause of enzootic pneumonia which is considered the most important respiratory disease of swine worldwide[7]. It is easy to isolate in the lab, on artificial media, from organs and nasal swabs and can now be readily identified by PCR testing. At Gallant, *Pasteurella multocida* we isolated in approximately 21% of our respiratory cases in 2011 and as high as 26% in 2009 (Table 1). *P. multocida* isolation as high as 77% (114/148) in cranioventrally located bronchopneumonia cases, has been reported and of these, 85% were in pure culture[4]. In the same study, they indicated success at isolating *P. multocida* in the pericardial sac and kidney. Gallant did not receive kidney tissue with our submissions so are unable to comment

on isolation success from this organ. An Ontario[5] study reported that *P. multocida* was isolated in 27% (108/395) of tonsils from swine sampled at a federally inspected Ontario abattoir from June to Dec 2008, indicating that *P. multocida* is part of the commensal flora in the pig tonsil. Further studies could reveal if this commensal strain of *Pasteurella multocida* is capable of initiating disease such as bronchopneumonia. Since most of our cases were respiratory it is not unexpected that the most common organ for isolation was the lung at 91%, however, we also found it in the heart, spleen, and brain (see Table 2). The presence of *Pasteurella multocida* is not new to swine medicine and was recognized as a contributor to fatal swine pneumonia at least 120 years ago.[1] Our lab often isolates it with other respiratory organisms such as *Haemophilus parasuis, Bordetella bronchiseptica, Actinobacillus suis, and Streptococcus suis* and this is typical of other reports[1]. *P. multocida* is thought to be a secondary infection that is very common in the final stage of enzootic pneumonia[1,2,3,4] with colonization developing after the aggravation of the lung tissue due to the primary invaders. Systemic infections are not often reported but studies[3,4] show that systemic spreading of *P. multocida* does occur and this would concur with Gallant's findings of *P. multocida* isolation in the heart, spleen and brain.

There are 4 capsular types of *Pasteurella multocida* although A and D are most commonly associated with swine disease. Although Type A is most often associated with pneumonia and Type D with atrophic rhinitis, through PCR molecular analysis[3,4], strain and virulence factor differences have also been found between the strains that cause atrophic rhinitis and pneumonia. The virulent capabilities of Pasteurella *multocida* is something to consider when interpreting diagnostic reports and designing treatment options.

Treatment

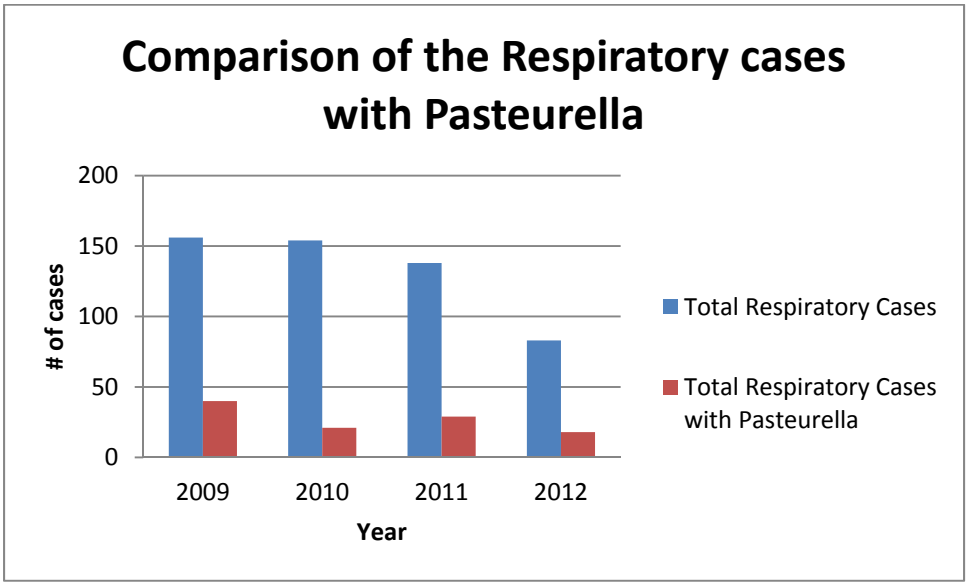
Generally, *P. multocida* is sensitive to cephalosporins, quinolines, penicillins, aminoglycosides, tetracyclines and erythromycin and resistant to tylosin, vancomycin, metronidazole, dapsone and tiamulin [1]. Commercially available combination *P. multocida* and *B. bronchiseptica* bacterins have been successfully used in prevention of atrophic rhinitis but the differences in the strains capable of causing pneumonia may create a different challenge for bacterin success against *P. multocida* pneumonia.

Conclusion

P. multocida is an important swine pathogen that is implicated in atrophic rhinitis, enzootic pneumonia and systemic infection. It is routinely cultured during laboratory analysis from multiple sample sites and should be considered a significant finding. Damage to turbinates from atrophic rhinitis and lung tissue from bronchopneumonia would result in poor performance or death[7]. Understanding its role in the clinical signs is essential to controlling the damage and the losses.

Table 1: Comparison of respiratory cases at Gallant Labs with isolation of *Pasteurella multocida*

	Total # of cases	Total # of respiratory cases	% Respiratory cases	Total # of cases with *Pasteurella multocida*	% of Respiratory cases with *Pasteurella multocida*
Year					
2009	454	156	34%	40	26%
2010	412	154	37%	21	14%
2011	401	138	34%	29	21%
2012	275	83	30%	18	22%



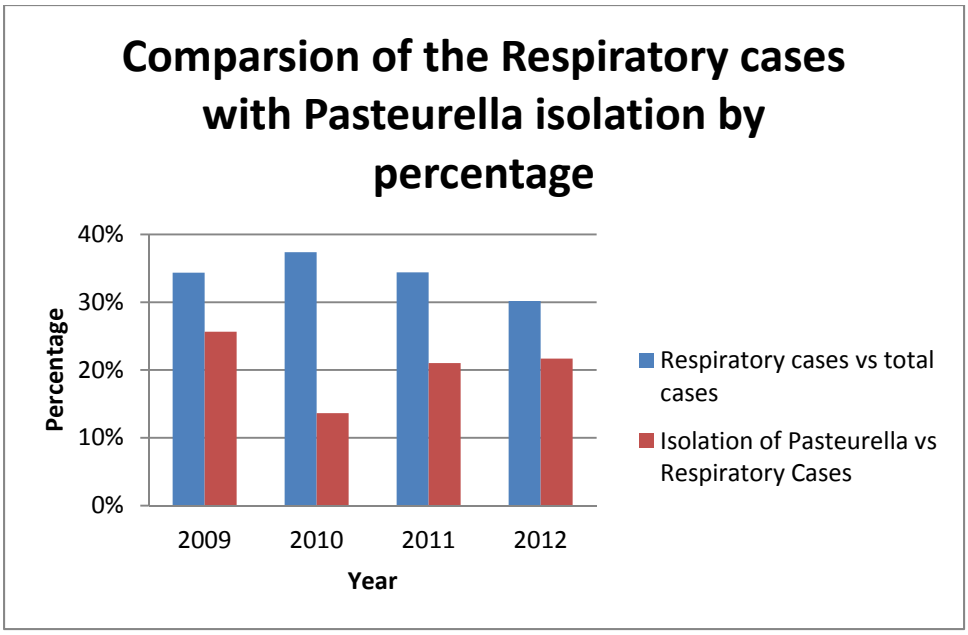


Table 2: Comparison of frequency of isolation of *P. multocida* from swine tissue samples at Gallant

Isolation Frequency in Organs	Organ Submissions			
	Lung	Heart	Spleen	Brain
Total Cases	64	52	9	12
Specimens positive for *P. multocida* isolation	58	36	5	5
% of total cases	91%	69%	56%	42%

Staphylococcus hyicus- not just Greasy Pig

Exudative epidermitis (Greasy Pig) is an infection of the skin by *Staphylococcus hyicus* and appears as greasy exudation, exfoliation and vesicle formation[6]. The exfoliation is caused by an exotoxin produced by the organism. Strains of *Staphylcoccus hyicus* are considered virulent or non-virulent depending on the presence or absence of this exotoxin gene. Through molecular analysis it is known that there are different types of this toxin[6,10] including ExhA, ExhB, ExhC and ExhD, ShetA and ShetB. PCR testing can be used to screen isolates for these exotoxin genes, distinguishing between positive and negative strains[8]. *Staphylococcus hyicus* is easily isolated during routine diagnostic testing and can be found in skin, joint, lung, heart, and brain samples. Recent problems with treatment failures revealed strains with widespread resistance to Penicillin G and Ampicilin[9]. Reports of *S. hyicus* implicated in arthritis and lameness[11] would indicate that the organism can gain entry through the skin and travel to internal organs and joints.

Gallant bacteriology data, gathered through routine testing, showed that *Staphylococcus hyicus* can also be readily isolated in multiple organs including lung, heart and brain- as well as joints. A study [11] showed that *Staphylococcus hyicus* was implicated as the causative agent in 25% of the arthritic cases examined.

Case Study:

In a Gallant diagnostic case 3 brain swabs, 3 joint swabs and 12 tissue samples were submitted from three -6 week old nursery pigs. The clinical signs included slow growing pigs and polyarthritis (no mention of greasy pig) and we were requested to rule out Strep and Glasser's. Results showed predominance of *Staphylococcus hyicus* in one brain swab, all 3 of the joint swabs, the 3 hearts, 1 liver/kidney/spleen sample, the 3 lymph nodes, and the 3 lungs. *Streptococcus suis* was also found in 2 joints and 2 lymph nodes (serotypes 3, 11) and an autoagglutinating isolate -and were considered insignificant due to the overwhelming *Staphylococcus* infection. There was no isolation of *Haemophilus parasuis* (Glasser's) in any of the samples. This *Staphylococcus hyicus* isolate was also resistant to Penicillin G and Ampicillin.

Table 4 compares *Staphylococcus hyicus* strains isolated at Gallant either from organs or skin to determine if there were differences in their sensitivity to antimicrobial agents. Although the sample size is small, it is worth noting that regardless of origin, all isolates were sensitive to Amoxicillin, Amikacin, Apramycin, Ceftiofur, and Trimethoprim Sulphamethoxazole . Conversely, all isolates were resistant to Lincomycin and Spectinomycin. Similar to the previous study[9], there was little sensitivity (1 isolate out of each set) to Penicillin or Ampicillin, however, this same study found 71% of isolates resistant to Ceftiofur which is opposite to our findings. This may be due to regional differences as our data includes isolates from Western Canada. Further studies of isolates from both skin and organ sites along with evaluating any regional differences are necessary to understand the implications of exudative epidermitis and septicemia caused by *Staphylococcus hyicus*.

Table 3: Incidence of *Staphylococcus hyicus* isolation in positive cases submitted to Gallant, based on organ location.

	Ear	Skin	Joint	lung	Heart	Brain
S. hyicus +/cases	3/3	19/19	13/16	14/28	8/26	7/8

The incidence of finding *Staphylococcus hyicus* in multiple internal organs and joints is not unusual as Table 3 indicates. This table shows the frequency of isolation in organs in cases

positive for *Staphylococcus hyicus*. These findings would suggest that greater attention should be given to this organism when recovered from organs and joints of pigs and the associated resistance pattern to antimicrobials when developing treatment strategies.

Treatment

Antimicrobials are widely used for treatment but, due to diverse sensitivity and resistance, the decision should be based on bacterial isolation and susceptibility testing. Currently, there is no commercially available bacterin for *Staphylococcus hyicus* but autogenous bacterins can be made from the herd isolates.

Table 4: Comparative sensitivity of *Staphylococcus hyicus* isolated at Gallant from organs vs skin.

Antimicrobial Sensitivity- *Staphylococcus hyicus*	Organ/joint		Skin/ear	
	sens/total	% sens	sens/total	% sens
Aureomycin	1/6	16%	1/8	13%
Amoxicillin	6/6	100%	8/8	100%
Amikacin	6/6	100%	8/8	100%
Ampicillin	1/6	16%	4/8	50%
Apramycin	6/6	100%	8/8	100%
Ceftiofur (XNL)	6/6	100%	8/8	100%
Lincomycin	0/6	0%	0/7	0%
Clindamycin	1/6	16%	0/7	0%
Penicillin	1/6	16%	1/8	13%
Florfenicol	4/6	66%	7/8	88%
Tylosin	1/6	16%	0/7	0%
Gentamicin	5/6	83%	8/8	100%
Neomycin	5/6	83%	8/8	100%
Spectinomycin	0/6	0%	0/7	0%
Trimethoprim Sulphamethoxazole (SXT)	6/6	100%	8/8	100%
Tetracycline	1/6	16%	1/8	13%
Triple Sulfa	2/6	33%	1/8	13%
Tilmicosin	4/6	66%	3/8	38%

Conclusion

Staphylococcus hyicus is well known for causing greasy pig disease in swine and due to its ability for developing antimicrobial resistance, treatment may be a challenge and require diagnostic testing to target appropriate treatment. The systemic capabilities of this organism as a primary pathogen should also be considered when found in multiple organs and joints and attention paid to the possible strain differences (antibiotic or toxin gene) depending on the site of isolation.

Escherichia coli- Not just an intestinal problem

Escherichia coli is a commensal bacterial organism inhabiting the intestinal tract of warm blooded creatures, both human and animal. While most strains work synergistically with their host, some virulent strains are capable of causing intestinal (ETEC –**E**ntero**T**oxigenic ***E. C****oli)* and extraintestinal (ExPEC- **Ex**traintestinal **P**athogenic ***E. C****oli)* disease, depending on associated virulence factors. The virulence genes for ExPEC strains include an adhesion (P fimbriae, S/F1C fimbriae, F165 fimbriae, Afa/Dr adhesions and type 1 fimbriae), toxins (hemolysin, cytotoxic nectrotizing factor (CNF), and cytolethal distending toxin), surface antigens (group II, group III capsules and lipopolysaccharide), invasins (an invasin responsible for invasion of brain endothelium -IbeA), iron uptake systems (Aerobactin-Aero), and secretion systems (type III secretion system)[12]. These virulence factors facilitate the colonization of the organism, evasion of the host immune system, and subsequent invasion and tissue damage. PCR based testing is used to routinely distinguish ExPEC strains from normal flora and/or post mortem contamination. Table 5 shows the frequency of four virulence factors tested at Gallant compared to the number of positive cases. Aerobactin is the most commonly identified at 78% with P fimbriae and TSH (Temperature Sensitive Hemagglutinin) secondary at 44% each. The cytotoxic nectrotizing factor (CNF) was least frequent at 22%.

Table 5: Frequency of virulence factor detection of system ExPEC strains at Gallant.

Virulence Factor			
P fimbriae	CNF	Aero	TSH
P+/case	CNF+/case	Aero+/case	TSH+/case
4/9	2/9	7/9	4/9
44%	22%	78%	44%

In any one isolate these four factors can be found alone or in combination. For example, one isolate could be just Aero+ as shown in Table 6 while another is P+CNF+Aero+.

Table 6: Summary of E. coli strains identified at Gallant. Each isolate is routinely tested for all 4 virulence factors (VF) and reported at ExPEC with the positive factors.

VF_1	VF_2	VF_3	VF_4	ExPEC Virotype
P	.	Aero	TSH	P+Aero+TSH+
P	CNF	Aero	.	P+CNF+Aero
.	.	Aero	.	Aero+
P	CNF	.	.	P+CNF+
.	.	Aero	TSH	Aero+ TSH+
.	.	Aero	TSH	Aero+TSH+
.	.	.	.	P+ Aero+
P	.	Aero	.	P+ Aero+
.	.	Aero	TSH	Aero+ TSH+

There is wide diversity of the virulence genes (factors) associated with ExPEC strains and these are thought to give it a competitive advantage in the pig gut and allow it to colonize and even dominate the flora of the gut[13]. Although not causing intestinal disease in the host, the gut could be a reservoir.

ExPEC strains can be highly pathogenic with mortality up to 100% and manifested by gross lesions of polyserositis and septicemia[12]. In addition, various strains are capable of causing urinary tract infections, pyelonephritis, pericarditis, meningitis, and septicemia in humans and animals. The genome of *E. coli* has a conserved core of genes that operate the functions of the cell plus a "flexible" gene pool that allows the organism to adapt[14]. The flexible gene pool can gain or lose genomic DNA such as plasmids, transposons etc. that will affect its pathogenicity, antimicrobial resistance and competitiveness in the environment. This means that a commensal *E. coli* strain may transform into a virulent strain if able to gain virulence factors enabling it to colonize, invade and escape the host immune system.

With readily available PCR testing, *E. coli* strains isolated from extraintestinal organs can be identified as potential pathogenic agents and could be compared to isolates identified in the gut. PCR has allowed us to differentiate between significant pathogens and commensal *E. coli* found during routine diagnostic testing. Knowing the significance of the extraintestinal *E. coli* strains will lead to more targeted treatment strategies.

Treatment

Escherichia coli, as proven by its adaptability and diversity, is a challenge to treat using antimicrobials. Table 7 lists some of the common antimicrobial agents and the susceptibility pattern of ExPEC strains isolated at Gallant. The ExPEC strains tested were all resistant to five of the seventeen antimicrobials tested and susceptible to only Apramycin, Gentamycin and Neomycin. A thorough diagnostic evaluation, complete with PCR identification and susceptibility testing is necessary to determine the appropriate treatment strategy. There are no commercially available bacterins available specifically for ExPEC .

Table 7: List of percentage of sensitive ExPEC strains isolated at Gallant for common antimicrobial agents.

Antimicrobial Sensitivity- *Escherichia coli (ExPEC)*	Heart/peritoneum	
	sens/total	% sens
Aureomycin	0/7	0%
Amoxicillin	2/7	29%
Amikacin	6/7	86%
Ampicillin	0/7	0%
Apramycin	7/7	100%
Ceftiofur (XNL)	1/7	14%
Lincomycin	0/7	0%
Clindamycin	0/7	0%
Florfenicol	5/7	71%
Tylosin	0/7	0%
Gentamicin	7/7	100%
Neomycin	7/7	100%
Spectinomycin	1/7	14%
Trimethoprim Sulphamethoxazole SXT	5/7	71%
Tetracycline	1/7	14%
Triple Sulfa	1/7	14%
Tilmicosin	3/7	43%

Conclusion

Escherichia coli are a master at adaptation and capable of causing severe losses in swine due to enteric and extraintestinal disease. PCR methods have improved our ability to determine whether the *E. coli* strain is virulent or non-virulent and this is particularly useful to determine the significance since it can be part of the normal flora or post mortem contamination.

Prevention and treatment will remain challenging due to the variability amongst the strains. The strain capable of causing scours is not the same strain as the one capable of systemic infection and both must be considered significant. Virulent *Escherichia coli* are capable of causing severe losses due to scours (ETEC) but ExPEC strains must also be considered an important pathogen in extraintestinal disease.

Summary

Bacterial and viral microorganisms continue to evolve as environmental pressures from antimicrobial and bacterin/vaccine use, movement and mixing of animals, and the exchange of DNA/RNA fragments occurs. Diligent observations in the lab and development of specific identification test methods such as PCR will assist the veterinarian in understanding the strain variation and subsequent significance in clinical disease.

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